

# SCIENTIFIC PROGRAM & ABSTRACTS

68<sup>th</sup> Annual Meeting

July 6-9, 2021



Boston Marriott Copley Place  
Boston, MA, USA

In support of improving patient care, this activity has been planned and implemented by Amedco LLC and Society for Reproductive Investigation. Amedco LLC is jointly accredited by the Accreditation Council for Continuing Medical Education (ACCME), the Accreditation Council for Pharmacy Education (ACPE), and the American Nurses Credentialing Center (ANCC), to provide continuing education for the healthcare team.



# Society for Reproductive Investigation

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Dear Colleagues,

We are delighted to announce the 68th Annual Meeting of the Society for Reproductive Investigation (SRI) July 6 – 9, 2021 in Boston, MA, USA. **For the first time, the 2021 SRI Annual meeting will be held in a hybrid format—providing options for both in-person and virtual attendance!** Based on current vaccination rates in the US, levels of projected COVID-19 immunity and achievement of herd immunity, and expected relaxation of restrictions for hotel occupancy, many of us can gather together in person this week.

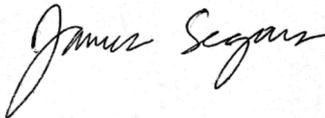
The theme of the 2021 meeting is, “*Navigating the Future for Reproductive Science.*” With Irina Burd, MD, PhD, as Program Director, the Program Committee has developed an outstanding program that includes President’s Distinguished Researchers, career development activities, Oral Sessions, invited Mini-Symposia, invited Lunch and Learn Symposia and a Hot Topics Plenary Session. The meeting will feature inspiring talks from two outstanding President’s Distinguished Researcher Awardees: Joan Steitz, PhD, 2018 Lasker-Koshland Awardee from the Yale School of Medicine and Gregg Semenza, MD, PhD, 2019 Nobel Laureate from Johns Hopkins. In addition, Susan Fisher, PhD, the 2020 March of Dimes Developmental Biology Prize Awardee from University of California, San Francisco will deliver an exciting lecture on her research.

Several career development activities and networking opportunities will be offered, including Career Development Workshops, “Your Grant in One Page Clinic,” and “Connection Corners.” Also *NEW* this year, the Diversity, Equity, and Inclusion Forum will be held on Thursday, July 8, over the lunch hour, giving all participants an option to attend.

To allow all abstract submitters to participate and share their science the poster sessions will be virtual this year. You may find a link to the virtual abstract site on the virtual platform. Participants were given the option to hang their poster in the poster hall if they wish, and so please visit during the scheduled times to support them. We understand that COVID-19 may have delayed research and therefore once again offered **Late Breaking Abstract submissions for the 2021 Annual Meeting.** We have added new abstract categories this year including *Women’s Health Disparities and Inequities and COVID-19.*

The 2021 Annual Meeting will be held in Boston, MA, USA at the Boston Marriott Copley Place. The SRI leadership strongly encourages all in-person attendees to get vaccinated. As always, the 68th Annual SRI Meeting will be highly collegial and provide a forum for outstanding science and the opportunity for fruitful global networking. *On behalf of the SRI Council, Program Committee, and Staff, we are pleased to offer this NEW option for participation! We look forward to networking and sharing science throughout the week at SRI!*

Yours,



Jim Segars, MD  
SRI President, 2019 – 2021




Irina Burd, MD, PhD  
SRI Program Director, 2021



# Society for Reproductive Investigation

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## Executive Committee March 2020 – July 2021

<b>President</b> James H. Segars, MD Baltimore, MD, USA	<b>President Elect</b> Nanette F. Santoro, MD Aurora, CO, USA	<b>President Nominee</b> Stephen Matthews, PhD Toronto, ON, Canada
<b>Immediate Past President</b> Murray D. Mitchell, DPhil, DSc Brisbane, QLD, Australia	<b>President Nominee</b> Carole Mendelson, PhD Dallas, TX, USA	<b>Secretary Treasurer</b> Paul J. Rozance, MD Aurora, CO, USA

## Council Members March 2020 – July 2021

Ayman Al-Hendy, MD, PhD (2022) Chicago, IL, USA	Asgerally T. Fazleabas, PhD (2021) Grand Rapids, MI, USA	Virginia Winn, MD, PhD (2023) Stanford, CA, USA
Irina Burd, MD, PhD (2023) Baltimore, MD, USA	Thomas Jansson, MD, PhD (2021) Aurora, CO, USA	In-Training <i>ad-hoc</i> Council Member Jessica Hebert, PhD Portland, OR, USA
	Amy P. Murtha, MD (2022) San Francisco, CA, USA	

## Program Committee Members

<b>2021 Program Director</b> Irina Burd, MD, PhD Baltimore, MD, USA	Nardhy Gomez-Lopez, PhD Detroit, MI, USA	Leslie Myatt, PhD, FRCOG Portland, OR, USA
<b>Program Committee Chair</b> Emre Seli, MD New Haven, CT, USA	Jessica Hebert, PhD Portland, OR, USA	Mana Parast, MD, PhD San Diego, CA, USA
<b>Incoming Program Chair</b> Bo Rueda, PhD Boston, MA, USA	Thomas Jansson, MD, PhD Aurora, CO USA	Nanette Santoro, MD Aurora, CO USA
Charles Ducsay, PhD Loma Linda, CA, USA	Dineo Khabele, MD St. Louis, MO, USA	James Segars, MD Baltimore, MD, USA
Asgi Fazleabas, PhD Grand Rapids, MI, USA	Shannon Laughlin-Tommaso, MD Rochester, MN, USA	Shannon Whirlledge, MD Boston, MA, USA
Dino A. Giussani, PhD, ScD, FRCOG Cambridge, England, United Kingdom	Carole Mendelson, PhD Dallas, TX, USA	

## SRI Administration

**Executive Director**  
Leah Miller  
Email: lmiller@sri-online.org

**Meetings Manager**  
Jamie Brouws  
Email: jbrouws@sri-online.org

**Program Manager**  
Morgan Derby  
Email: mderby@sri-online.org

**Administrative Coordinator**  
Ciera Arias  
Email: carias@sri-online.org

# Society for Reproductive Investigation

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## Presidents' Page

<b>Year</b>	<b>President</b>	<b>Year</b>	<b>President</b>
1953+	William J. Diekmann	1987	Edward E. Wallach
1954+	William J. Diekmann	1988+	Daniel R. Mishell, Jr.
1955+	Ernest W. Page	1989+	Howard L. Judd
1956+	Nicholas S. Assali	1990+	Carl J. Pauerstein
1957*		1991	Norman F. Gant, Jr.
1958+	Allan C. Barnes	1992	Frederick Naftolin
1959+	Russell R. deAlvarez	1993	W. Ann Reynolds
1960+	Louis M. Hellman	1994+	Gary D. Hodgen
1961+	James T. Bradbury	1995	Alan H. DeCherney
1962+	Leon C. Chesley	1996	Anne Colston Wentz
1963+	Charles E. McLellan	1997	James M. Roberts
1964+	Roger B. Scott	1998	Rogerio A. Lobo
1965+	Jack A. Pritchard	1999	Joe Leigh Simpson
1966+	Ben M. Peckham	2000	Eli Y. Adashi
1967+	J. George Moore	2001	Jennifer R. Niebyl
1968+	Charles H. Hendricks	2002+	Sherman Elias
1969+	Andre E. Hellegers	2003	John R.G. Challis
1970	Edward J. Quilligan	2004	Jerome F. Strauss, III
1971+	Joseph Seitchik	2005	Steven G. Gabbe
1972+	T. Terry Hayashi	2006	Gerson Weiss
1973+	William M. Paul	2007	Linda C. Giudice
1974+	C. Donald Christian	2008	Charles J. Lockwood
1975+	Walter L. Herrmann	2009	Felice Petraglia
1976	Robert B. Jaffe	2010	Leslie Myatt
1977+*	Kenneth J. Ryan	2011	Robert N. Taylor
1978+*	Paul C. McDonald	2012	Stephen J. Lye
1979+	Thomas H. Kirschbaum	2013	Sarah L. Berga
1980+	A. Brian Little	2014	Kelle H. Moley
1981+	Pentti K. Siiteri	2015	Serdar Bulun
1982+	Samuel S.C. Yen	2016	Hugh Taylor
1983+	Lawrence D. Longo	2017	Yoel Sadovsky
1984	James C. Warren	2018	Sandy T. Davidge
1985+	William N. Spellacy	2019	Murray D. Mitchell
1986	Roy M. Pitkin	2020	James H. Segars*
		2021	James H. Segars

\*No Meeting

+Deceased

# Society for Reproductive Investigation

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## SRI WOULD LIKE TO THANK THE 2021 ABSTRACT REVIEWERS

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Laxmi Baxi	Gloria Huang	Michael Ross
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Loic Blanchon	Juan Irwin	Yoel Sadovsky
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Marilyn Cipolla	Jamie Lo	Amy Valent
Charles Coddington	Andrea Loewendorf	Ignatia Van den Veyver
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Nataki Douglas	Ramkumar Menon	Robert Wild
Charles Ducsay	Audrey Merriam	Virginia Winn
Annie Dude	Torri Metz	Amy Wooldridge
Brett Einerson	Manju Monga	Liliya Yamaleyeva
Elizabeth Enninga	Sara Morelli	Xiaohua Yang
Michael Fassett	Terry Morgan	Lynn Yee
Asgerally Fazleabas	Kenichiro Motomura	Yoshio Yoneyama
Maisa Feghali	Louis Muglia	Jie Yu
Jorge Figueroa	Amy Murtha	Tamas Zakar
Antonina Frolova	Indira Mysorekar	Jianhong Zhang
Deepika Garg	Manubai Nagamani	Jing Zheng
Caroline Gargett	Shanmugasundaram Nallasamy	Krina Zondervan
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Nardhy Gomez-Lopez	Perrie O'Tierney-Ginn	
Bernard Gonik	George Osol	
Frank Gonzalez	Anna Palatnik	
Styliani Gouloupoulou	Angela Palumbo	
Michael Gravett	W. Tony Parks	

# Society for Reproductive Investigation

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## On-Site Meeting Registration

All scientific sessions, satellite sessions and poster sessions will be held at the Boston Marriott Copley Place. Entry to all sessions (including guests at the poster sessions) requires a paid registration and the wearing of a name badge.

## Registration Hours

Monday, July 5	3:00 p.m. – 6:30 p.m.
Tuesday, July 6	7:00 a.m. – 7:30 p.m.
Wednesday, July 7	7:00 a.m. – 6:00 p.m.
Thursday, July 8	7:00 a.m. – 7:00 p.m.

## Registration Fees

	<b>ON SITE</b>
Member	\$300.00
Associate Member	\$300.00
In-Training Member	\$100.00
Non-Member	\$400.00
*In-Training Non-Member (Must have support letter)	\$150.00
Emeritus Member	\$300.00
Emeritus Member 70+	Complimentary ( <i>SRI registration only</i> )

## Session Fees:

<b>Satellite Meetings</b> (Tuesday, July 6, 2021)	\$70.00
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\* All In-Training Non Member registrations must be accompanied by a letter from the trainee's supervisor confirming the status of the registrant.

## Speaker Ready Room

Participants and speakers who are scheduled to give oral presentations may preview their presentations prior to their lectures. The Speaker Ready Room is located at the Boston Marriott Copley Place, in the Vineyard. **Please note that all presentations must be saved in widescreen 16x9 Microsoft PowerPoint format. USB Flash Drives, USB Thumb Drives and USB Pen Drives containing presentations must be provided to the AV Technicians in the Speaker Ready Room at least 4 hours prior to the oral session or the day before for morning presentations.**

## SRI Cancellation Policy:

Meeting registration cancellations must be in writing to the SRI administrative office in Milwaukee, WI

555 East Wells Street, Suite 1100  
Milwaukee, WI 53202

Registration fees will be refunded, less a \$50.00 administrative fee if the request is made prior to the online registration deadline date of the current year. **No Refunds** will be made on registration cancellations after the online registration deadline date of the current year.

## Host Hotel

**Boston Marriott Copley Place**  
110 Huntington Avenue  
Boston, MA 02116  
Phone: +1-617-236-5800

## Awards and Their Meanings

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**The DeCherney Society Lifetime Distinguished Service Award** is intended to recognize an individual who has made outstanding service contributions to the Society for Reproductive Investigation and significant contributions to the field of reproductive medicine and women's health. In 2018, current and former trainees of SRI Past President, Dr. Alan DeCherney, provided a generous donation of \$50,000 to endow this award for future recipients. Past awardees include Alan DeCherney (2016); Joe Leigh Simpson; MD (2017); James M. Roberts, MD (2017); John Challis, PhD, FRCOG, FRSC (2018); Norman Gant, MD (2019); Sarah L. Berga, MD (2020).

**The SRI Distinguished Scientist Award** is made annually to a senior member of the Society who has made significant and lasting contributions to the Society and to scientific research in reproductive medicine. The recipient is selected by the President, with the advice of officers and other members. As of 2012, this award is generously sponsored by the Giorgio Pardi Foundation in honor of Professor Giorgio Pardi, a distinguished former member of the SRI. Past awardees include Nicholas S. Assali (1982); Willard M. Allen (1983); Edward H. G. Hon (1984); Donald H. Barron (1985); James T. Bradbury and Leon Chesley (1986); Louis Hellman and Elizabeth Ramsey (1987); Jack A. Pritchard (1988); Kenneth J. Ryan (1989); Paul C. MacDonald (1990); Giacomo Meschia (1991); Samuel S. C. Yen (1992); Robert B. Jaffe (1993); Daniel R. Mishell, Jr. (1994); Paul G. McDonough (1995); Lawrence D. Longo (1996); Pentti K. Siiteri (1997); Edward J. Quilligan (1998); Orlando J. Miller (1999); Howard W. Jones, Jr. (2000); Georgeanna Seegar Jones (2000); James R. Scott (2000); Robert K. Creasy (2000); Frederick P. Zupan (2001); Joe Leigh Simpson (2002); Frederick Naftolin (2003); Luigi Mastroianni, Jr. (2004); Maria Delivoria-Papadopoulous (2005); Jerome F. Strauss, III (2006); D. Michael Nelson (2007); Linda C. Giudice (2008); John R.G. Challis (2009); Gautam Chaudhuri (2010); James M. Roberts (2011); Peter Nathanielsz (2012); Charles J. Lockwood (2013); Carole Mendelson (2014); Eli Adashi (2015); Roberto Romero (2016); Leslie Myatt (2017); Lucilla Poston, PhD (2018); Roger Smith, AM, MBBS, FRACP, PhD (2019); Hugh S. Taylor, MD (2020).

**The President's Achievement Award** is made annually to a member of the Society whose record in scientific investigation is outstanding and assures a continued productive career in research. The recipient is selected by the President, with the advice of officers and other members. Past awardees include Gary D. Hodgen (1983); Evan R. Simpson (1984); John R.G. Challis (1985); Joe Leigh Simpson (1986); A. H. DeCherney (1987); Aaron J.W. Hsueh (1988); Eli Y. Adashi (1989); Jerome F. Strauss (1990); Murray D. Mitchell (1991); Roberto Romero (1992); Maria Delivoria-Papadopoulos (1993); Robert Schenken and M. Linette Casey (1994); Rogerio A. Lobo (1995); Linda C. Giudice (1996); Stephen J. Lye (1997); Deborah J. Anderson (1998); Mark I. Evans (1999); Ricardo Azziz (2000); Sarah L. Berga (2000); Richard S. Legro (2000); Katherine D. Wenstrom (2001); Serdar Bulun (2002); Sandra Davidge (2003); Yoel Sadovsky (2004); Philip Baker (2005); Sam Mesiano (2006); Kelle Moley (2007); Hugh Taylor (2008); Fiona Lyall and Jane Norman (2009); Marilyn Cipolla (2010); Lisa M. Halvorson (2011); Stephen Matthews (2012); Caroline Gargett (2013); Anil Sood (2014); Ayman Al-Hendy and Emily Su (2015); Emre Seli (2016); Jennifer Condon, PhD and Thomas Jansson (2017); Mana Parast, MD, PhD (2018); Sarah England, PhD (2019); Kjersti Aagaard, MD, PhD (2020).

**The SRI Mentorship Award** was established in 2003 to recognize the contributions of a member of the society to training and career development of investigators in the field of reproductive science and women's health. The first recipient of this award was Lawrence D. Longo (2004); Edward J. Quilligan (2005); Mortimer Levitz (2006); Philip J. DiSaia (2007); James Roberts (2008); B.C.J.M. Fauser (2009); Louis Peeters (2010); James C. Rose (2011); John R.G. Challis (2012); Joan Hunt (2013); Leslie Myatt (2014); Linda Giudice (2015); Jerome F. Strauss (2016); Carole Mendelson (2017); Gautam Chaudhuri, MD, PhD (2018); Jane Norman, MD (2019).

**The President's Plenary Award** Recognizes the four highest ranked abstracts chosen for presentation at the President's New Investigator Plenary Session. The recipients of the award will each be presented with a \$1,000 award at the Annual Meeting Awards Ceremony, Tuesday evening. These awards are supported by the SRI Past Presidents Fund.

**The Kusum Lata Award** Established in 2020 and supported by Dr. Bhuchitra Singh, will provide a \$1,000 award to two worthy In Training Investigators, coming from Indian Universities and currently residing in India. The award is based on high quality of their abstract submitted and accepted for the Annual Meeting, chosen by the SRI Program Committee. The award winners will be presented a certificate and monetary award at the Award Ceremony on Tuesday evening during the SRI Annual Scientific Meeting.

**SRI Chinese Trainee Travel Award** Established in 2020, provides a \$1,000 award each to Five worthy In Training Investigators coming from Chinese Universities. The award is based on high quality of abstracts submitted and accepted (decided by the SRI Program Committee). The award winners will be presented a certificate and monetary award at the Award Ceremony on Tuesday evening during the SRI Annual Scientific Meeting.

**The SRI President's Presenter's Award** Originally established in 1996 as the Wyeth President's Presenter Award to recognize the 25 most meritorious oral abstracts submitted by individuals still in training. Fellows and those in both pre and post-doctoral training are eligible to receive the award. The 25 awardees will receive a certificate and a \$1,000 award, and will be recognized during the meeting. The Society has always sought a means by which to encourage young investigators to present their research at our meeting. We anticipate the SRI President's Presenter's Award will encourage more abstract submissions and higher quality abstracts by the very people who need encouragement to consider a research career.

**The Early Career Investigator Travel Award** The Early Career Investigator Travel Award, with support from the National Institutes of Health, was established to enhance the career development of early investigators by facilitating interaction with other scientists in the field, development of research collaborations, and exposure to the latest technologies and scientific developments in reproductive biology and medicine. Early career investigators are defined as individuals who have completed their terminal research degree or end of post-graduate clinical training, whichever date is later, within the past 10 years and who has not previously competed successfully as PD/PI for a substantial NIH independent research award. Up to four \$1,000 travel awards and certificates will be given beginning in 2019 to awardees who will be selected by competitive review of abstracts submitted to each annual SRI meeting by the Program Committee, with special regard paid to representation of women and minorities.

**The Laxmi Baxi Award** Established in 2013 and will be awarded to PhD individuals only, who are either graduate students still in training or postdoctoral fellows within five years of their PhD degree. Two \$1,000 travel awards will be given to the top abstracts in basic reproductive science and in translational reproductive science. The awardees will receive a plaque, award and they will be honored at the SRI Annual Meeting Awards Ceremony. This award is made possible by a generous, long-standing member of SRI, Dr. Laxmi Baxi, and was created to encourage young PhD trainees to present their research at our meeting.

**The Thomas McDonald Award** Acknowledges the highest ranked abstract by an in training investigator within the field of fetal neuroscience. This award honors the legacy of Dr. McDonald, whose immense contributions to the field of obstetrics and gynecology focused upon the neuroendocrinology of the developing fetus, placental function, fetal brain development, and the uterine contractility. The \$750 monetary award and certificate will be presented at the Award Ceremony during the SRI Annual Scientific Meeting.

**The Giorgio Pardi Foundation Awards** Provide a \$1,000 monetary award to a Junior Scientist as well as the three best young worthy investigators coming from Italian Universities. The award winners will be presented at the Award Ceremony during the SRI Annual Scientific Meeting.

**Underrepresented Minority Awards** To both encourage global participation and further diversify the Annual Meeting, SRI offers Awards to allow discounted membership and Annual Meeting registration for 25 individuals who are underrepresented in their country or originate from a low-income, lower-middle-income, or upper-middle-income country. The awardees will be recognized at the Annual Meeting.





**SRI Annual Scientific Meeting**  
*Navigating the Future for Reproductive Science*  
**July 6 – 9, 2021**  
**Boston, MA, USA**

	Monday, July 5	Tuesday, July 6	Wednesday, July 7	Thursday, July 8	Friday, July 9
<b>Morning</b>		<p>7:00 a.m. – 7:15 p.m. Registration</p> <p>8:30 a.m. – 12:00 p.m. Satellite Sessions</p>	<p>7:00 a.m. – 6:30 p.m. Registration</p> <p>8:00 a.m. – 10:00 a.m. Concurrent Oral Presentations I</p> <p>10:00 a.m. – 10:30 a.m. Coffee Break</p> <p>10:30 a.m. – 12:00 p.m. Concurrent Mini Symposia I</p>	<p>7:00 a.m. – 6:30 p.m. Registration</p> <p>7:00 a.m. – 8:00 a.m. SRI Business Meeting <i>*Breakfast will be served and ALL SRI Members are invited to attend.</i></p> <p>8:00 a.m. – 9:00 a.m. President's Distinguished Researchers Award II</p> <p>9:00 a.m. – 9:45 a.m. Hot Topics and Awards Ceremony</p> <p>9:45 a.m. – 10:15 a.m. Coffee Break</p> <p>10:15 a.m. – 11:45 a.m. Concurrent Oral Presentations III</p>	<p>9:00 a.m. – 1:00 p.m. Virtual Career Development Workshops</p>
<b>Afternoon</b>	<p>3:00 p.m. – 6:30 p.m. Registration</p>	<p>1:15 p.m. – 4:30 p.m. Satellite Sessions Continued</p>	<p>12:45 p.m. – 1:45 p.m. Lunch and Learn Symposia I <i>*Ticketed and includes lunch</i></p> <p>12:45 p.m. – 1:45 p.m. Lunch and Learn Symposia II <i>*Ticketed and includes lunch</i></p> <p>12:45 p.m. – 1:45 p.m. Past President's Lunch <i>*Invitation Only</i></p> <p>2:00 p.m. – 3:30 p.m. Concurrent Oral Presentations II</p> <p>3:30 p.m. – 4:00 p.m. Coffee Break</p> <p>4:00 p.m. – 5:30 p.m. Poster Session I</p>	<p>12:00 p.m. – 1:00 p.m. Diversity, Equity and Inclusion Forum <i>*Ticketed and includes lunch</i></p> <p>12:00 p.m. – 1:00 p.m. Lunch and Learn Symposium III <i>*Ticketed and includes lunch</i></p> <p>12:00 p.m. – 1:00 p.m. Lunch and Learn Symposium IV <i>*Ticketed and includes lunch</i></p> <p>1:15 p.m. – 2:45 p.m. Concurrent Oral Presentations IV</p> <p>2:45 p.m. – 4:15 p.m. Poster Session II</p> <p>4:15 p.m. – 6:15 p.m. Mini Symposia II</p>	<p>1:30 p.m. – 2:30 p.m. Virtual Grant Writing Clinic <i>*Application required</i></p>
<b>Evening</b>		<p>5:00 p.m. – 5:20 p.m. Presidential Address</p> <p>5:25 p.m. – 6:00 p.m. March of Dimes Developmental Biology Prize Lecture</p> <p>6:00 p.m. – 7:00 p.m. President's New Investigator Plenary <i>Supported by the Past Presidents' Fund</i></p> <p>7:00 p.m. – 7:30 p.m. Awards Ceremony</p> <p>7:30 p.m. – 8:30 p.m. Welcome Reception</p>	<p>5:45 p.m. – 6:30 p.m. President's Distinguished Researchers Award I</p> <p>6:30 p.m. – 7:10 p.m. Awards Ceremony</p> <p>7:30 p.m. – 9:00 p.m. Connection Corners <i>*Ticketed</i></p>		

Plenary/Oral Sessions	Abstract Numbers	Title Pages	Abstract Pages
<b>Tuesday, July 6, 2021, 6:00 PM - 7:00 PM</b>			
President's Plenary	O-001 - O-004	3A	49A
<b>Wednesday, July 7, 2021, 8:00 AM - 10:00 AM</b>			
Clinical Perinatology I	O-005 - O-012	4A	50A
Gynecology I	O-013 - O-020	4A	53A
Parturition I	O-021 - O-028	5A	56A
Placenta I	O-029 - O-036	6A	59A
Maternal Biology and Health	O-037 - O-044	6A	62A
Reproductive Biology I	O-045 - O-052	7A	65A
<b>Wednesday, July 7, 2021, 2:00 PM - 3:30 PM</b>			
Developmental Programming I	O-053 - O-056 O-155 - O-156	7A	68A 109A
Fetus I	O-057 - O-062	8A	70A
Preeclampsia I: Immune Function/Cytokines	O-063 - O-068	8A	73A
Gynecology II	O-069 - O-074	9A	75A
Parturition II	O-075 - O-080	9A	78A
Reproductive Endocrinology I	O-081 - O-086	10A	81A
<b>Thursday, July 8, 2021, 10:15 AM - 11:45 AM</b>			
Epidemiology	O-087 - O-092	10A	83A
Development Programming II	O-093 - O-098	11A	85A
Reproductive Biology II	O-099 - O-104	11A	88A
Placenta II	O-105 - O-110	12A	90A
Reproductive Endocrinology II	O-111 - O-114 O-157 - O-158	12A	92A 110A
Clinical Perinatology II	O-115 - O-120	13A	94A
<b>Thursday, July 8, 2021, 1:15 PM - 2:45 PM</b>			
Fetus II	O-121 - O-126	13A	96A
Health Disparities and COVID	O-127 - O-132	14A	99A
Placenta III	O-133 - O-136 O-159 - O-160	14A	101A 110A
Reproductive Biology III	O-137 - O-142	15A	102A
Preeclampsia II: Preeclampsia/Related Disorders	O-143 - O-148	15A	105A
Gynecologic Oncology	O-149 - O-152 O-161 - O-162	16A	107A 111A

Poster Sessions	Abstract Numbers	Title Pages	Abstract Pages
<b>Wednesday, July 7, 2021, 4:00 PM - 5:30 PM</b>			
Basic Parturition	W-001 - W-033	16A	112A
Gynecology	W-034 - W-060	18A	124A
Clinical Perinatology	W-061 - W-095	19A	134A
Developmental Programming	W-096 - W-107	21A	148A
Fetus	W-108 - W-125	22A	153A
Gynecologic Oncology	W-127	23A	160A
Maternal Biology	W-128 - W-146	23A	160A
Placenta	W-147 - W-182	24A	168A
Preeclampsia	W-183 - W-199	26A	182A
Reproductive Endocrinology	W-200 - W-216	27A	188A
Reproductive Biology	W-217 - W-238	28A	195A
Epidemiology	W-239 - W-247	29A	203A
Population Health	W-248 - W-251	30A	206A
Women's Health Disparities and Inequities	W-252 - W-254	30A	207A
COVID-19	W-255 - W-263	30A	208A
<b>Thursday, July 8, 2021, 2:45 PM - 4:15 PM</b>			
Basic Parturition	T-001 - T-033	31A	211A
Gynecology	T-034 - T-060	33A	224A
Clinical Perinatology	T-061 - T-095	34A	233A
Developmental Programming	T-096 - T-107	36A	249A
Fetus	T-108 - T-124	37A	253A
Gynecologic Oncology	T-125 - T-126	38A	261A
Maternal Biology	T-127 - T-144	38A	262A
Placenta	T-145 - T-179	39A	271A
Preeclampsia	T-180 - T-195	41A	284A
Reproductive Endocrinology	T-196 - T-211	42A	291A
Reproductive Biology	T-212 - T-232	43A	296A
Epidemiology	T-233 - T-241	44A	304A
Population Health	T-242 - T-244	45A	307A
Women's Health Disparities and Inequities	T-245 - T-247	45A	308A
COVID-19	T-248 - T-256	45A	309A



# **Scientific Program Schedule**

## **68<sup>th</sup> Annual Scientific Meeting**

*Underline represents presenting author; Asterisk represents senior author; Dagger represents an in-training author.*



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**Tuesday, July 6, 2021 - Plenary**

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**6:00 PM - 7:00 PM****Plenary****PRESIDENT'S PLENARY****Salon E-F**

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- 6:00 PM O-001** **Simvastatin Inhibits Estrogen Signaling by Modulating Receptor Trafficking in Leiomyoma Cells**  
*Sadia Afrin<sup>†</sup>, Malak El Sabeh<sup>†</sup>, Mostafa Borahay\*. Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- 6:15 PM O-002** **Genetically-Induced Placental Endocrine Malfunction Alters the Maternal Liver and Whole Body Metabolic Function in Pregnancy**  
*J Lopez-Tello<sup>†</sup>, E Salazar, T Napso, HEJ Yong, ER Christoforou, I Sandovici, M Constancia, Amanda N Sferruzzi-Perri\*. University of Cambridge, Cambridge, United Kingdom.*
- 6:30 PM O-003** **Low-Dose of IL-2 Normalizes Hypertension and Mitochondrial Function in Response to Placental Ischemia**  
*Evangeline Deer<sup>†</sup>, Lorena Amaral, Nathan Campbell, Sarah Fitzgerald, Owen Herroock, Tarek Ibrahim, Babbette LaMarca\*. University of Mississippi Medical Center, Jackson, MS, United States.*
- 6:45 PM O-004** **DHES0815A, a Novel Antibody-Drug Conjugate Targeting HER2/neu, Is Highly Active Against Uterine Serous Carcinomas *In Vitro* and *In Vivo***  
*Joan Rose Tymon-Rosario<sup>†</sup>, Elena Bonazzoli, Bellone Stefania, Aranzazu Manzano, Silvia Pelligra, Adele Guglielmi, Barbara Gnutti, Burak Zeybek, Paola Manara, Luca Zammataro, Justin Harold, Dennis Mauricio, Natalia Buza, Pei Hui, Gary Altwerger, Gulden Menderes, Ratner Elena, Mitchell Clark, Vaagn Andikyan, Gloria Huang, Masoud Azodi, Peter E Schwartz, Alessandro D Santin\*. Yale, New Haven, CT, United States.*

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**Wednesday, July 7, 2021 - Concurrent Session I**


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**8:00 AM - 10:00 AM****Oral****CLINICAL PERINATOLOGY I****Salon AB**

- 8:00 AM O-005** **Molecular Mechanisms Underlying Selective Sorting of miRNAs into Small Extracellular Vesicles in Placental Cells in Gestational Diabetes Mellitus (GDM)**  
Soumyalekshmi Nair†, Andrew Lai, Nanthini Jayabalan, Dominic Guanzon, Katherin Scholz-Romero, David McIntyre, Martha Lappas, Carlos Salomon.<sup>1,5</sup> <sup>1</sup>University of Queensland Centre for Clinical Research, Brisbane, Australia; <sup>2</sup>University of Queensland, Mater Health, South Brisbane, Australia; <sup>3</sup>University of Melbourne, Melbourne, Australia; <sup>4</sup>Mercy Hospital for Women, Victoria, Australia; <sup>5</sup>University of Concepcion, Concepción, Chile.
- 8:15 AM O-006** **Associations of Dietary Glycemic Index and Load during Pregnancy with Blood Pressure, Placental Hemodynamic Parameters and the Risk of Gestational Hypertensive Disorders**  
Clarissa J. Wiertsema†, Rama J. Wahab, Annemarie G.M.G.J. Mulders, Romy Gaillard. *Erasmus MC, Rotterdam, Netherlands.*
- 8:30 AM O-007** **Metabolomic Signatures of Low and High Adiposity Neonates Differ Based on Maternal BMI**  
Begum Aydogan Mathykt†, Brian Piccolo, Kartik Shankar, Perrie O'Tierney-Ginn\*. <sup>1</sup>Brandon Regional Hospital, Brandon, FL, United States; <sup>2</sup>USDA-ARS Arkansas Children's Nutrition Center, Little Rock, AR, United States; <sup>3</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>4</sup>Tufts Medical Center, Boston, MA, United States.
- 8:45 AM O-008** **Adult Offspring of Obese Mice Have Reduced Resistance Artery Vasodilator Responses**  
Ramón A. Lorca\*, Julie A Houck, Jerad H Dumolt, Owen R Vaughan, Kelsey Barner, Theresa L Powell, Thomas Jansson, Lorna G Moore, Colleen G Julian. *University of Colorado Anschutz Medical Campus, Aurora, CO, United States.*
- 9:00 AM O-009** **The Impact of Pre-Pregnancy Maternal Lipid Metabolism on Neonatal Adiposity**  
Raziel Rojas-Rodriguez†, Patrick M Catalano\*. *Tufts Medical Center, Boston, MA, United States.*
- 9:15 AM O-010** **Highly Atherogenic Lipid Particles Are Associated with Preeclampsia (Pre-E) in Obese Women with Unexplained Infertility Who Conceived during Ovarian Stimulation with Intrauterine Insemination (OS-IUI)**  
Robert A. Wild, Rodney K Edwards, David S Wrenn, Yan D Zhao, Karl R Hansen. <sup>1</sup>University of Oklahoma HSC, Oklahoma City, OK, United States; <sup>2</sup>Quest Diagnostics, Secaucus, NJ, United States.
- 9:30 AM O-011** **Frequency and Correlates of Severe Chronic Hypertension (CHTN) 5 - 7 Years after Pregnancy Complicated by Mild CHTN.**  
Ayamo Gina Oben†, Jeff Szychowski, Rachel Sinkey\*, Peter Ketch†, Cooper Elkins†, William Andrews\*, Alan Tita\*. *University of Alabama at Birmingham, Birmingham, AL, United States.*
- 9:45 AM O-012** **A Prognostic Model for Early Risk Stratification of Spontaneous Preterm Birth.**  
Anadeijda Landman†, Marjon de Boer\*, Marije Lamain-de Ruiter\*, Martijn Heymans\*, Arie Franx\*, Martijn Oudijk\*, Mireille Bekker\*, Wendy Koster\*. <sup>1</sup>Amsterdam UMC - VUmc, Amsterdam, Netherlands; <sup>2</sup>University Medical Center Utrecht, Wilhelmina Children's Hospital, Utrecht, Netherlands; <sup>3</sup>Erasmus MC, Rotterdam, Netherlands; <sup>4</sup>Amsterdam UMC - AMC, Amsterdam, Netherlands.

**8:00 AM - 10:00 AM****Oral****GYNECOLOGY I****Salon CD**

- 8:00 AM O-013** **Loss of MIG-6 Results in Endometrial Progesterone Resistance and Endometriosis-Related Infertility through ERBB2 Overexpression.**  
Tae Hoon Kim, Jung-Yoon Yoo, Jung-Ho Shin, Ryan Marquardt, Ulrich Müller, Asgerally Fazleabas, Steven Young, Bruce Lessey, Ho-Geun Yoon, Jae-Wook Jeong\*. <sup>1</sup>Michigan State University, Grand Rapids, MI, United States; <sup>2</sup>Yonsei University College of Medicine, Seoul, Korea, Republic of; <sup>3</sup>Guro Hospital, Korea University Medical Center, Seoul, Korea, Republic of; <sup>4</sup>Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>5</sup>University of North Carolina, Chapel Hill, NC, United States; <sup>6</sup>Wake Forest Health, Winston-Salem, NC, United States.
- 8:15 AM O-014** **The Role of ARID1a in Mediating Endometriosis Disease Progression.**  
Valerie Flores†, Tran Dang, Hugh S Taylor\*, Gloria Huang. *Yale School of Medicine, New Haven, CT, United States.*
- 8:30 AM O-015** **Endometriosis Is a Possible Risk Factor for Premature Cardiovascular Disease.**  
Jessica N Blom†, Maria P Velez\*, Chad McClintock†, Jessica Pudwell\*, Susan Brogly\*, Olga Bougie\*. <sup>1</sup>Kingston Health Sciences, Dept OBGYN, Queen's University, Kingston, ON, Canada; <sup>2</sup>Kingston Health Sciences, ICES Queen's, Kingston, ON, Canada; <sup>3</sup>Kingston Health Sciences, Dept Surgery, Queen's University, Kingston, ON, Canada.
- 8:45 AM O-016** **Randomized, Placebo-Controlled Trial of Botulinum Toxin for Endometriosis-Associated Chronic Pelvic Pain: A Longitudinal Assessment.**  
Pamela Stratton\*, Hannah K Tandon†, Vy Phan†, Jacqueline V Aredo†, Ninet Sinaii, Jay P Shah, Barbara I Karp\*. <sup>1</sup>NIH, Bethesda, MD, United States; <sup>2</sup>University of Nebraska Medical Center, Omaha, NE, United States; <sup>3</sup>Stanford University, Stanford, CA, United States.
- 9:00 AM O-017** **Bleeding Patterns in Women with Endometriosis-Associated Pain Treated with Relugolix Combination Therapy: SPIRIT Program.**  
Andrea S Lukes, Sawsan As-Sanie, Christian M Becker, Mauricio S Abrao, Claudia Mehedintu, Galyna Reznichenko, Linda Giudice, Furong Wang, Viatcheslav G Rakov, Qurratul A Warsi. <sup>1</sup>Carolina Woman's Wellness Center, Durham, NC, United States; <sup>2</sup>University of Michigan, Ann Arbor, MI, United States; <sup>3</sup>Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom; <sup>4</sup>Sao Paulo University, Sao Paulo, Brazil; <sup>5</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; <sup>6</sup>Clinical Maternity Hospital # 4, Zaporizhzhya, Ukraine; <sup>7</sup>University of California, San Francisco, CA, United States; <sup>8</sup>Myovant Sciences, Inc., Brisbane, CA, United States; <sup>9</sup>Myovant Sciences GmbH, Basel, Switzerland.
- 9:15 AM O-018** **Single-Cell Sequencing Reveals Novel Cell Type and Heterogeneous Non-Monoclonal Cell Populations in Human Leiomyomas.**  
Jyoti Goad†, Joshua Rudolph, Jian-Jun Wei, Serdar E Bulun, Debabrata Chakravarti, Aleksandar Rajkovic\*. <sup>1</sup>University of California, San Francisco, CA, United States; <sup>2</sup>Northwestern University, Chicago, IL, United States.

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**Wednesday, July 7, 2021 - Concurrent Session I**


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**9:30 AM**  
**O-019** **Long Noncoding RNA MIAT Modulates the Extracellular Matrix Deposition in Leiomyomas via Sponging MiR-29 Family.**

Tsai-Der Chuang, Derek Quintanilla, Drake Boos, Omid Khorram\*. *LA Biomed at UCLA Medical Center, Torrance, CA, United States.*

**9:45 AM**  
**O-020** **Reduction in Menstrual Blood Loss in Patients Treated with Relugolix Combination Therapy: LIBERTY Long-Term Extension Study.**

Ayman Al-Hendy<sup>1</sup>, Andrea S Lukes<sup>2</sup>, Alfred Poindexter III<sup>3</sup>, Roberta Venturella<sup>4</sup>, Claudio Villarreal<sup>5</sup>, Rachel B Wagman<sup>6</sup>, Yulan Li<sup>6</sup>, Laura McKain<sup>6</sup>, Elizabeth A Stewart<sup>7</sup>. <sup>1</sup>*University of Chicago, Chicago, IL, United States*; <sup>2</sup>*Carolina Woman's Wellness Center, Durham, NC, United States*; <sup>3</sup>*Baylor College of Medicine and St. Luke's Episcopal Hospital, Houston, TX, United States*; <sup>4</sup>*University Magna Graecia, Catanzaro, Italy*; <sup>5</sup>*University of Chile, Santiago, Chile*; <sup>6</sup>*Myovant Sciences, Inc., Brisbane, CA, United States*; <sup>7</sup>*Mayo Clinic, Rochester, MN, United States.*

**8:00 AM - 10:00 AM**

**Oral**

**PARTURITION I**

**Salon HI**

**8:00 AM**  
**O-021** **Upregulation of miR-877-3p and Downregulation of miR-186-3p in the Placentas of Patients with Idiopathic Preterm Birth.**

Duygu Mutluay, Xiaofang Guo†, Ozlem Guzeloglu Kayisli, Frederick Schatz, Umit Kayisli, Charles Lockwood. *University of South Florida, Morsani College of Medicine, Tampa, FL, United States.*

**8:15 AM**  
**O-022** **Iron-Dependent Apoptosis Causes Embryotoxicity in Inflamed and Obese Pregnancy.**

Allison L Fisher†<sup>1</sup>, Veena Sangkhae<sup>2</sup>, Tomas Ganz<sup>2</sup>, Elizabeta Nemeth\*. <sup>1</sup>*Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States*; <sup>2</sup>*Center for Iron Disorders, UCLA, Los Angeles, CA, United States.*

**8:30 AM**  
**O-023** **A Broad Spectrum Chemokine Inhibitor Prevents Infection-Induced Myometrial Inflammation and Activation.**

Adam Boros-Rausch†<sup>1,2</sup>, Tsung-Yen Wu<sup>3</sup>, Kristina Adams Waldorf<sup>3</sup>, Oksana Shynlova\*,<sup>1,2,4</sup> Stephen Lye\*.<sup>1,2,4</sup> <sup>1</sup>*The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada*; <sup>2</sup>*Department of Physiology, The University of Toronto, Toronto, ON, Canada*; <sup>3</sup>*The University of Washington, United States, Washington, WA, Canada*; <sup>4</sup>*Department of Obstetrics & Gynecology, The University of Toronto, Toronto, ON, Canada.*

**8:45 AM**  
**O-024** **Exposure to Experimental Fetal Inflammatory Response Syndrome Results in IL-6 Dependent Upregulation of Interferon Lambda Expression and Subsequent Loss of Neonatal Paneth Cells.**

Sarah Nichole Watson†, Huiyu Gong, Brian Jubert†, Steven Mcelroy\*. *University of Iowa, Iowa City, IA, United States.*

**9:00 AM**  
**O-025** **Establishment of a Mouse Uterine Explant Model to Study Inflammation in Pregnancy and Parturition.**

Madeline Snedden<sup>1</sup>, Chandrashekar Kyathanahalli,<sup>1,2</sup> Emmet Hirsch\*.<sup>1,2</sup> <sup>1</sup>*NorthShore University HealthSystem, Evanston, IL, United States*; <sup>2</sup>*University of Chicago, Chicago, IL, United States.*

**9:15 AM**  
**O-026** **Inflammatory-Related Mir-612 Is Increased in Circulating Exosome-Like Vesicles from Women Undergoing Preterm Birth.**

Bruna Ribeiro de Andrade Ramos\*<sup>1</sup>, Júlia Abbade Tronco†<sup>1</sup>, Márcio de Carvalho\*,<sup>1</sup> Patrícia Pintor dos Reis\*,<sup>1</sup> Juliano Coelho da Silveira\*,<sup>2</sup> Márcia Guimarães da Silva\*.<sup>1</sup> <sup>1</sup>*Sao Paulo State University, Botucatu, Brazil*; <sup>2</sup>*Sao Paulo University, Pirassununga, Brazil.*

**9:30 AM**  
**O-027** **Dose-Response Profile of a Novel Anti-Interleukin-1 Therapeutic, Rytvela, for Prevention of Preterm Birth.**

Tiffany Habelrin†<sup>1,2</sup>, Sarah-Eve Loisel†<sup>1,2</sup>, France Côté†<sup>1,2</sup>, Xin Hou\*,<sup>2</sup> Christiane Quiniou\*,<sup>2</sup> Sylvain Chemtob\*.<sup>1,2</sup> <sup>1</sup>*Université de Montréal, Montreal, QC, Canada*; <sup>2</sup>*CHU Sainte-Justine, Montreal, QC, Canada.*

**9:45 AM**  
**O-028** **Placenta Serum Amyloid A-2 (SAA-2) Plays an Important Role in Maternal Inflammation-Induced Adverse Fetal Outcomes.**

Yang Liu†, Jin Liu†, Anguo Liu†, Irina Burd\*, Lei Jun\*. *Johns Hopkins University, Baltimore, MD, United States.*

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**Wednesday, July 7, 2021 - Concurrent Session I**


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**8:00 AM - 10:00 AM****Oral****PLACENTA I****Salon KJ**

- 8:00 AM O-029** **A Microphysiological Model of Human Trophoblast Invasion during Early Embryo Implantation.**  
Sneha Mani,<sup>1</sup> Ju Young Park,<sup>1</sup> Jeremy Clair,<sup>2</sup> Cassidy Blundell,<sup>1</sup> Rachel Young,<sup>1</sup> Jessica Kanter,<sup>1</sup> Scott Gordon,<sup>3</sup> Yoon-Suk Yi,<sup>1</sup> Dan Donggeun Huh\*,<sup>1</sup> Monica Mainigi\*,<sup>1</sup> <sup>1</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>2</sup>Pacific Northwest National Laboratory, Richland, WA, United States; <sup>3</sup>The Children's Hospital of Philadelphia, Philadelphia, PA, United States.
- 8:15 AM O-030** **The Utero-Placental Vascular Skeleton: A New Image Processing Technique to Estimate Utero-Placental Vascular Morphologic Development and the Association with Maternal Hemodynamic Adaptation to Pregnancy.**  
Eline S de Vos†, Anton HJ Koning\*, Régine PM Steegers-Theunissen\*, Sten P Willemsen\*, Bas B van Rijn\*, Eric AP Steegers\*, Annemarie GMGJ Mulders\*. Erasmus Medical Centre, Rotterdam, Netherlands.
- 8:30 AM O-031** **Placental Cell-Specific Extracellular Vesicle Profiles from Syncytiotrophoblasts and Extravillous Trophoblasts in the First Trimester.**  
Terry Morgan, Mayu Morita, Leslie Myatt. Oregon HS University, Portland, OR, United States.
- 8:45 AM O-032** **Impaired Cell Polarity and Bi-Directional Endothelial Cell-Matrix Interactions Mediate Disrupted Fetoplacental Angiogenesis.**  
Shuhan Ji\*, Diane Gumina†, Emily J Su\*. University of Colorado School of Medicine, Aurora, CO, United States.
- 9:00 AM O-033** **Activation of Amnion Signaling during Intrauterine Inflammation Is TNF-Dependent.**  
Pietro Presicce†, M Cappelletti†, M Morselli†, P Senthamaraikannan†, L Miller\*, M Pellegrini\*, A Jobe\*, C Chougnet\*, S Kallapur\*. <sup>1</sup>UCLA David Geffen School of Medicine, Los Angeles, CA, United States; <sup>2</sup>Inst for Quantitative and Computational Biosciences UCLA, Los Angeles, CA, United States; <sup>3</sup>Cincinnati Children's Hospital, Cincinnati, OH, United States; <sup>4</sup>UCD, Davis, CA, United States.
- 9:15 AM O-034** **Role of GATA2 and GATA3 in Syncytiotrophoblast Development during Mammalian Placentation.**  
Ananya Ghosh†. University of Kansas Medical Center, Kansas City, KS, United States.
- 9:30 AM O-035** **Females Are Not Just 'Protected' Males: Sex-Specific Vulnerabilities in Placenta and Brain after Prenatal Immune Disruption.**  
Amy E Braunt†, Pamela A Carpentier†, Brooke A Babineau†, Aditi R Narayan†, Michelle L Kielhold†, Hyang Mi Moon†, Jennifer Su†, Vidya Saravanapandian†, Theo D Palmer\*. <sup>1</sup>Stanford, Stanford, CA, United States; <sup>2</sup>Northwestern, Chicago, IL, United States; <sup>3</sup>Trinity Biosciences, Brisbane, CA, United States; <sup>4</sup>Oregon Health & Science University, Portland, OR, United States; <sup>5</sup>University of California San Diego, La Jolla, CA, United States.
- 9:45 AM O-036** **Derivation of Human Trophoblast Stem Cells from Pluripotent Stem Cells to Model Early Placental Development.**  
Francesca Soncin, Mariko Horii, Rob Morey†, Tony Buy, Daniela Requena, Virginia Chu Cheung†, Omar A Farah, Don Pizzo, Mana M Parast. University of California San Diego, La Jolla, CA, United States.

**8:00 AM - 10:00 AM****Oral****MATERNAL BIOLOGY AND HEALTH****Wellesley**

- 8:00 AM O-037** **Percutaneous Electrical Stimulation of Skeletal Muscle Attenuates Insulin Resistance in Pregnant Rats with Diet-Induced Obesity.**  
David Coggin-Carr†, Keara McElroy-Yaggy\*, Paul D Taylor\*, Anna L David\*, Elisabet Stener-Victorin\*, Tom Jetton\*. <sup>3</sup>University of Vermont, Burlington, VT, United States; <sup>2</sup>University College London, London, United Kingdom; <sup>3</sup>University of Vermont, Colchester, VT, United States; <sup>4</sup>Kings College London, London, United Kingdom; <sup>5</sup>Karolinska Institutet, Stockholm, Sweden.
- 8:15 AM O-038** **Seasonality and the Immune System during Pregnancy: Impact of Seasonal Affective Disorder.**  
Cindy Xin Wen Zhang†, Robert Levitan, Stephen Matthews. University of Toronto, Toronto, ON, Canada.
- 8:30 AM O-039** **Estradiol Stimulates Pregnancy-Dependent H<sub>2</sub>S Biosynthesis in Human Uterine Artery Endothelial Cells via ERα/ERβ-Mediated Upregulation of CBS Transcription.**  
Jin Bai†, Thomas J Lechuga†, Qian-rong Qi†, Yi-hua Yang†, Quan-wei Zhang†, Yan Li†, Dong-bao Chen\*. University of California, Irvine, CA, United States.
- 8:45 AM O-040** **Placental miRNAs Predicted to Target Insulin Signaling Pathways Are Associated with Maternal Insulin Resistance in Late Pregnancy.**  
Fernanda L Alvarado Flores†, William Beyer, Tomoko Kaneko-Tarui, Tianjiao Chu, Yoel Sadovsky, Patrick Catalano, Perrie O'Tierney-Ginn\*. <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, United States.
- 9:00 AM O-041** **Clot Formation Potential in the Late Pregnant and Postpartum Woman.**  
Elizabeth Barker†, Ira Bernstein, Thomas Orfeo, Kelley McLean, Maria Cristina Bravo. Larner College of Medicine, University of Vermont, Burlington, VT, United States.
- 9:15 AM O-042** **Identification and Isolation of Rare Microchimeric Cells in Maternal and Cord Blood.**  
Yonghou Jiang, John Houck, Marc Carlson, Stephen McCartney†, Kelsey Olerich†, Sami B Kanaan, J Lee Nelson, Raj Shree, Whitney Harrington\*. <sup>1</sup>University of Washington, Seattle, WA, United States; <sup>2</sup>Seattle Children's Research Institute, Seattle, WA, United States; <sup>3</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, United States.
- 9:30 AM O-043** **Women with Adverse Pregnancy Outcomes Have Higher Risk of Midlife Stroke: The PATH Study.**  
Eliza C Miller, Natalie A. Bello, Rindcy Davis, Alexander M Friedman, Mitchell S.V. Elkind, Ronald Wapner, Sarah E. Tom\*. Columbia University, New York, NY, United States.
- 9:45 AM O-044** **Isolating Mechanisms on Future Cardio-Renal Risk in Mothers with Complicated Pregnancy.**  
Kimberley J. Botting, Wen Tong†, Youguo Niu†, Tessa A Garrud†, Lin Zhang†, Sage G Ford†, Qiang Lyu†, Olga V Patey†, Dino A Giussani\*. University of Cambridge, Cambridge, United Kingdom.

## Wednesday, July 7, 2021 - Concurrent Session II

8:00 AM - 10:00 AM

Oral

### REPRODUCTIVE BIOLOGY I

Suffolk

- 8:00 AM O-045** **Small NonCoding RNA Biotypes in the Human Preimplantation Embryo.**  
*Sophie Petropoulos\**,<sup>1,2,3</sup> Stewart J Russell†,<sup>4</sup> Cheng Zhao†,<sup>2</sup> Karen Menezes†,<sup>4</sup> Clifford L Librach\*,<sup>4,5,6</sup>  
<sup>1</sup>University of Montreal, Montreal, QC, Canada; <sup>2</sup>Karolinska Institutet, Stockholm, Sweden; <sup>3</sup>Centre de recherche du CHUM, Montreal, QC, Canada; <sup>4</sup>CReATe Fertility Centre, Toronto, ON, Canada; <sup>5</sup>University of Toronto, Toronto, ON, Canada; <sup>6</sup>Women's College Hospital, Toronto, ON, Canada.
- 8:15 AM O-046** **Screening Putative Haplo-Essential Translation Genes for Contribution to Early Reproductive Failure.**  
*Luwam Ghidai\**,<sup>1</sup> Denise Lanza,<sup>1</sup> Lauryl M. J. Nutter,<sup>2</sup> Pilar Cacheiro,<sup>3</sup> Violeta Munoz-Fuentes,<sup>4</sup> Jason Heaney,<sup>1</sup>  
<sup>1</sup>Baylor College of Medicine, Houston, TX, United States; <sup>2</sup>The Hospital for Sick Children, Toronto, ON, Canada; <sup>3</sup>Queen Mary University, London, United Kingdom; <sup>4</sup>Wellcome Genome Campus, Hinxton, United Kingdom.
- 8:30 AM O-047** **Elucidating the Role of FOXO3 in Regulating Ovarian Reserve and Function in Humans.**  
*Caterina Clementi*, Karen Hunter Cohn, Genevieve Galarneau, Piraye Yurttas Beim\*. *Celmatix Inc., New York, NY, United States.*
- 8:45 AM O-048** **Loss of Ovarian Expression of the Noncoding RNA H19 Promotes Susceptibility to Doxorubicin-Induced DNA Damage.**  
*Amanda N. Kallen\**,<sup>1</sup> Pingping Lu,<sup>2</sup> Jing Wang,<sup>3</sup> Joshua Johnson,<sup>4</sup> <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>Women's Hospital of Zhejiang University School of Medicine, Hangzhou, China; <sup>3</sup>Department of Oncology, Beijing Friendship Hospital, Capital Medical University, Beijing, China; <sup>4</sup>University of Colorado Denver, Aurora, CO, United States.
- 9:00 AM O-049** **Novel Anti-Mullerian Hormone Receptor 2 Binding Peptide (AMHR2BP) Modulates Oocyte Function In Vivo.**  
*Laura Detti\**,<sup>1</sup> Ghassan M Saed\*,<sup>2</sup> <sup>1</sup>Cleveland Clinic, Cleveland, OH, United States; <sup>2</sup>Wayne State University, Detroit, MI, United States.
- 9:15 AM O-050** **Aneuploidy in Human Embryos May Elicit a Premature Differentiation Response in the Inner Cell Mass.**  
*Angel Martin†*,<sup>1</sup> Francisco Dominguez,<sup>1</sup> Alicia Quiñonero,<sup>1</sup> Carmina Vidal,<sup>2</sup> Amparo Mercader,<sup>1,2</sup> Fernanda Insua,<sup>2</sup> Maria Jose De los Santos,<sup>1,2</sup> <sup>1</sup>IVI Foundation-IIS La Fe, Valencia, Spain; <sup>2</sup>IVI RMA, Valencia, Spain.
- 9:30 AM O-051** **Bromodomain Extraterminal (BET) Family Inhibitor JQ1 Inhibits Nuclear Maturation and Cytoplasmic Organization of Oocytes.**  
*Keerthana Karunakar Poojary†*, Sandhya Kumari†, Satish K Adiga, Guruprasad Kalthur\*. *Kasturba Medical College, Udupi, India.*
- 9:45 AM O-052** **Opposite Effects of LH/PKA/MTOR and AMPK Signaling on Induction of Autophagy in Luteal Cells.**  
*Emilia Przygodzka†*, Michele R Plewest†, Guojuan Li, John S Davis\*. *University of Nebraska Medical Center, Omaha, NE, United States.*

2:00 PM - 3:30 PM

Oral

### DEVELOPMENTAL PROGRAMMING I

Salon AB

- 2:00 PM O-053** **Epigenetic Effects of N-acetylcysteine on H<sub>2</sub>S-Mediated Protection Against Fetal Origins of Vascular Disease.**  
*A. A. Paz†*,<sup>1</sup> T. A. Garrud†,<sup>2</sup> E. Peñaloza†,<sup>1</sup> F. Vega-Tapia†,<sup>1</sup> S. G. Ford,<sup>2</sup> Y. Niu,<sup>2,3,4</sup> B. J. Krause\*,<sup>1</sup> D. A. Giussani\*,<sup>2,3,4</sup> <sup>1</sup>Universidad de O'Higgins, Rancagua, Chile; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom; <sup>3</sup>Centre for Trophoblast Research, University of Cambridge, Cambridge, United Kingdom; <sup>4</sup>Cambridge Cardiovascular Strategic Research Initiative, University of Cambridge, Cambridge, United Kingdom.
- 2:15 PM O-054** **Placental Endocrine Malfunction Causes Sex Specific Changes in Metabolic Organs of Fetal and Adult Mouse Offspring.**  
*Efthimia Christoforou†*, Panayiotis Laouris†, Jorge Lopez-Tello†, Marta Ibanez Lligona†, Hannah Yong, Alison Forhead, Amanda Sferruzzi-Perri\*. *University of Cambridge, Cambridge, United Kingdom.*
- 2:30 PM O-055** **Maternal Obesity Upregulates Fetal Heart Pparg Expression with Consequences for Later Life Cardiac Metabolic and Contractile Function in Mice.**  
*O. R. Vaughan*,<sup>1</sup> J. Chan,<sup>2</sup> L. Cox,<sup>2</sup> V. Ferchaud-Roucher,<sup>1</sup> F. Rosario,<sup>1</sup> J. E. B. Reusch,<sup>1</sup> A. C. Keller,<sup>1</sup> T. L. Powell,<sup>1</sup> T. Jansson.<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>Wake Forest School of Medicine, Winston-Salem, NC, United States.
- 2:45 PM O-056** **Defining the Role of the Hypothalamic Pituitary Adrenal Axis in the Relationship between Fetal Growth and Adult Cardiometabolic Outcomes.**  
*Wriyu N Martin†*,<sup>1,2</sup> Carol A Wang,<sup>1,3</sup> Stephen J Lye,<sup>4</sup> Rebecca M Reynolds,<sup>5</sup> Stephen G Matthews,<sup>4,6</sup> Carly E McLaughlin,<sup>7</sup> Christopher Oldmeadow,<sup>1,3</sup> Roger Smith,<sup>1,3</sup> Craig E Pennell\*,<sup>1,3</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter New England Local Health District, New South Wales, Australia; <sup>3</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>4</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>5</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>6</sup>University of Toronto, Toronto, ON, Canada; <sup>7</sup>Curtin University, Western Australia, Australia.
- 3:00 PM O-155** **Zika Virus Vertical Transmission Dynamics in the Pregnant Rhesus Macaque.**  
*Michelle R Koenig†*,<sup>1</sup> A Mitzey,<sup>1</sup> L T Keding,<sup>1</sup> T A Treadway,<sup>1</sup> H Simmons,<sup>1</sup> A Mejia,<sup>1</sup> M I Bliss,<sup>1</sup> A M Weiler,<sup>1</sup> T Friedrich,<sup>1</sup> X Zeng,<sup>2</sup> D H O'Connor,<sup>1</sup> E L Mohr,<sup>1</sup> T G Golos\*,<sup>1</sup> <sup>1</sup>University of Wisconsin Madison, Madison, WI, United States; <sup>2</sup>Fort Detrick, Frederick, MD, United States.
- 3:15 PM O-156** **Tracking Postnatal Behavioral Outcomes in a Novel Mouse Model of Congenital CMV Infection.**  
*Gregory Wohl Kirschen†*,<sup>1</sup> Anguo Liu,<sup>1</sup> Yang Liu,<sup>1</sup> Ashley Coggins,<sup>1</sup> Jun Lei,<sup>1</sup> Karen Racicot,<sup>2</sup> Andrew Thagard,<sup>3</sup> Irina Burd\*,<sup>1</sup> <sup>1</sup>Johns Hopkins, Baltimore, MD, United States; <sup>2</sup>Michigan State University, East Lansing, MI, United States; <sup>3</sup>Uniformed Services University, Bethesda, MD, United States.

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**Wednesday, July 7, 2021 - Concurrent Session II**


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**2:00 PM - 3:30 PM****Oral****FETUS I****Salon CD**

- 2:00 PM O-057** **Hypoxemia Prevents Insulin Mediated Suppression of Gluconeogenic Gene Expression in the Fetal Liver during Hypoglycemia.**  
Priya Mukherjee†, Amanda Jones,<sup>1</sup> Paul Rozance,<sup>1</sup> Brown Laura,<sup>1</sup> Sean Limesand,<sup>2</sup> Stephanie Wesolowski.<sup>1</sup>  
<sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University of Arizona, Tuscon, AZ, United States.
- 2:15 PM O-058** **Maternal Obesity Induces Fetal Sheep Hepatic Oxidative Stress (OS) and Mitochondrial Damage Predominantly in the Right Lobe.**  
Susana P Pereira†,<sup>1,2</sup> Luis F Grilo†,<sup>2</sup> Mariana S Diniz†,<sup>3</sup> Carolina Tocantins†,<sup>2</sup> João D Martins†,<sup>2</sup> Stephen Ford\*,<sup>4</sup> Peter W Nathanielsz\*,<sup>4</sup> Paulo J Oliveira\*,<sup>2</sup> <sup>1</sup>University of Porto, Porto, Portugal; <sup>2</sup>University of Coimbra, Coimbra, Portugal; <sup>3</sup>University of Coimbra, Porto, Portugal; <sup>4</sup>University of Wyoming, Laramie, WY, United States.
- 2:30 PM O-059** **Impaired Autonomic Control of Heart Rate Variability during Acute Stress in the Chronically Hypoxic Fetus.**  
N. Hafiz†,<sup>1</sup> B. J Allison,<sup>2</sup> N. Itani,<sup>2</sup> K. J Botting,<sup>2</sup> Y. Niu,<sup>2</sup> C. C Lees,<sup>1</sup> C. J Shaw\*,<sup>1</sup> D. A. Giussani\*,<sup>2</sup> <sup>1</sup>Imperial College London, London, United Kingdom; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.
- 2:45 PM O-060** **RNAseq of Amniotic Fluid Reveals Activation of the Innate Immune Response and Differential Expression of Brain Transcripts in Fetuses with Cytomegalovirus Infection.**  
Lisa Hui\*,<sup>1,2,3,4</sup> Luc de Catte,<sup>5</sup> Neeta Vora,<sup>6</sup> Sally Beard,<sup>1</sup> Jovana Maksimovic,<sup>7</sup> Alicia Oshlack,<sup>7</sup> Susan P Walker,<sup>1,2</sup> Natalie Hannan\*,<sup>1</sup> <sup>1</sup>University of Melbourne, Melbourne, Australia; <sup>2</sup>Mercy Hospital for Women, Heidelberg, Australia; <sup>3</sup>Murdoch Children's Research Institute, Parkville, Australia; <sup>4</sup>Northern Health, Epping, Australia; <sup>5</sup>Universitair Ziekenhuis, Leuven, Belgium; <sup>6</sup>University of North Carolina, Chapel Hill, NC, United States; <sup>7</sup>Peter MacCallum Cancer Centre, Parkville, Australia.
- 3:00 PM O-061** **Uterine Fibroids Associated with Lower Fetal Fraction and Indeterminate NIPS Results in Non-Obese Subjects.**  
Teodora Kolarova†, Hayley MacKinnon†, Jaclynnne Hedge, Christina Lockwood, Raj Shree\*. *University of Washington, Seattle, WA, United States.*
- 3:15 PM O-062** **Cervical Gene Delivery of the Antimicrobial Peptide Human  $\beta$  Defensin 3 (HBD3) Reduces Perinatal Neuroinflammation in a Mouse Model of Ascending Infection-Associated Preterm Birth.**  
Ashley K Boyle†,<sup>1</sup> Natalie Suff,<sup>2</sup> Simon N Waddington,<sup>1</sup> Donald Peebles\*,<sup>1</sup> <sup>1</sup>University College London, London, United Kingdom; <sup>2</sup>King's College London, London, United Kingdom.

**2:00 PM - 3:30 PM****Oral****PREECLAMPSIA I: IMMUNE FUNCTION/ CYTOKINES****Salon HI**

- 2:00 PM O-063** **Functional Enrichment Analysis of Differentially Expressed Transcripts/Genes of Medium/Large STBEVs Identified Potentially Dysregulated Pathways in Early Onset Preeclampsia (EOPE).**  
Toluwalase Awoyemi†, Adam Cribbs, Wei Zhang, Chris Redman, Manu Vatish\*. *University of Oxford, Oxford, United Kingdom.*
- 2:15 PM O-064** **Proinflammatory and Anti-Inflammatory Cytokine Responses by Decidua T Cells in Preeclampsia.**  
Ai-ris Yonekura Collier, Dan H Barouch\*. *Beth Israel Deaconess Medical Center, Boston, MA, United States.*
- 2:30 PM O-065** **Impact of Preeclampsia on Polarization and Functionality of Hofbauer Cells.**  
Monika Horvat Mercknik†, Carolin Schlieffsteiner\*, Christian Wadsack\*. *Medical University of Graz, Graz, Austria.*
- 2:45 PM O-066** **CD4 T Cells Deficiency Enhances Internal Carotid Artery Constriction in Postpartum Mice: The Role of Nitric Oxide.**  
Natalia I Gokina, Rebecca I Fairchild, Kirtika Prakash, Nicole M DeLance, Elizabeth A Bonney\*. *University of Vermont, Burlington, VT, United States.*
- 3:00 PM O-067** **Noninvasive Prediction of Preeclampsia in Pregnancy with Circulating RNA.**  
Mira N Moufarrej†,<sup>1</sup> Ronald J Wong,<sup>1</sup> Ana A Campos,<sup>1</sup> Cecele C Quaintance,<sup>1</sup> Rene V Sit,<sup>2</sup> Michelle Tan,<sup>2</sup> Norma F Neff,<sup>2</sup> Maurice L Druzin,<sup>1</sup> Virginia D Winn,<sup>1</sup> Gary M Shaw,<sup>1</sup> David K Stevenson,<sup>1</sup> Stephen R Quake\*,<sup>1,2</sup> <sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>Chan Zuckerberg Biohub, Stanford, CA, United States.
- 3:15 PM O-068** **Circulating SIGLEC6 Is Deranged in Preeclampsia and May Be a Biomarker of Disease Severity.**  
Tu'uhevaha J Kaitu'u-Lino,<sup>1</sup> Susan P Walker,<sup>1</sup> Teresa M MacDonald,<sup>1</sup> Catherine Cluver,<sup>2</sup> Roxanne Hastie,<sup>1</sup> Lina Bergman,<sup>3</sup> Lesley McCowan,<sup>4</sup> Rennae Taylor,<sup>4</sup> Emerson Keenan,<sup>1</sup> Natalie J Hannan,<sup>1</sup> Ping Cannon,<sup>1</sup> Tuong-Vi Nguyen,<sup>1</sup> Manju Kandel,<sup>1</sup> Stephen Tong.<sup>1</sup> <sup>1</sup>University of Melbourne, Melbourne, Australia; <sup>2</sup>Stellenbosch University, Cape Town, South Africa; <sup>3</sup>Uppsala University, Uppsala, Sweden; <sup>4</sup>University of Auckland, Auckland, New Zealand.

## Wednesday, July 7, 2021 - Concurrent Session II

2:00 PM - 3:30 PM

Oral

### GYNECOLOGY II

Salon KJ

**2:00 PM O-069** **Pregnancy Outcomes Following Routine Early Provision of IUD after First Trimester Induced Abortion - 5-year Follow-Up of a Randomized Controlled Trial.**

Oskari M. Heikinheimo\*,<sup>1</sup> Elina Pohjoranta†,<sup>1</sup> Satu Suhonen\*,<sup>2</sup> Maarit Mentula\*,<sup>1</sup> Mika Gissler.<sup>3,4</sup> <sup>1</sup>*Helsinki University Hospital, Helsinki, Finland*; <sup>2</sup>*Centralized Family Planning, Helsinki, Finland*; <sup>3</sup>*National Institute for Health and Welfare, Helsinki, Finland*; <sup>4</sup>*Karolinska Institute, Stockholm, Sweden*.

**2:15 PM O-070** **Towards 3-D Imaging of the Murine Vagina.**

Diego R Gatica†, Li Guang†, J. Quincy Brown, Kristin S Miller. *Tulane University, New Orleans, LA, United States*.

**2:30 PM O-071** **A Meta-Analysis Investigation of Anti-Mullerian Hormone Trends in Survivors of Childhood Cancer.**

Allison Kumnick†, Veronica Gomez-Lobo, Ninet Sinai, Jacqueline Maher\*. *National Institutes of Health, Bethesda, MD, United States*.

**2:45 PM O-072** **Euploid Miscarriage Is Associated with *Lactobacillus* spp. Deplete Vaginal Microbial Composition and Local Inflammation.**

Karen Grewal†,<sup>1</sup> Yun S Lee,<sup>1</sup> Ann Smith,<sup>2</sup> Jan J Brosens,<sup>3</sup> Tom Bourne,<sup>1</sup> Maya Al-Memar,<sup>1</sup> Samit Kundu,<sup>1</sup> David A MacIntyre,<sup>1</sup> Phillip Bennett.<sup>1</sup> <sup>1</sup>*Imperial College London, London, United Kingdom*; <sup>2</sup>*University West of England, Bristol, United Kingdom*; <sup>3</sup>*University of Warwick, Warwick, United Kingdom*.

**3:00 PM O-073** **Anemia and Abnormal Uterine Bleeding Prevalence in the Environment, Leiomyomas, Latina and Adiposity Study (ELLAS).**

Torie C Plowden,<sup>1</sup> Anne Waldo,<sup>2</sup> Anita Malone,<sup>2</sup> Felix M Valbuena,<sup>3</sup> Charo Ledon,<sup>4</sup> Donna D Baird,<sup>5</sup> Erica E Marsh\*,<sup>2</sup> <sup>1</sup>*Womack Army Medical Center, Fort Bragg, NC, United States*; <sup>2</sup>*University of Michigan, Ann Arbor, MI, United States*; <sup>3</sup>*(CHASS) Center, Detroit, MI, United States*; <sup>4</sup>*Buenos Vecinos, Ann Arbor, MI, United States*; <sup>5</sup>*NIEHS, Research Triangle Park, NC, United States*.

**3:15 PM O-074** **Route of Myomectomy and Probability of Pregnancy or Live Birth.**

Sophia Anderson†,<sup>1</sup> Laine Thomas,<sup>1</sup> Lauren Wise,<sup>2</sup> Elizabeth A Stewart\*,<sup>3</sup> *COMPARE-UF Team*. <sup>1</sup>*DCRI, Durham, NC, United States*; <sup>2</sup>*Boston University, Boston, MA, United States*; <sup>3</sup>*Mayo Clinic, Rochester, MN, United States*.

2:00 PM - 3:30 PM

Oral

### PARTURITION II

Wellesley

**2:00 PM O-075** **Cell Free Fetal DNA (cffDNA) from Human Amnion Epithelial Cells Increase Inflammatory Load in Human Fetal Membrane Tissue.**

Chelsea Saito Reis, Samantha Oetjen, Claire Kendall-Wright. *Chaminade University of Honolulu, Honolulu, HI, United States*.

**2:15 PM O-076** **Microbial Metabolites Compromise Epithelial Barrier Integrity: Potential Mechanisms by Which Non-Optimal Cervicovaginal Microbiota Lead to Preterm Birth.**

Kristin D Gerson†, Yusra Gimie, Lauren Anton, Michal A Elovitz\*. *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States*.

**2:30 PM O-077** **Single Cell Transcriptomics Identify Distinct Epithelial Populations in Early versus Late Pregnancy.**

Shanmugapriya Madhukaran†, Anne Cooley, Gary Hon\*, Mala Mahendroo\*. *UTSW Medical Center, Dallas, TX, United States*.

**2:45 PM O-078** **Essential Roles of Uterine Peristalsis by Cav1.2 in Reproduction and Adenomyosis in Mice.**

Mingzi Qu†, Ping Lu, Christina Baer, Lawrence Lifshitz, Ronghua ZhuGe\*. *University of Massachusetts Medical School, Worcester, MA, United States*.

**3:00 PM O-079** **Differential Regulation of SOX Family Transcription Factors in the Pregnant and Labouring Mouse Myometrium.**

Nawrah Khader†,<sup>1</sup> Virlana Shchuka†,<sup>1</sup> Anna Dorogin,<sup>2</sup> Oksana Shynlova\*,<sup>2</sup> Jennifer Mitchell\*,<sup>1</sup> <sup>1</sup>*University of Toronto, Toronto, ON, Canada*; <sup>2</sup>*Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada*.

**3:15 PM O-080** **Intrauterine Infection Induced Preterm Labor Is Associated with Site-Specific Phosphorylation of Progesterone Receptor A in Myometrial Cells.**

Rachel A Wilson†,<sup>1</sup> Pietro Presicce,<sup>2</sup> Monica Cappelletti,<sup>2</sup> Alan Jobe,<sup>3</sup> Senad Divanovic,<sup>3</sup> Sing Sing Way,<sup>3</sup> Claire Chougnet,<sup>3</sup> Sam Mesiano,<sup>1</sup> Suhas Kallapur.<sup>2</sup> <sup>1</sup>*Case Western Reserve University, Cleveland, OH, United States*; <sup>2</sup>*University of California, Los Angeles, Los Angeles, CA, United States*; <sup>3</sup>*Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States*.

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**Thursday, July 8, 2021 - Concurrent Session III**


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**2:00 PM - 3:30 PM****Oral****REPRODUCTIVE ENDOCRINOLOGY I****Suffolk**

**2:00 PM O-081** **Cumulus Cells Transcriptomic Biomarkers of Euploid Human Embryo Implantation.**  
Cynthia Scott†, <sup>1</sup>Shiny Titus†, <sup>2</sup>Emre Seli\*, <sup>3</sup><sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>The Foundation for Embryonic Competence, Basking Ridge, NJ, United States; <sup>3</sup>Yale School of Medicine, New Haven, CT, United States.

**2:15 PM O-082** **Rapid Aneuploidy Testing in Reproduction Using Nanopore-Based Sequencing.**  
 Shan Wei, <sup>1</sup>Alexandre Djandji, <sup>1</sup>Nataly Hoffman, <sup>1</sup>Claudia Cujar, <sup>1</sup>Refik Kayali, <sup>2</sup>Cegniz Cinnioglu, <sup>3</sup>Ronald Wapner, <sup>1</sup>Mary D'Alton, <sup>1</sup>Brynn Levy\*, <sup>1</sup>Zev Williams\*. <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>Genomix Los Angeles, Torrance, CA, United States; <sup>3</sup>NextGen Genetics, Santa Clara, CA, United States.

**2:30 PM O-083** **Characterization of the Non-Classical Progesterone Receptor Membrane Component 2 (PGRMC2) during the Human Menstrual Cycle and *In Vitro* Decidualization.**  
Yassmin Medina-Laver†, <sup>1</sup>Indra Diaz-Hernandez†, <sup>2</sup>Pilar Alama\*, <sup>1</sup>Roberto Gonzalez-Martin†, <sup>1</sup>Andrea Palomar†, <sup>2</sup>Alicia Quiñonero\*, <sup>1</sup>Francisco Dominguez\*. <sup>1,2</sup><sup>1</sup>IVI Foundation-RMA Global, Valencia, Spain; <sup>2</sup>IIS La Fe, Valencia, Spain.

**2:45 PM O-084** **Human Placenta Mesenchymal Stem Cells Derived Exosomes Successfully Reverse Infertility in Chemotherapy Induced Premature Ovarian Insufficiency Mouse Model.**  
Esra Cetin, Hang-soo Park, Hiba Siblani, Ayman Al-Hendy. *University of Chicago, Chicago, IL, United States.*

**3:00 PM O-085** **An In Vivo Model of Human FSH Dysregulation in Obesity: Obese Women Exhibit Lower Serum FSH Levels in Response to Intravenous Recombinant FSH after GnRH Suppression.**  
Katherine Kuhn\*, <sup>1</sup>Thanh-Ha Luu†, <sup>1</sup>Andrew P Bradford, <sup>1</sup>Luke Wittenberg, <sup>2</sup>Nne-Omoji Nwobodo, <sup>3</sup>Polotsky J Alex\*, <sup>1</sup>Michael Wemple. <sup>4</sup><sup>1</sup>U. Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>2</sup>U. California Davis, Davis, CA, United States; <sup>3</sup>UCHealth, Aurora, CO, United States; <sup>4</sup>University of Colorado, Aurora, CO, United States.

**3:15 PM O-086** **Different Immunoregulatory Components at the Decidua Basalis of Oocyte Donation Pregnancies.**  
Kim van Bentem†, Manon Bos, Carin van der Keur, Hanneke Kapsenberg, Lisa Lashley\*, Michael Eikmans\*, Marie-Louise van der Hoorn\*. *Leiden University Medical Center, Leiden, Netherlands.*

**10:15 AM - 11:45 AM****Oral****EPIDEMIOLOGY****Salon AB**

**10:15 AM O-087** **Associations of Maternal Urinary Bisphenol and Phthalate Urine Concentrations in Pregnancy with Offspring Pubertal Development.**  
Sophia Blaauwendraad†, Romy Gaillard\*, Vincent Jaddoe\*. *Erasmus Medical Center Rotterdam, Rotterdam, Netherlands.*

**10:30 AM O-088** **Maternal Levels of Perfluoroalkyl Substances (PFAS) during Early Pregnancy in Relation to Preeclampsia Subtypes and Biomarkers of Preeclampsia Risk.**  
Paige A Bommarito†, <sup>1</sup>Kelly K Ferguson, <sup>1</sup>John D Meeker, <sup>2</sup>Thomas F McElrath, <sup>3</sup>David E Cantonwine. <sup>3</sup>*National Institute of Environmental Health Sciences, Durham, NC, United States; <sup>2</sup>University of Michigan School of Public Health, Ann Arbor, MI, United States; <sup>3</sup>Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States.*

**10:45 AM O-089** **Maternal Preconception Platelet Activation and Pregnancy Outcomes.**  
Ashley Shea†, <sup>1,2</sup>Lauren Theilen\*, <sup>1,2</sup>Heather Campbell, <sup>1,2</sup>Erica Johnstone, <sup>1</sup>Meredith Humphreys, <sup>1</sup>Sunni Mumford, <sup>3</sup>Alexandra Purdue-Smithe, <sup>3</sup>Lindsey Sjaarda, <sup>3</sup>Neil Perkins, <sup>3</sup>Robert Silver, <sup>1,2</sup>Enrique Schisterman. <sup>3</sup>*University of Utah, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States; <sup>3</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States.*

**11:00 AM O-090** **The Association between Living in a Food Desert and the Likelihood of Initiating Breastfeeding.**  
Adriana Campos†, <sup>1</sup>Jean Paul Tanner, <sup>1</sup>Ronee E Wilson, <sup>1</sup>Jason L Salemi, <sup>2</sup>Peeraya Sawangkum, <sup>1</sup>Kimberly Fryer, <sup>1</sup>Adetola Louis-Jacques\*. <sup>1</sup>*University of South Florida, Tampa, FL, United States; <sup>2</sup>Baylor College of Medicine, Houston, TX, United States.*

**11:15 AM O-091** **How Late Is Too Late to Reverse the Effects of the Developmental Origins of Health and Disease?**  
Craig E Pennell, <sup>1,2</sup>Carol A Wang, <sup>1,2</sup>Wendy H Oddy, <sup>3</sup>Claire E Meyerkort, <sup>4</sup>Stephen G Matthews, <sup>5,6</sup>Stephen J Lye. <sup>5</sup>*University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>University of Tasmania, Hobart, Australia; <sup>4</sup>Sir Charles Gairdner Hospital, Western Australia, Australia; <sup>5</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>6</sup>University of Toronto, Toronto, ON, Canada.*

**11:30 AM O-092** **Predictors of Teenage Pregnancy in Zambia between 2007 and 2018.**  
Claire H Packer†, <sup>1,2,3</sup>Nelly-Claire Muntalima, <sup>2</sup>Ana M Langer, <sup>1</sup>Michael T Mbizvo\*. <sup>2</sup>*Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>2</sup>Population Council, Lusaka, Zambia; <sup>3</sup>Oregon Health & Science University, Portland, OR, United States.*

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**Thursday, July 8, 2021 - Concurrent Session III**


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**10:15 AM - 11:45 AM****Oral****DEVELOPMENTAL PROGRAMMING II****Salon CD**

- 10:15 AM O-093** **Maternal Exposure to  $\Delta 9$ -Tetrahydrocannabinol Results in Symmetrical IUGR Associated with Cardiac Dysfunction in Postnatal Life.**  
Kendrick Lee†,<sup>1,2</sup> Kristian McCarthy,<sup>1</sup> Steven R Laviolette,<sup>1</sup> Qingping Feng,<sup>1,3</sup> Daniel B Hardy\*.<sup>1,2</sup>  
<sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>Children's Health Research Institute, London, ON, Canada; <sup>3</sup>Children's Health Research Institute, London, ON, United States.
- 10:30 AM O-094** **Under-Expression of Placental Endocrine-Specific Igf2 Programmes Cardiovascular Disease in the Adult Male Offspring.**  
A. N. Sferruzzi-Perri, W. Ching†, Y. Niu, T. A. Garrud†, H. Yong, E. R. Christoforou†, J. Lopez-Tello, D. A. Giussani\*, A. N. Sferruzzi-Perri\*. *University of Cambridge, Cambridge, United Kingdom.*
- 10:45 AM O-095** **Accumulation of Suboptimal Periconceptional Social, Lifestyle and Medical Exposures as Markers for the Maternal Vulnerable Condition and Impairment of Embryonic Growth: The Rotterdam Periconceptional Cohort (Predict Study).**  
Sofie K.M. van Zundert†, Lenie van Rossem\*, Sten P. Willemsen\*, Lindsey van der Meer†, Hiske E. Ernst-Smelt\*, Régine P.M. Steegers-Theunissen\*. *Erasmus MC, University Medical Center, Rotterdam, Netherlands.*
- 11:00 AM O-096** **Maladaptive Cardiomyocyte Calcium Handling in Adult Offspring of Hypoxic Pregnancy: Protection by Antenatal Maternal Melatonin.**  
Mitchell C Lock†,<sup>1</sup> Kerri LM Smith,<sup>1</sup> Yougou Niu,<sup>2</sup> Olga V Patey,<sup>2</sup> Sage G Ford,<sup>2</sup> Andrew W Trafford,<sup>1</sup> Dino A Giussani\*,<sup>2</sup> Gina LJ Galli\*.<sup>1</sup> *The University of Manchester, Manchester, United Kingdom; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.*
- 11:15 AM O-097** **Vascular Disorders of Pregnancy Increase Susceptibility to Neonatal and Infantile Pulmonary Hypertension in High-Altitude Populations.**  
Alexandra Heath,<sup>1</sup> Colleen G Julian\*,<sup>2</sup> Lilian Toledo-Jaldin,<sup>3</sup> Inge von Alvensleben,<sup>1</sup> Litzzi Lazo Vega,<sup>3</sup> Hussna Yasini,<sup>2</sup> Jesus Dorado Madera,<sup>2</sup> Margaret Stalker,<sup>2</sup> Julie A. Houck,<sup>2</sup> Lorna G. Moore.<sup>2</sup> *<sup>1</sup>Kardiozentrum, La Paz, Bolivia, Plurinational State of; <sup>2</sup>University of Colorado School of Medicine, Aurora, CO, United States; <sup>3</sup>Hospital Materno-Infantil, La Paz, Bolivia, Plurinational State of.*
- 11:30 AM O-098** **Reduced TGF $\beta$  Responsiveness in Skeletal Muscle Satellite Cells from Lambs with Fetal Growth Restriction.**  
Rosa I Luna Ramirez†, Miranda J Anderson, Ravi Goyal, Sean W Limesand\*. *The University of Arizona, Tucson, AZ, United States.*

**10:15 AM - 11:45 AM****Oral****REPRODUCTIVE BIOLOGY II****Salon HI**

- 10:15 AM O-099** **Polycomb Repressive Complex 2 Antagonizes Wound Healing Responses in the Decidual Stroma by Modulating the Transforming Growth Factor Beta Pathway.**  
Ivan Osokine†, Damon Rideaux, Tara McIntyre, Johan Siewiera, Adrian Erlebacher\*. *University of California, San Francisco, San Francisco, CA, United States.*
- 10:30 AM O-100** **Proteomic Analysis of Extracellular Vesicles Secreted by Primary Endometrial Epithelial Cells from Fertile Women Reveals Functions Related to Embryo Implantation Not Present in an Endometrial Epithelial Cell Line.**  
Marina Segura-Benítez†,<sup>1,2</sup> María Cristina Carbajo-García†,<sup>1,2</sup> Ana Corachán†,<sup>1,2</sup> Amparo Faus,<sup>1</sup> Antonio Pellicer\*,<sup>1,3</sup> Hortensia Ferrero\*.<sup>1</sup> *IVI Foundation - IIS La Fe, Valencia, Spain; <sup>2</sup>University of Valencia, Valencia, Spain; <sup>3</sup>IVIRMA Rome, Rome, Italy.*
- 10:45 AM - 11:00 AM O-101** **Jagged1 Regulates Endometrial Receptivity in Both Humans and Mice.**  
Wei Zhou, Ellen Menkhorst, Evdokia Dimitriadis. *University of Melbourne, Melbourne, Australia.*
- 11:00 AM O-102** **Antiphospholipid Antibodies Accelerate Endometrial Stromal Cell Decidualization and Senescence and Induce Inflammation via TLR4, p38 MAPK and ROS Signaling.**  
Mancy Tong†,<sup>1</sup> Teimur Kayani†,<sup>1</sup> Deidre M Jones,<sup>2</sup> Lawrence W Chamley,<sup>2</sup> Vikki M Abrahams\*.<sup>1</sup> *<sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>The University of Auckland, Auckland, New Zealand.*
- 11:15 AM O-103** **The Long-Term Impact of Selective Progesterone Receptor Modulator (SPRM) Ulipristal Acetate (UPA) on the Cell Cycle and Cell Proliferation in Human Endometrium as Assessed by RNA Sequencing.**  
Aleksandra Tsolova†, Rohan Chodankar, Alison Murray, Lucy Whitaker, Moira Nicol, Alistair Williams, Hilary Critchley. *University of Edinburgh, Edinburgh, United Kingdom.*
- 11:30 AM O-104** **Next Generation of Human Endometrial Organoids: Bioengineering-Based Strategies for Preserving Tissue-Specific Extracellular Environment.**  
Emilio Francés-Herrero†,<sup>1,2</sup> Elena Juárez-Barber†,<sup>2</sup> Hannes Campo†,<sup>2,3</sup> Sara López-Martínez†,<sup>2</sup> Lucía de Miguel-Gómez†,<sup>1,2</sup> Amparo Faus,<sup>2</sup> Antonio Pellicer\*,<sup>4</sup> Hortensia Ferrero\*,<sup>2</sup> Irene Cervelló\*.<sup>2</sup> *<sup>1</sup>Universitat de València, Valencia, Spain; <sup>2</sup>IVI Foundation-IIS La Fe, Valencia, Spain; <sup>3</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States; <sup>4</sup>IVIRMA-Rome, Rome, Italy.*

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**Thursday, July 8, 2021 - Concurrent Session III**


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**10:15 AM - 11:45 AM****Oral****PLACENTA II****Salon KJ**

- 10:15 AM O-105** **Fibronectin-Integrin Interactions Regulate Placental Endothelial Cell Migration in Severe Fetal Growth Restriction.**  
Diane L Gumin†, Shuhan Ji, Kathryn McPeak, Emily J Su\*. *University of Colorado Anschutz Medical Campus, Aurora, CO, United States.*
- 10:30 AM O-106** **Characterization of Regulation of NLRP3 Inflammasome Activity in Placental Hofbauer Cells.**  
Magnolia G Wang†, Seth Guller\*,<sup>2</sup> *University of Pennsylvania, Philadelphia, PA, United States;* <sup>2</sup>*Yale School of Medicine, New Haven, CT, United States.*
- 10:45 AM O-107** **AKT Signaling Controls Trophoblast Development and Placentation.**  
Keisuke Kozai, Mae-Lan Winchester†, Mikaela E Simon†, Khursheed Iqbal, Masanaga Muto, Regan L Scott†, Chad Slawson\*, Michael J Soares\*. *University of Kansas Medical Center, Kansas City, KS, United States.*
- 11:00 AM O-108** **Changes in Lipid Profiles of Placental Exosomes in Maternal Plasma Characterize Pregnancies with a Small-for-Gestational Age Fetus.**  
Miira M Klemetti†, Ante BV Pettersson†,<sup>2</sup> Porter R Tyler,<sup>1</sup> Aafaque Khan,<sup>3</sup> Premy Shan,<sup>3</sup> Hannes Röst\*,<sup>3</sup> Martin Post\*,<sup>2</sup> Isabella Caniggia\*.<sup>1</sup> *Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada;* <sup>2</sup>*Peter Gilgan Centre for Research and Learning, Hospital for Sick Children, Toronto, ON, Canada;* <sup>3</sup>*Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada.*
- 11:15 AM O-109** **Velocity-Selective Arterial Spin Labeling Perfusion Measurements in 2<sup>nd</sup> Trimester Human Placenta.**  
Daniel Seiter,<sup>1</sup> Ruiming Chen,<sup>1</sup> Kai Ludwig,<sup>1</sup> Ante Zhu,<sup>2,1</sup> Dinesh Shah,<sup>1</sup> Sean Fain,<sup>1</sup> Oliver Wieben,<sup>1</sup> Kevin M Johnson\*.<sup>1</sup> *UW-Madison, Madison, WI, United States;* <sup>2</sup>*GE Healthcare, Niskayuna, NY, United States.*
- 11:30 AM O-110** **Placental NRF2 May Serve a Key Role in Maternal-Fetal Tolerance during Pregnancy.**  
Kyunghee Hong†, Youn-Tae Kwak, Sribalashubashini Muralimanoharan, Carole R Mendelson\*. *UT Southwestern Medical Center, Dallas, TX, United States.*

**10:15 AM - 11:45 AM****Oral****REPRODUCTIVE ENDOCRINOLOGY II****Wellesley**

- 10:15 AM O-111** **Premenstrual Dysphoric Disorder (PMDD) Is Associated with Estradiol-Dependent Aberrations in Cellular Ca<sup>2+</sup> Homeostasis and the Endoplasmic Reticulum Stress Response.**  
Howard Li†,<sup>1,2</sup> Neelima Dubey†,<sup>2</sup> Jessica F Hoffmann†,<sup>2</sup> David R Rubinow,<sup>3</sup> Peter J Schmidt\*,<sup>2</sup> David Goldman\*.<sup>4</sup> *<sup>1</sup>Yale School of Medicine, New Haven, CT, United States;* *<sup>2</sup>National Institute of Mental Health (NIMH), Bethesda, MD, United States;* *<sup>3</sup>UNC Chapel Hill, Chapel Hill, NC, United States;* *<sup>4</sup>National Institute of Alcohol Abuse and Alcoholism (NIAAA), Bethesda, MD, United States.*
- 10:30 AM O-112** **Examination of Androgen Effects on Female Reproductive Axis: Hypothalamus-Pituitary-Ovary.**  
Sheng Wu\*, Olubusayo Awe†, Mingxiao Feng†, James Segars. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- 10:45 AM O-113** **Disparities in Seeking Infertility Care: Data from the 2017-2019 CDC National Survey of Family Growth.**  
Lauren E Barrison†,<sup>1</sup> Allison R Kumnick†,<sup>2</sup> Veronica Gomez-Lobo,<sup>2</sup> Jaqueline Y Maher\*.<sup>2</sup> *MedStar Washington Hospital Center/Georgetown University Hospital, Washington, DC, United States;* *<sup>2</sup>National Institutes of Health, Bethesda, MD, United States.*
- 11:00 AM O-114** **Normal Weight Women with Polycystic Ovary Syndrome (PCOS) Exhibit Oxidative Stress in Response to Saturated Fat Ingestion Even in the Absence of Abdominal Adiposity (AA).**  
Frank González\*,<sup>1</sup> Robert V. Considine,<sup>2</sup> Ola A. Abdelhadi,<sup>2</sup> Jiaping Xue,<sup>1</sup> Anthony J. Acton.<sup>2</sup> *<sup>1</sup>University of Illinois at Chicago College of Medicine, Chicago, IL, United States;* *<sup>2</sup>Indiana University School of Medicine, Indianapolis, IN, United States.*
- 11:15 AM O-157** **Adopting 3D Methods to Culture Endometrial Stromal Cells Enhances Their Decidualization Response.**  
Kira Buttrey†, Juan S Gnecco, Alexander Brown, Clara Ives, Linda G Griffith\*. *Massachusetts Institute of Technology, Cambridge, MA, United States.*
- 11:30 AM O-158** **Dysregulation of Primordial Follicle Growth Activation Regulatory Factor Expression in a Granulosa Cell Model of Galactosemic Primary Ovarian Insufficiency.**  
John Rushing†,<sup>1</sup> Evelyn Llerena Cari,<sup>1</sup> Synneva Hagen-Lillevik,<sup>2</sup> Amanda Kallen,<sup>3</sup> Alex J. Polotsky,<sup>1</sup> Kent Lai,<sup>2</sup> Joshua Johnson\*.<sup>1</sup> *<sup>1</sup>University of Colorado School of Medicine (AMC), Aurora, CO, United States;* *<sup>2</sup>University of Utah School of Medicine, Salt Lake City, UT, United States;* *<sup>3</sup>Yale University School of Medicine, New Haven, CT, United States.*

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**Thursday, July 8, 2021 - Concurrent Session IV**


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**10:15 AM - 11:15 AM****Oral****CLINICAL PERINATOLOGY II****Suffolk**

- 10:15 AM O-115** **Preferred Timing of Efficacy of a New Therapeutic Candidate, Rytvela, for Prevention of Inflammation-Induced Preterm Birth and Fetal Growth Restriction.**  
Sarah-Eve Loisellet<sup>1,2</sup>, Renay Poupart<sup>2</sup>, Xin Hou<sup>\*,1</sup>, Mathieu Nadeau-Vallée<sup>2</sup>, France Côté<sup>1,2</sup>, Tiffany Habelriht<sup>1,2</sup>, Christiane Quiniou<sup>\*,1</sup>, Sylvain Chemtob<sup>\*,1,2</sup>  
<sup>1</sup>CHU Sainte-Justine, Montreal, QC, Canada; <sup>2</sup>Université de Montréal, Montreal, QC, Canada.
- 10:30 AM O-116** **Comparative Adverse Effects of Antenatal Glucocorticoid Formulations: Studies in the Chicken Embryo.**  
T A Garrud<sup>†,1</sup>, N Teulings<sup>†,1</sup>, F G Conlon<sup>†,1</sup>, W Tong<sup>†,1</sup>, S G Ford<sup>†,1</sup>, Y Niu<sup>†,1</sup>, L M Nicholas<sup>†,1</sup>, J B Derks<sup>\*,2</sup>, S E Ozanne<sup>\*,1</sup>, D A Giussani<sup>\*,1</sup>  
<sup>1</sup>University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>University Medical Centre, Utrecht, Netherlands.
- 10:45 AM O-117** **Maternal Leukocyte DNA Methylation during Asymptomatic Pregnancy and Future Spontaneous Preterm Birth among Black American Women.**  
Shannon Gillespie, Chenggong Han, Zilu Liu<sup>†</sup>, Cindy Anderson, Shili Lin. *The Ohio State University, Columbus, OH, United States.*
- 11:00 AM O-118** **Impact of Progesterone on Mechanism of Preterm Premature Rupture of Membrane.**  
Heejoong Lee<sup>1</sup>, Banghyun Lee<sup>\*,2</sup>  
<sup>1</sup>The Catholic University of Korea, College of Medicine, Uijongbu, Korea, Republic of; <sup>2</sup>Inha University, College of Medicine, Incheon, Korea, Republic of.
- 11:15 AM O-119** **A Novel Murine Model of Cytomegalovirus Infection in Pregnancy.**  
Angela Shaddeau<sup>†,1</sup>, Gregory Kirschen<sup>†,1</sup>, Anna Chudnovets<sup>1</sup>, Quan Na<sup>1</sup>, Ayan Ghosh<sup>2</sup>, Halli Miller<sup>2</sup>, Jun Lei<sup>1</sup>, Ravit Boger<sup>2</sup>, Andrew Thagard<sup>3</sup>, Karen Racicot<sup>2,4</sup>, Irina Burd<sup>\*,1</sup>  
<sup>1</sup>Johns Hopkins School of Medicine, Baltimore, MD, United States; <sup>2</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>3</sup>Naval Medical Center Portsmouth, Portsmouth, VA, United States; <sup>4</sup>Michigan State University, East Lansing, MI, United States.
- 11:30 AM O-120** **Psychological Distress during Pregnancy and Adverse Maternal and Perinatal Health Outcomes; the Role of Socioeconomic Status.**  
Leonie A. Daalderop<sup>†,1</sup>, Jacqueline Lagendijk<sup>†,1</sup>, Eric A.P. Steegers<sup>\*,1</sup>, Hanan El Marroun<sup>\*,1,2</sup>, Anke G. Posthumus<sup>†,1</sup>  
<sup>1</sup>Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Erasmus School of Social and Behavioural Sciences, Erasmus University Rotterdam, Rotterdam, Netherlands.

**1:15 PM - 2:45 PM****Oral****FETUS II****Salon AB**

- 1:15 PM O-121** **MAP4K4: Identification of a Novel Candidate Gene Using Prenatal Exome Sequencing Data and Functional Modeling in Zebrafish.**  
Neeta L. Vora<sup>1</sup>, John Griffin<sup>†,2</sup>, Kelly Gilmore<sup>1</sup>, Julie K. Holsclaw<sup>†,2</sup>, Elizabeth Bhoj<sup>3</sup>, Erica E. Davis<sup>\*,4</sup>  
<sup>1</sup>UNC-Chapel Hill, Chapel Hill, NC, United States; <sup>2</sup>Duke University, Durham, NC, United States; <sup>3</sup>Children's Hospital of Pennsylvania, Philadelphia, PA, United States; <sup>4</sup>Northwestern University, Chicago, NC, United States.
- 1:30 PM O-122** **An Aberrant Endothelial Cell Response to Flow: Mechanistic Implications for Congenital Heart Defects in the Feto-Placental Unit.**  
Yalda Afshar<sup>1</sup>, Anhyo Jeong<sup>1</sup>, Christine Jang<sup>1</sup>, Gary Satou<sup>1</sup>, Mark Sklansky<sup>1</sup>, M. Luisa Iruela-Arispe<sup>2</sup>  
<sup>1</sup>University of California, Los Angeles; <sup>2</sup>David Geffen School of Medicine, Los Angeles, CA, United States; <sup>3</sup>Northwestern University, Chicago, IL, United States.
- 1:45 PM O-123** **Measuring In Vivo Arterial Stiffness in Fetal Life.**  
Nima Moghaddas<sup>†,1</sup>, Beth J Allison<sup>2</sup>, Youguo Niu<sup>1</sup>, Kimberley J Botting<sup>1</sup>, Carmel M McEniery<sup>1</sup>, Dino A Giussani<sup>1</sup>  
<sup>1</sup>University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>Monash University, Melbourne, Australia.
- 2:00 PM O-124** **In Utero e-Cigarettes Exposure Enhances Neonatal Brain Ischemic Damage: Role of the miRNA-30c.**  
Andrew Walayat<sup>†</sup>, Yanyan Zhang, Yong Li, Asanjan Hosseini Maryam, Daliao Xiao<sup>\*,</sup>  
*Loma Linda University, Loma Linda, CA, United States.*
- 2:15 PM O-125** **Early and Mid-Gestation Zika Virus (Zikv) Infection in the Olive Baboon (*Papio anubis*) Leads to Neurological Birth Defects Due to Congenital Zika Syndrome (czs).**  
Sunam G Dockins<sup>†</sup>, Darlene N Reuter, Marta E Macted, Ashley A Martin, Molly E Dubois, James F Papin, Dean A Myers<sup>\*,</sup>  
*OUHSC, Oklahoma City, OK, United States.*
- 2:30 PM O-126** **Thyroid Hormone Dominates IGF1 Expression and Actions in Fetal Cardiomyocytes.**  
Natasha N Chattergoon<sup>1</sup>, Samantha Louey<sup>1</sup>, Sonnet Jonker<sup>1</sup>, George Giraud<sup>2,1</sup>, Kent L Thornburg<sup>\*,1</sup>  
<sup>1</sup>Oregon Health and Science University, Portland, OR, United States; <sup>2</sup>Portland VA Medical Center, Portland, OR, United States.

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**Thursday, July 8, 2021 - Concurrent Session IV**


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**1:15 PM - 2:45 PM****Oral****HEALTH DISPARITIES AND COVID****Salon CD**

- 1:15 PM O-127** **Black and White Women in the United States Military Have Comparable Rates of Preeclampsia and Post-Preeclamptic Cardiovascular Disease.**  
 Andrea I Loewendorf\*,<sup>1</sup> Emily A Stone,<sup>2</sup> Lee Ann Zarzabal,<sup>2</sup> Thornton Mu,<sup>3</sup> Amelia Duran-Stanton.<sup>4</sup>  
<sup>1</sup>ImmunoVation, Pasadena, CA, United States; <sup>2</sup>Defense Health Agency, San Antonio, TX, United States; <sup>3</sup>Brooke Army Medical Center, San Antonio, TX, United States; <sup>4</sup>5Chief, Ready and Resilient Integration Branch/Deputy Surgeon, Houston, TX, United States.
- 1:30 PM O-128** **Racial and Ethnic Disparities in Complications among Patients Undergoing a Trial of Labor after Cesarean Section at Mount Sinai Hospital.**  
 Ayisha Brielle Buckley†, Stephanie Sestito†, Tonia Ogundipe†, Natalie Cohen†, Mitchell Rosenberg†, Kelly Wang, Jill Berkin\*, Stoffels Guillaume, Chelsea DeBolt†, Jessica Peterson†, Joanne Stone\*, Angela Bianco\*, Luciana Vieira\*. *Icahn School of Medicine at Mount Sinai Hospital, Manhattan, NY, United States.*
- 1:45 PM O-129** **Potential Risk of Infection of First Trimester Placentas by SARS-CoV2.**  
 Sampada Kallol,<sup>1</sup> Laura Martin-Sancho,<sup>2</sup> Donald Pizzo,<sup>1</sup> Sumit K Chanda,<sup>2</sup> Mana Parast,<sup>1</sup> Francesca Soncin.<sup>1</sup>  
<sup>1</sup>UCSD, La Jolla, CA, United States; <sup>2</sup>SBP, La Jolla, CA, United States.
- 2:00 PM O-130** **Impact of SARS-CoV-2 Infection during Pregnancy by Maternal Race-Ethnicity.**  
 Darios Getahun,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiaxiao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Michael J Fassett.<sup>7</sup> <sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States.
- 2:15 PM O-131** **Stressors in Fertility Treatment: The Impact of the COVID-19 Pandemic.**  
 Sarah Dynia†, Caroline Peschansky†, Safina Usmani, Sonia Patel†, Kayla Vitale†, Jawaria Amir, Royi Lynn†, Lauren Grimm, Erica Loudon, Roohi Jeelani, Angie Beltsos. *Vios Fertility Institute, Chicago, IL, United States.*
- 2:30 PM O-132** **Expression of SARS-CoV-2 Entry Molecules at Maternal-Fetal Interface and Regulation of Proinflammatory Cytokines and Coagulation Factor III in Human Placental Microvascular Endothelial Cell by Spike Protein.**  
 Xiaofang Guo, Umit A. Kayisli, Asli Ozmen, Nihan Semerci, Kellie Larsen, Zhi Tian, Frederick Schatz, Diane Allen-Gipson, Ozlem Guzeloglu-Kayisli, Charles J. Lockwood. *University of South Florida, Tampa, FL, United States.*

**1:15 PM - 2:45 PM****Oral****PLACENTA III****Salon HI**

- 1:15 PM O-133** **Chorionic Somatomammotropin RNA Interference Reduces Global Nutrient Uptake and Umbilical Blood Flow Resulting in Intrauterine Growth Restriction.**  
 Amelia R Tanner†,<sup>1</sup> Cameron S Lynch,<sup>1</sup> Victoria C Kennedy,<sup>1</sup> Asghar Ali,<sup>1</sup> Quinton A Winger,<sup>1</sup> Paul J Rozance,<sup>2</sup> Russell V Anthony\*.<sup>1</sup> <sup>1</sup>Colorado State University, Fort Collins, CO, United States; <sup>2</sup>University of Colorado School of Medicine, Aurora, CO, United States.
- 1:30 PM O-134** **Trophoblast-Specific Knockdown of the System A Amino Acid Transporter, Slc38a2/SNAT2, Causes Fetal Growth Restriction in Mice.**  
 Owen R Vaughan,<sup>1,2</sup> Elena Silva,<sup>1</sup> Kenneth Barentsen,<sup>1</sup> Russell V Anthony,<sup>3</sup> Thomas L Brown,<sup>4</sup> Theresa L Powell,<sup>1</sup> Thomas Jansson.<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University College London, London, United Kingdom; <sup>3</sup>Colorado State University, Fort Collins, CO, United States; <sup>4</sup>Wright State University, Dayton, OH, United States.
- 1:45 PM O-135** **An In Vitro Placenta Model with a Native Stroma for Mechanistic Transport Studies.**  
 Katherine M. Nelson†, Sarah A. Geissler, Jason P. Gleghorn\*. *University of Delaware, Newark, DE, United States.*
- 2:00 PM O-136** **Obesity Downregulates Lipid Metabolism Genes in First Trimester Placenta.**  
 Aisha Rasool†,<sup>1</sup> Begum Aydogan Mathyk,<sup>2</sup> Danielle Roncari,<sup>1</sup> Perrie O'Tierney-Ginn\*.<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>Brandon Regional Hospital, Brandon, FL, United States.
- 2:15 PM O-139** **A Multiomics Approach to Placental Dysfunction in Common Obstetrical Syndromes.**  
 Oren Barak†,<sup>1,2</sup> Samantha Piekos†,<sup>3</sup> Tianjiao Chu,<sup>1,2</sup> Elena Sadovsky,<sup>1</sup> Jean-Francois Moulliet,<sup>1,2</sup> Yingshi Ouyang,<sup>1,2</sup> Lee Hood,<sup>3</sup> Nathan Price,<sup>3,4</sup> Yoel Sadovsky\*.<sup>1,2</sup> <sup>1</sup> Magee Womens Research Institute, Pittsburgh, PA, United States; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, United States; <sup>3</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>4</sup>Onegevity Health, New York, NY, United States.
- 2:30 PM O-160** **Determining the Impact of Environmental Toxin Cadmium at the Feto-Maternal Interface Using an Organ-on-Chip (FMi-OOC) Device.**  
 Sungjin Kim†,<sup>1</sup> Lauren Richardson†,<sup>2</sup> Enkhtuya Radnaat,<sup>2</sup> Zunwei Chen†,<sup>1</sup> Ivan Rusyn,<sup>1</sup> Ramkumar Menon\*,<sup>2</sup> Arum Han\*.<sup>1</sup> <sup>1</sup>TAMU, College Station, TX, United States; <sup>2</sup>UTMB, Galveston, TX, United States.

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**Thursday, July 8, 2021 - Concurrent Session IV**


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**1:15 PM - 2:45 PM****Oral****REPRODUCTIVE BIOLOGY III**

Salon KJ

- 1:15 PM O-137** **Thymic Progesterone Receptor Expression Regulates Maternal Thymic Involution in Murine Pregnancy.**  
Soo Hyun Ahn†, Sean L Nguyen,<sup>1</sup> Tae-Hoon Kim,<sup>2</sup> Jae-Wook Jeong,<sup>2</sup> Ripla Arora,<sup>1</sup> Margaret G Petroff\*.<sup>1</sup>  
<sup>1</sup>Michigan State University, East Lansing, MI, United States; <sup>2</sup>Michigan State University, Grand Rapids, MI, United States.
- 1:30 PM O-138** **A Role for the IL-33-ILC2 Axis in Driving Uterus-Intrinsic Parturition Pathways in Mice.**  
Johan Siewiera†, Madelene Dahlgren, Kelly Cautivo, Damon Rideaux, Ari Molofsky, Adrian Erlebacher. *UCSF, San Francisco, CA, United States.*
- 1:45 PM O-139** **Single-Cell RNA Sequencing Identifies Novel Uterine Macrophage Population Increased by Regulatory T Cells and Associated with Decreased Fetal Loss.**  
Emma L Lewis†, Paige M Porrett,<sup>2</sup> Michal A Elovitz\*.<sup>1</sup>  
<sup>1</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>2</sup>University of Alabama, Birmingham, AL, United States.
- 2:00 PM O-140** **Pluripotent Stem Cell Derived Endometrial Stromal Fibroblasts Co-Culture with Endometrial Epithelial Organoids to Form a 3-Dimensional Model of the Human Endometrium.**  
Virginia Chu Cheung†, Chian-yu Peng,<sup>1</sup> Mirna Marinic,<sup>2</sup> Noboru J Sakabe,<sup>2</sup> Ivy Aneas,<sup>2</sup> Vincent J Lynch,<sup>3</sup> Carole Ober,<sup>2</sup> Marcelo A Nobrega,<sup>2</sup> John A Kessler\*.<sup>1</sup>  
<sup>1</sup>Northwestern University, Chicago, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States; <sup>3</sup>University of Chicago, Chicago, IL, United States.
- 2:15 PM O-141** **The Vertical Transfer of Maternal Immune Cells during Pregnancy Promotes Neonatal Immunity against Viral Infections in Mice.**  
Ina Stelzer<sup>1,2</sup> Christopher Urbschat,<sup>1</sup> Kristin Thiele,<sup>1</sup> Ioanna Trivai,<sup>1</sup> Julian Kottlau,<sup>1</sup> Felix Stahl,<sup>1</sup> Maria Emilia Solano\*,<sup>1</sup> Petra Arck\*.<sup>1</sup> <sup>1</sup>University Medical Center Hamburg-Eppendorf, Hamburg, Germany; <sup>2</sup>Stanford University, Stanford, CA, United States.
- 2:30 PM O-142** **Differentiation of Mouse iPSCs into Functional Oocytes.**  
Raymond M. Anchan\*, Nicholas W Ng, Kha U Dam, Ankrish Milne, Emily R Disler, Nicole Dunn, Kevin M Elias, Elizabeth Ginsburg. *Brigham & Women's Hospital, Harvard Medical School, Boston, MA, United States.*

**1:15 PM - 2:45 PM****Oral****PREECLAMPSIA II: PREECLAMPSIA/RELATED DISORDERS**

Wellesley

- 1:15 PM O-143** **HO-1 Genetic Variants Display Racial Diversity and May Impact Hypertensive Disorders in Pregnancy.**  
Tianyanxin Sun†, Nima Mousavi†,<sup>2</sup> Ronald J Wong,<sup>1</sup> Nazish Sayed,<sup>1</sup> Joseph C Wu,<sup>1</sup> David K Stevenson,<sup>1</sup> Melissa Gymrek,<sup>2</sup> Virginia D Winn\*.<sup>1</sup> <sup>1</sup>Stanford University School of Medicine, Palo Alto, CA, United States; <sup>2</sup>University of California San Diego, San Diego, CA, United States.
- 1:30 PM O-144** **Surgically Increasing Uteroplacental Impedance Results in Attenuated Uterine Vascular Remodeling and Preeclampsia-Related Placental Secretomics during Pregnancy.**  
Nga Ling Ko\*,<sup>1</sup> Narmin Mukhtarova,<sup>1</sup> Catrina Hood,<sup>2</sup> Ying Wai Lam,<sup>2</sup> George Osol.<sup>1</sup> <sup>1</sup>The University of Vermont, Burlington, VT, United States; <sup>2</sup>Proteomics Facility, Vermont Genetics Network, The University of Vermont, Burlington, VT, United States.
- 1:45 PM O-145** **The New Generation Antiplatelet Agent Prasugrel Represents an Exciting Novel Candidate Therapy for Preeclampsia.**  
Natalie Hannan\*,<sup>1</sup> Natasha De Alwis†,<sup>2</sup> Natalie Binder,<sup>1</sup> Sally Beard,<sup>1</sup> Vi Nguyen,<sup>1</sup> Kaitu'u-Lino Tu'uhevaha,<sup>1</sup> Stephen Tong.<sup>1</sup> <sup>1</sup>University of Melbourne, Parkville, Australia; <sup>2</sup>University of Melbourne, Heidelberg, Australia.
- 2:00 PM O-146** **Sildenafil Improves Placental Function and Remodeling in Preeclamptic Rats.**  
Dylan J Lawrence†, Carolyn L Bayer\*. *Tulane University, New Orleans, LA, United States.*
- 2:15 PM O-147** **Differential Distribution of Tryptophan-Metabolites in Fetal and Maternal Circulations during Pregnancy: Preeclampsia-Elevated Aryl Hydrocarbon Receptor Ligands.**  
 Ying-jie Zhao†,<sup>1</sup> Chi Zhou,<sup>1</sup> Ying-ying Wei,<sup>2</sup> Hui-hui Li,<sup>1</sup> Kai Wang\*,<sup>2</sup> Jing Zheng\*.<sup>1</sup> <sup>1</sup>University of Wisconsin-Madison, Madison, WI, United States; <sup>2</sup>Tongji Univ. School of Medicine, Shanghai, China.
- 2:30 PM O-148** **Can WS1442 Ameliorate Maternal Vascular Dysfunction in Preeclampsia?**  
Stephanie A Worton†, Yasmin Mills†, Susan L Greenwood\*, Jenny E Myers\*. *University of Manchester, Manchester, United Kingdom.*

## Thursday, July 8, 2021 - Concurrent Session IV

1:15 PM - 2:45 PM

Oral

## GYNECOLOGIC ONCOLOGY

Suffolk

- 1:15 PM O-149** **Evaluation of ATR Inhibitors for Targeted Therapy of Ovarian Clear Cell Carcinoma.**  
Jing Ji,<sup>1</sup> Zhigui Li†,<sup>1</sup> Emily Sherman†,<sup>1</sup> Olorunfoba Osagiet†,<sup>1</sup> Shijun Mi,<sup>3</sup> Whitney Soble,<sup>1</sup> Jessie Li†,<sup>1</sup> Gloria Huang\*.<sup>1</sup> <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>Xi'an Jiaotong University, Xi'an, China; <sup>3</sup>Albert Einstein College of Medicine, Bronx, NY, United States.
- 1:30 PM O-150** **Endoplasmic Reticulum Stress Response to Hyperinsulinemia in Endometrial Epithelial Cells Depends on PIK3CA Mutation.**  
Mike R Wilson†, Ronald L Chandler\*. Michigan State University, Grand Rapids, MI, United States.
- 1:45 PM O-151** **ACS NSQIP - Personalised Risk Prediction Tool for Postoperative Complications in Gynaecology Surgery?**  
Lusine Sevinyan†,<sup>1</sup> Sadie Jones†,<sup>2</sup> Jonathan Horne†,<sup>3</sup> Rasiyah Bharathan,<sup>3</sup> Anil Tailor,<sup>1</sup> Simon Butler-Manuel,<sup>1</sup> Peter Williams,<sup>4</sup> Thumuluru Kavitha Madhuri.<sup>1,5</sup> <sup>1</sup>Royal Surrey NHS Foundation Trust, Guildford, United Kingdom; <sup>2</sup>Cardiff University, Cardiff, United Kingdom; <sup>3</sup>University Hospitals of Leicester NHS Trust, Leicester, United Kingdom; <sup>4</sup>University of Surrey, Guildford, United Kingdom; <sup>5</sup>University of Brighton, Brighton, United Kingdom.
- 2:00 PM O-152** **High NLRP7 Expression Promotes Choriocarcinoma Development: Proof of Concept from Clinical and Preclinical Studies.**  
Deborah Reynaud†,<sup>1</sup> Roland Abi Nahed†,<sup>1</sup> Nicolas Lemaire†,<sup>1</sup> Pierre-Adrien Bolze\*,<sup>1</sup> Touria Aboussaouira\*,<sup>2</sup> Padma Murthi\*,<sup>3</sup> Rima Slim\*,<sup>4</sup> Mohamed Benharouga\*,<sup>1</sup> Nadia Alfaidy\*.<sup>1</sup> <sup>1</sup>INSERM U1292, Grenoble, France; <sup>2</sup>Hassan II University, Casablanca, Morocco; <sup>3</sup>Monash Biomedicine Discovery Institute, Victoria, Australia; <sup>4</sup>McGill University, Montreal, QC, Canada.
- 2:15 PM O-161** **Entinostat Increases Sensitivity to Olaparib in a Homologous Recombination Proficient Syngeneic Mouse Model of Ovarian Cancer.**  
Vijayalaxmi G Gupta\*,<sup>1</sup> Yosklay L Fernandez,<sup>2</sup> Katherine F Roby,<sup>2</sup> Fiona Yull,<sup>3</sup> Marta A Crispens,<sup>4</sup> Andrew J Wilson,<sup>4</sup> Harsh B Pathak,<sup>2</sup> Andrew K Godwin,<sup>2</sup> Andrea Jewell,<sup>2</sup> Dineo Khabele.<sup>1</sup> <sup>1</sup>Washington University St. Louis, St. Louis, MO, United States; <sup>2</sup>University of Kansas Medical Center, Kansas City, KS, United States; <sup>3</sup>Vanderbilt School of Medicine, Nashville, TN, United States; <sup>4</sup>Vanderbilt University Medical Center, Nashville, TN, United States.
- 2:30 PM O-162** **Factors Associated with Referral-Based Receipt of Fertility Consultation Among Reproductive Age Women with Pre-invasive or Invasive Gynecologic Malignancies.**  
Ruoxi Yu†, Anna L Beavis\*, Mindy S Christianson\*, Kala Viswanathan\*, Akila N Viswanathan\*, Rebecca L Stone\*. Johns Hopkins University School of Medicine, Baltimore, MD, United States.

4:00 PM - 5:30 PM

Poster

## BASIC PARTURITION

- W-001** **Association between Daily Melatonin and Cortisol Rhythms and Gestation Length.**  
Ronald T. McCarthy,<sup>1</sup> Peinan Zhao,<sup>1</sup> Anjana Delhi,<sup>1</sup> Nandini Raghuraman,<sup>1</sup> Emily S Jungheim,<sup>1</sup> Justin C Fay,<sup>2</sup> Erik D Herzog,<sup>3</sup> Sarah K England\*.<sup>1</sup> <sup>1</sup>Washington University School of Medicine, St. Louis, MO, United States; <sup>2</sup>University of Rochester, New York, NY, United States; <sup>3</sup>Washington University in St. Louis, St. Louis, MO, United States.
- W-002** **Neurosteroids and Steroid Hormones in Preterm Birth.**  
Gabriella Mayne†,<sup>1</sup> Peter E. DeWitt,<sup>2</sup> Brandy Ringham,<sup>2</sup> Anna Warrenner,<sup>1</sup> Uwe Christians,<sup>2</sup> Dana Dabelea,<sup>2</sup> K. Joseph Hurt\*.<sup>3</sup> <sup>1</sup>University of Colorado, Denver, CO, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>University of Colorado School of Medicine, Aurora, CO, United States.
- W-003** **Adverse Events Due to Inflammation Are Successfully Prevented by an Allosteric Modulator of IL-6R in a LPS Mouse Model of Preterm Birth.**  
Elizabeth Prairie,<sup>1</sup> France Côté†,<sup>1</sup> Laurence Gobeil,<sup>2</sup> Sarah-Eve Loisel,<sup>1</sup> Xin Hou,<sup>1</sup> Christiane Quiniou,<sup>1</sup> David Olson,<sup>3</sup> Sylvain Chemtob\*.<sup>1</sup> <sup>1</sup>Université de Montréal, Montreal, QC, Canada; <sup>2</sup>Université de Sherbrooke, Sherbrooke, QC, Canada; <sup>3</sup>University of Alberta, Montreal, QC, Canada.
- W-004** **Progesterone Withdrawal-Induced Inflammatory Drive of Cervix Ripening and Preterm Birth Is Linked to Morphology of Macrophage Phenotypes.**  
Olivia G Beck, Michael A Kirby, Steven M. Yellon\*. Loma Linda University, Loma Linda, CA, United States.
- W-005** **The Anti-Inflammatory Properties of MicroRNA-125 Limit the Vasoobliteration in a Rat Model of Oxygen-Induced Retinopathy.**  
Maëlle Wirth†,<sup>1,2</sup> Michel Desjarlais,<sup>1</sup> Isabelle Lahaie,<sup>1</sup> Samy Omri,<sup>1</sup> Rabah Dabouz,<sup>1</sup> José-Carlos Rivera,<sup>1</sup> Sylvain Chemtob.<sup>1,3</sup> <sup>1</sup>Maison-Neuve-Rosemont Hospital Research Center, Montréal, QC, Canada; <sup>2</sup>Université de Montréal, Montréal, QC, Canada; <sup>3</sup>Centre Hospitalier Universitaire Sainte-Justine Research Center, Montréal, QC, Canada.
- W-006** **Mid-Trimester Changes in Cervicovaginal Metabolites Are Distinct in Women with and without Preterm Birth.**  
Megan Cavanagh†, Emmanuel Amabebe†, Neha Kulkarni†, Dilly Anumba\*. University of Sheffield, Sheffield, United Kingdom.
- W-007** **Prostaglandin F2α Does Not Induce an Inflammatory Response in Murine Macrophages and Pregnant Mouse Uterine Explants Ex Vivo.**  
Madeline Snedden,<sup>1</sup> Chandrashekara Kyathanahalli,<sup>1,2</sup> Emmet Hirsch\*.<sup>1,2</sup> <sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States.
- W-008** **A Systematic Review of Prenatal Interventions in Preclinical Infection and Inflammation Preterm Birth Models.**  
Faith Miller, Anna L David, Ashley K Boyle†\*. University College London, London, United Kingdom.
- W-009** **Estrous Cycle-Dependent Differential Inflammatory Profiles and Responses to Bacterial Lipopolysaccharide of Mouse Peritoneal Macrophages.**  
Chandrashekara N Kyathanahalli,<sup>1,2</sup> Madeline Snedden,<sup>1</sup> Emmet Hirsch.<sup>1,2</sup> <sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States.

## Wednesday, July 7, 2021 - Poster Session I - Back Bay Conference and Exhibition Center

- W-010**     **The Vaginal Microbiome and the Risk of Preterm Birth: A Systematic Review and Network Meta-Analysis.**  
Unnur Gudnadottir†, Justine Debelius, Juan Du, Luisa W. Hugerth, Hanna Danielsson, Ina Schuppe-Koistinen, Emma Fransson\*, Nele Brusselaers\*. *Karolinska Institutet, Stockholm, Sweden.*
- W-011**     **Bromodomain and Extra-Terminal (BET) Epigenetic Reader Expression and Function in Decidual Stromal Cells.**  
Tamás Zakár\*,<sup>1,2,3</sup> Sandeep Ajaonkar†,<sup>1</sup> Jonathan J Hirst\*,<sup>1,3</sup> <sup>1</sup>*University of Newcastle, Callaghan, Australia;* <sup>2</sup>*John Hunter Hospital, New Lambton Heights, Australia;* <sup>3</sup>*Hunter Medical Research Institute, New Lambton Heights, Australia.*
- W-012**     **Cervicovaginal Microbiota and Immune Output: Potential Determinants of Spontaneous Preterm Birth in Black Women.**  
Kristin D Gerson†, Clare McCarthy, Heather H Burris, Michal A Elovitz\*. *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*
- W-013**     **The Use of Peripheral Blood Neutrophil Counts in the Prediction of Funisitis Following Preterm Prelabour Rupture of Membranes.**  
Lara Budwig†,<sup>1</sup> Richard Brown,<sup>1</sup> Yun S Lee,<sup>1,2</sup> Katherine Mountain†,<sup>1,2</sup> Belen Gimeno-Molina†,<sup>1,2</sup> Malko Adan,<sup>1,2</sup> Erna Bayar,<sup>1,2</sup> David A MacIntyre,<sup>1,2</sup> Phillip R Bennett,<sup>1,2</sup> Lynne Sykes\*,<sup>2,1</sup> <sup>1</sup>*Imperial College, London, United Kingdom;* <sup>2</sup>*Imperial College College March of Dimes PRC, London, United Kingdom.*
- W-014**     **Dampened Response to Bacterial Lipopolysaccharide by Mouse Peritoneal Macrophages during Pregnancy.**  
Chandrashekar N Kyathanahalli,<sup>1,2</sup> Madeline Snedden,<sup>1</sup> Emmet Hirsch,<sup>1,2</sup> <sup>1</sup>*NorthShore University HealthSystem, Evanston, IL, United States;* <sup>2</sup>*University of Chicago, Chicago, IL, United States.*
- W-015**     **Human Leukocytes Express Different Chemokine Receptors at Term and Preterm Labor.**  
Han Lee†,<sup>1,2</sup> Nanlin Yin\*,<sup>2</sup> Zheng Liu†,<sup>2</sup> Lulu Wang†,<sup>2</sup> Yuxin Ran†,<sup>2</sup> Jenelle Chen†,<sup>1</sup> Hongbo Qi\*,<sup>2</sup> David Olson\*,<sup>1,1</sup> <sup>1</sup>*University of Alberta, Edmonton, AB, Canada;* <sup>2</sup>*Chongqing Medical University, Chongqing, China.*
- W-016**     **Impact of Mild Restraint Stress on Placental Pathology in Murine Model.**  
Ethelin Cammock†, Jennifer J Barr†, Abigail Combs†, Mauro Schenone\*, Giancarlo Mari\*. *University of Tennessee Health Sciences Center, Memphis, TN, United States.*
- W-017**     **Phenotypic Analysis of Human Choriodecidua in Relation with Parturition.**  
Léa Chicoisne†, Vaarany Karunanithy\*, Céline Bertholle\*, Brigitte Izac\*, Franck Letourneur\*, Daniel Vaiman\*, Francisco Miralles\*, Muriel Andrieu\*, Céline Mehats\*. *Institut Cochin, Paris, France.*
- W-018**     **Human Fetal Membranes Secrete Proinflammatory Cytokines Upon Toll-Like Receptor 9 and Cell-Free Fetal DNA Stimulation.**  
Samantha P Oetjen†,<sup>1</sup> Chelsea A Saito Reist†,<sup>1</sup> Claire E Kendal-Wright\*,<sup>1,2</sup> <sup>1</sup>*Chaminade University of Honolulu, Honolulu, HI, United States;* <sup>2</sup>*John A. Burns School of Medicine University of Hawaii, Honolulu, HI, United States.*
- W-019**     **Progesterone (P4) Is Locally Induced by Inflammatory Signals at the Maternal-Fetal Interface in Fetal Membranes.**  
Robert Moore\*, Deepak Kumar\*, Joseph Mansour\*, Brian Mercer\*, Sam Mesiano\*, John Moore\*. *Case Western Reserve University, Cleveland, OH, United States.*
- W-020**     **Cigarette Smoke Condensate Exposure Induces RAGE-Dependent Sterile Inflammation in Amniotic Epithelial Cells.**  
Helena Choltus†,<sup>1</sup> Corinne Belville,<sup>1</sup> Denis Gallot,<sup>1,2</sup> Régine Minet-Quinard,<sup>1,2</sup> Julie Durif,<sup>3</sup> Loïc Blanchon,<sup>1</sup> Vincent Sapin\*,<sup>1,2</sup> <sup>1</sup>*Clermont Auvergne University, Clermont-Ferrand, France;* <sup>2</sup>*Clermont-Ferrand Hospital, Clermont-ferrand, France;* <sup>3</sup>*Clermont-Ferrand Hospital, Clermont-Ferrand, France.*
- W-021**     **Initial Validation of Cervix Microstructure Imaging Using Quantitative Histology.**  
Wenjie Wu†,<sup>1</sup> Zhexian Sun†,<sup>1</sup> Hui Wang,<sup>1</sup> Xiao Ma†,<sup>1</sup> Hansong Gao†,<sup>1</sup> Sicheng Wang†,<sup>1</sup> Zichao Wen†,<sup>1</sup> Qing Wang,<sup>1</sup> Peinan Zhao,<sup>1</sup> Pamela K Woodard,<sup>1</sup> Hannah R Krigman,<sup>1</sup> Alison G Cahill,<sup>2</sup> Yong Wang\*,<sup>1</sup> <sup>1</sup>*Washington University School of Medicine, Saint Louis, MO, United States;* <sup>2</sup>*Dell Medical School, University of Texas, Austin, TX, United States.*
- W-022**     ***Gardnerella Vaginalis* Is Associated with Increased Tryptophan Metabolism in the Cervicovaginal Space: A Potential Role for Microbial Metabolites in Spontaneous Preterm Birth.**  
Kristin D Gerson†, Clare McCarthy, Heather H Burris, Michal A Elovitz\*. *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*
- W-023**     **Interrogation of Collagen Degradation Pathways for Cervical Extracellular Matrix (ECM) Remodeling through Pregnancy.**  
Mariano Colon-Caraballo†, Mala Mahendroo. *UT Southwestern Medical Center, Dallas, TX, United States.*
- W-024**     **Stretch Preconditioning of the Uterine Unfolded Protein Response Promotes Uterine Quiescence and Prevents Preterm Labor: In Vivo Observations in the Non-Human Primate.**  
Chandrashekar N Kyathanahalli,<sup>1</sup> Arren Simpson,<sup>1</sup> Judith Ingles,<sup>1</sup> Miranda Li,<sup>2</sup> Hazel Huang,<sup>2</sup> Jeff Munson,<sup>2</sup> Lakshmi Rajagopal,<sup>2</sup> Mark R Johnson,<sup>3</sup> Pancharatnam Jeyasuria,<sup>1</sup> Kristina M Adams Waldorf,<sup>2</sup> Jennifer C Condon\*,<sup>1</sup> <sup>1</sup>*Wayne State University, Detroit, MI, United States;* <sup>2</sup>*University of Washington, Seattle, WA, United States;* <sup>3</sup>*Imperial College London, London, United Kingdom.*
- W-025**     **A Role for Mirabegron in the Management of Uterine Contraction.**  
Hazik Asift†, Scott Barnett†, Buxton Iain\*. *University of Nevada, Reno, Reno, NV, United States.*
- W-026**     **Pharmacological Chaperones Sensitize Cells to Oxytocin Treatment.**  
Manasi Malik†, Yingye Fang†, Michelle Roh†, Antonina I. Frolova, Princess I. Imoukhuede, Sarah K. England\*. *Washington University in St Louis, St Louis, MO, United States.*
- W-027**     **Combination Tocolysis of Dysregulated Myometrial Pathways for the Treatment of Preterm Labor.**  
Scott D Barnett†,<sup>1</sup> Mitchell Anderson,<sup>2</sup> Hazik Asift†,<sup>1</sup> Iain L.O. Buxton\*,<sup>1</sup> <sup>1</sup>*University of Nevada, Reno School of Medicine, Reno, NV, United States;* <sup>2</sup>*University of Nevada, Reno, Reno, NV, United States.*
- W-028**     **Micro-RNA 203 Regulates Myometrial Smooth Muscle Cell Expression of the Transient Receptor Vanilloid 4 Channel and Contractility.**  
Lihua Ying, Cristina M Alvira, David N Cornfield\*. *Stanford University, Stanford, CA, United States.*

## Wednesday, July 7, 2021 - Poster Session I - Back Bay Conference and Exhibition Center

- W-029** **M<sup>6</sup>a Posttranscriptional Modification Allows for an Adaptable Fluid Myometrial Proteome during Pregnancy.**  
Jenkins Lindsay,<sup>1</sup> Simpson Arren,<sup>1</sup> Jennifer Condon,<sup>2</sup> Jeyasuria Pancharatnam\*,<sup>1</sup> *Wayne State University School of Medicine, Detroit, MI, United States;* <sup>2</sup>*Michigan, Detroit, MI, United States.*
- W-030** **Uterine Stimulation Index: A Promising Technique for Personalized Use of Oxytocin.**  
Ponnala S Marinescu†,<sup>1</sup> Roger C Young,<sup>2</sup> David A Adair,<sup>3</sup> Braxton Hern,<sup>3</sup> Evelina Galas,<sup>3</sup> Eva K Pressman,<sup>1</sup> Neil S Seligman\*,<sup>1</sup> *University of Rochester, Rochester, NY, United States;* <sup>2</sup>*PreTeL, Inc., Chattanooga, TN, United States;* <sup>3</sup>*University of Tennessee College of Medicine, Chattanooga, TN, United States.*
- W-031** **Identification of Mundulone and Mundulone Acetate as Natural Products with Tocolytic Efficacy in Mono and Combination Therapy with Current Tocolytics.**  
Shajila Siricilla†,<sup>1</sup> Christopher J Hansen,<sup>1</sup> Jackson H Rogers,<sup>1</sup> Carolyn L Simpson,<sup>1</sup> Stacey L Crockett,<sup>1</sup> Jeff Reese,<sup>1</sup> Bibhash C Paria,<sup>1</sup> Jennifer L Herington\*,<sup>1,2</sup> *Vanderbilt University Medical Center, Nashville, TN, United States;* <sup>2</sup>*Vanderbilt University, Nashville, TN, United States.*
- W-032** **Novel Gold Electromyographic Area Sensors Detect Uterine Bioelectric Activity as Well as Standard Hydrogel-Silver Area Sensors.**  
Ponnala Sunderi Marinescu†,<sup>1</sup> Roger C Young,<sup>2</sup> Pulin Wang,<sup>3</sup> Eva K Pressman,<sup>4</sup> Neil S Seligman\*,<sup>4</sup> *University of Rochester, Rochester, NY, United States;* <sup>2</sup>*PreTeL, Inc., Chattanooga, TN, United States;* <sup>3</sup>*Stretch Med, Inc., Austin, TX, United States;* <sup>4</sup>*University of Rochester Medical Center, Rochester, NY, United States.*
- W-033** **Effects of Monocytes on Contractile Function and Inflammatory Response of UtSM Cells under Hypoxia.**  
Binsh Wu†, Xiaoyan Sha\*, Huishu Liu†. *Guangzhou Women & Children Medical Center, Guangzhou, China.*
- GYNECOLOGY**
- W-034** **Leveraging Human Genomics to Accelerate Next-Generation Female Contraceptive Drug Discovery.**  
Karen Hunter Cohn, Caterina Clementi, Genevieve Galarneau, Piraye Yurtas Beim\*. *Celmatix Inc, New York, NY, United States.*
- W-035** **Mass Cytometry Reveals Unique Clusters of Monocytes in Blood and Decreased Phagocytic Capacity of Eutopic Endometrial Macrophages in Women with Endometriosis.**  
Júlia Vallvé-Juanico†, Sushmita Sen†, Ashley F George†, Kim Chi Vo\*, Juan C Irwin\*, Alexis Combes\*, Nadia Roan\*, Linda C Giudice\*. *University of California San Francisco, San Francisco, CA, United States.*
- W-036** **Whole Transcriptome Sequencing of an Endometriosis Cohort and Generation of iPSC-Derived Models for Functional Screening.**  
Jeremy Y Huang†,<sup>1,2</sup> Songlei Liu,<sup>1</sup> Li Li,<sup>1</sup> Ian N Waldman,<sup>3</sup> Raymond M Anchan,<sup>3</sup> George M Church\*,<sup>1,2</sup> *Blavatnik Institute, Harvard Medical School, Boston, MA, United States;* <sup>2</sup>*Wyss Institute for Biologically Inspired Engineering, Boston, MA, United States;* <sup>3</sup>*Brigham and Women's Hospital, Boston, MA, United States.*
- W-037** **The Role of Estrogen Receptor Alpha in Mediating Progesterone Resistance and Disease Progression in Endometriosis.**  
Valerie A. Flores†, Tran Dang, Joshua Huttler, Hugh S Taylor\*. *Yale School of Medicine, New Haven, CT, United States.*
- W-038** **Tofacitinib Alters STAT3 Signaling and Leads to Endometriosis Lesion Regression.**  
Alexander Kotlyar†, Ramanaiiah Mamillapalli, Valerie Flores, Hugh Taylor. *Yale University, New Haven, CT, United States.*
- W-039** **Modeling the Adenomyotic Phenotype Using Endometrial Organoids and a Fully Defined Synthetic Extracellular Matrix.**  
Juan S Gnecco†,<sup>1</sup> Kira Buttrey†,<sup>1</sup> Alex Brown†,<sup>1</sup> Clara Ives,<sup>1</sup> Megan Loring,<sup>2</sup> Keith Isaacson,<sup>2</sup> Linda Griffith\*,<sup>1</sup> *MIT, Cambridge, MA, United States;* <sup>2</sup>*NWH, Newton, MA, United States.*
- W-040** **Clinical Presentation of Women with Adenomyosis Alone and Concurrently with Uterine Fibroids.**  
Nawras Zayat, Anthony Filipovic, Brittany Dey, Victor Mniarji, Cassandra Charles, Serin Seckin, Ozgul Muneyirci-Delale. *SUNY Downstate Medical Center, Brooklyn, NY, United States.*
- W-041** **The Role of Tfap2c in Endometriosis with Infertility.**  
Juan Yin†,<sup>1</sup> Kosina Wong,<sup>2</sup> Radu Apostol,<sup>2</sup> Huan Yang\*,<sup>2</sup> Anping Lin.<sup>3</sup> *The Ninth People's Hospital of Chongqing, Chongqing, China;* <sup>2</sup>*Coney Island Hospital, Brooklyn, NY, United States;* <sup>3</sup>*The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China.*
- W-042** **Should I Stay or Should I Go? The Effect of Ovarian Endometrioma on Ovarian Reserve and Pregnancy Outcomes.**  
Caroline Peschansky†, Safina Usmani, Sarah Dynia, Sonia Patel, Jawaria Amir, Royi Lynn, Kayla Vitale, Lauren Grimm, Erica Louden, Roohi Jeelani, Angie Beltsos. *Vios Fertility Institute, Chicago, IL, United States.*
- W-043** **Is Endometriosis Associated with Congenital Uterine Anomalies? A Systematic Review.**  
Bo Peng\*,<sup>1</sup> Erroll I. Byer\*,<sup>2</sup> Michael Moretti\*,<sup>2</sup> *American University of the Caribbean, School of Medicine, Cupecoy, Netherlands Antilles;* <sup>2</sup>*The Brooklyn Hospital Center, Department of Obstetrics and Gynecology, Brooklyn, NY, United States.*
- W-044** **Simvastatin Inhibits Progesterone Receptor Signaling in Uterine Leiomyoma Cells.**  
Sadia Afrin†, Malak El Sabeh, Mariko Miyashita-Ishiwata, Mostafa Borahay\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- W-045** **The Mediator Kinase-Dependent Myometrial Stem Cell Phosphoproteome.**  
Lindsey Barron, Subash Khadka, Thomas G Boyer\*. *University of Texas Health San Antonio, San Antonio, TX, United States.*
- W-046** **Pathological Reprogramming of Epitranscriptomics via METTL3 in Uterine Fibroids.**  
Qiwei Yang,<sup>1</sup> Karthigayan Shanmugasundaram,<sup>2</sup> Chuan He,<sup>3</sup> Ayman Al-Hendy,<sup>1</sup> Thomas G Boyer.<sup>2</sup> *University of Chicago, OB/GYN, Chicago, IL, United States;* <sup>2</sup>*University of Texas Health Science Center at San Antonio, San Antonio, TX, United States;* <sup>3</sup>*University of Chicago, Chemistry, Chicago, IL, United States.*
- W-047** **Tryptophan Catabolism Is Dysregulated in Leiomyomas.**  
Tsai-Der Chuang, Derek Quintanilla, Drake Boos, Omid Khorram\*. *The Lundquist Institute at UCLA Medical Center, Torrance, CA, United States.*
- W-048** **Simvastatin Induces the Expression of Matrix Metalloproteinase in Human Leiomyoma Cells.**  
Malak El Sabeh†, Sadia Afrin†, Mostafa Borahay\*. *Johns Hopkins University, Baltimore, MD, United States.*

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- W-049 Targeting Hippo Signaling by Epigallocatechin Gallate (EGCG) Reduces Cell Growth, Fibrosis, Inflammation, and Angiogenesis in Uterine Fibroid Cells.**  
Md Soriful Islam, Kamaria C Cayton Vaught, Joshua T. Brennan, James H Segars. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- W-050 Evidence for Homeotic Transformation in Uterine Fibroids: A Novel Paradigm.**  
Jaime A Roura-Monllor†, Minnie Malik, Paul H Driggers, Joy Britten, Anthony M DeAngelis, Erin F Wolff, William H Catherino\*. <sup>1</sup>Uniformed Services University of the Health Sciences, Bethesda, MD, United States; <sup>2</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States; <sup>3</sup>Pelex Med, McLean, VA, United States.
- W-051 Using Mendelian Randomization to Understand Uterine Leiomyomata and Its Associated Clinical Phenome.**  
Jacqueline A Piekos†, Jacklyn N. Hellwege\*, Nikhil K. KhanKari, Samantha Greenblatt†, Todd L. Edwards\*, Digna R Velez Edwards\*. <sup>1</sup>Vanderbilt University, Nashville, TN, United States; <sup>2</sup>Vanderbilt University Medical Center, Nashville, TN, United States; <sup>3</sup>Vanderbilt University Medical Center, Nashville, TN, United States.
- W-052 Cardiovascular Risks Factors and Uterine Leiomyoma: Is There an Association?**  
Serin Seckin, Cassandra Charles, Fadi Yacoub, Shukla Minakshi, Anthony Filipovic, Vanessa Pinard, Ozgul Muneyyirci-Delale. *SUNY Downstate Health Sciences University, Brooklyn, NY, United States.*
- W-053 Effectiveness of Microwave Endometrial Ablation Combined with Transcervical Resection in Treating Submucous Uterine Myoma.**  
Toshiyuki Kakinuma, Kakinuma Kaoru, Kaneko Ayaka, Kagimoto Masataka, Yanagida Kaoru, Matsuda Yoshio, Takeshima Nobuhiro, Ohwada Michitaka. *International University of Health and Welfare Hospital, Tochigi, Japan.*
- W-054 The Kinase Inhibitor Nintedanib Regulates Multiple Key Targets of Inflammation, Fibrosis, and Angiogenesis Involved in Uterine Fibroid Pathogenesis.**  
Md Soriful Islam, Sadia Afrin, Christina N Cordeiro Mitchell, Mostafa A Borahay, James H Segars\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- W-055 Extracellular Matrix Gene Expression in At-Risk Human Myometrial Stem Cells Align with Fibroid Tumor-Initiating Cells and Is Distinct from Normal Myometrial Stem Cells.**  
Maria Victoria Bariani\*, Mohamed Ali, Sandra L. Grimm, Cristian Coarfa, Qiwei Yang, Ayman Al-Hendy. <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>Ain Shams University, Cairo, Egypt; <sup>3</sup>Baylor College of Medicine, Houston, TX, United States.
- W-056 Myometrial Stem Cell Enrichment to Understand Uterine Fibroid Etiology.**  
Emmanuel N. X. Paul†, Tyler J. Carpenter, Joshua A. Grey, Jose M. Teixeira\*. *Michigan State University, Grand Rapids, MI, United States.*
- W-057 Discovery of Novel Molecular Mechanisms Underlying the Pathophysiology of Uterine Fibroids and Associated Heavy Menstrual Bleeding.**  
Chen-Yi Wang, Marina Maritati, Darragh P O'Brien, Kavita S Subramaniam, Adam Cribbs†, Jessica Malzahn, Thomas M Zollner, Bianca De Leo, Maik Obendorf, Joerg Mueller, Martin Fritsch, Benedikt M Kessler, Krina T Zondervan, Adrian Harris, Christian M Becker, Udo Oppermann, Martin Philpott\*. <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Bayer AG, Berlin, Germany.
- W-058 Abstract Withdrawn**
- W-059 Metastatic Crohn's Disease Involving the External Female Genitalia: A Review and Analysis of Published Cases.**  
Rachel L Leib†, Allison M Parrill†, Melissa A DeViney†, Ryan Raffelt†, David Adelstein\*, Bo Peng\*, Lisa Eng\*, Pierre Hindy\*, Aruna Mishra\*. <sup>1</sup>American University of the Caribbean School of Medicine, Cupecoy, Netherlands Antilles; <sup>2</sup>Nassau University Medical Center, East Meadow, NY, United States; <sup>3</sup>The Birthing Center of NY, Brooklyn, NY, United States; <sup>4</sup>Gastroenterology Associates of Brooklyn, Brooklyn, NY, United States; <sup>5</sup>BronxCare Health System, Bronx, NY, United States.
- W-060 Adipose Tissue Inflammation-Stimulated Adrenomedullin (ADM) Overexpression Contributes to Lipid Dysfunction in Diabetic Pregnancy.**  
Yuanlin Dong\*, Ancizar Betancourt, Michael Belfort, Chandra Yallampalli. *Baylor College of Medicine, Houston, TX, United States.*
- ### CLINICAL PERINATOLOGY
- W-061 McDonald versus Shirodkar Cerclage Technique in the Prevention of Preterm Birth: A Systematic Review and Meta-Analysis.**  
Liam McAuliffe, Ashad Issah, Rosanna Diacci, Kimberley P Williams, Anne-Marie Aubin, Jason Phung, Carol Wang, Alexander Maouris, Sebastian Leathersich, Panos Maouris, Craig E Pennell\*. <sup>1</sup>University of Newcastle, The Junction, NSW, Australia; <sup>2</sup>University of Newcastle, Newcastle, NSW, Australia; <sup>3</sup>Sir Charles Gairdner Hospital, Perth, WA, Australia; <sup>4</sup>King Edward Memorial Hospital, Subiaco, Western Australia, Australia; <sup>5</sup>Obstetrics and Gynaecology, Subiaco, Western Australia, Australia.
- W-062 Perivable Birth in the North Carolina Triad: Do Outcomes Vary by Birth Etiology?**  
Melissa L Kozakiewicz†, Kathleen V Ferry†, Jeff M Denney\*. *Wake Forest University School of Medicine, Winston Salem, NC, United States.*
- W-063 A Randomized, Placebo-Controlled, Proof-of-Concept Trial of Ebopiprant for the Treatment of Spontaneous Preterm Labor (PROLONG).**  
Ben W Mol, Anh Nguyen\*, Ildar Fatkullin\*, Hynek Heřman\*, Antonin Pařizek\*, Petr Janků\*, Tuong Ho\*, Tal Biron-Shental\*, Andrew Humberstone\*, Michel Brethous\*, Jean-Pierre Gotteland\*, Elizabeth Garner\*. <sup>1</sup>Monash University Monash Medical Centre, Melbourne, Australia; <sup>2</sup>University of Aberdeen, Aberdeen, United Kingdom; <sup>3</sup>Hanoi Obstetrics and Gynecology Hospital, Hanoi, Viet Nam; <sup>4</sup>Kazan State Medical University, Kazan, Russian Federation; <sup>5</sup>The Institute for the Care for Mother and Child, Prague, Czech Republic; <sup>6</sup>Charles University and General Faculty Hospital in Prague, Prague, Czech Republic; <sup>7</sup>Masaryk University, Brno, Czech Republic; <sup>8</sup>My Duc Hospital, Ho Chi Minh City, Viet Nam; <sup>9</sup>Tel Aviv University, Tel Aviv, Israel; <sup>10</sup>ObsEva SA, Geneva, Switzerland; <sup>11</sup>ObsEva Inc., Boston, MA, United States.
- W-064 Combined Vaginal Progesterone and Cervical Cerclage in the Prevention of Preterm Birth: A Systematic Review and Meta-Analysis.**  
Anne-Marie Aubin†, Kimberley P Williams†, Liam McAuliffe†, Ashad Issah†, Rosanna Diacci†, Jason Phung\*, Carol Wang\*, Craig Pennell\*. <sup>1</sup>University of Newcastle, Newcastle, Australia; <sup>2</sup>Hunter Medical Research Institute, Newcastle, Australia.

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- W-065**     **Enhancing Contraction Signals in Uterine Electromyography by the Wavelet-Based Denoising Algorithm SureShrink: Application in EMMI.**  
Zichao Wen, Hui Wang, Sicheng Wang, Yong Wang\*. *Washington University School of Medicine, St. Louis, MO, United States.*
- W-066**     **Differential Distribution of T Cell Subtypes at the Maternal-Fetal Interface May Contribute to Preterm Labor.**  
Lu Gao\*, Jianqiang Yuan†, Yuanyuan Liu†, Hongping Liu†. *Second Military Medical University, Shanghai, China.*
- W-067**     **Maternal and Neonatal Pregnancy Outcomes Following Fluoride Supplementation: A Pilot Randomized Controlled Trial .**  
Anna Maya Powell, Ramya Reddy, Kevin DeLong, Kimberly Jones-Beatty, Laura Ensign, Irina Burd\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*
- W-068**     **Adjunct Therapy at Time of Exam-Indicated Cervical Cerclage in Singleton Pregnancies: A Systematic Review and Meta-Analysis.**  
Ann M Bruno†, <sup>1,2</sup> Ashley E Benson, <sup>1,2</sup> Torri D Metz, <sup>1,2</sup> Nathan R Blue\*. <sup>1,2</sup> *University of Utah Health, Salt Lake City, UT, United States;* <sup>2</sup>*Intermountain Healthcare, Murray, UT, United States.*
- W-069**     **Preterm Premature Rupture of the Membranes after Cerclage Placement.**  
Maria Andrikopoulou, Liping Lu, Chen Cheng, Samsiya Ona, Joy Vink, Cynthia Gyamfi-Bannerman\*. *Columbia University Irving Medical Center, New York, NY, United States.*
- W-070**     **Survival of the Newborn with Diaphragmatic Hernia: EXIT versus Standard Procedure.**  
Daniele Francesco Lo Gerfo, Simona Lunardi, Marcello Bargione, Pietro Alimondi, Giulia Vellani, Antonio Vanella, Antonella Mercurio, Marzia Costanzo, Federica Cusimano, Roberta Vaccaro, Giorgia Ranieri, Domenico Incandela, Antonio Maiorana, Maria Chiara Di Liberto. *ARNAS Civico, Palermo, Italy.*
- W-071**     **Chaperon Mediated Autophagy and Macroautophagy in Placentas of Obese Women with or without Gestational Diabetes Mellitus: A Preliminary Study.**  
Chiara Mando, Cecilia Diceglie†, Gaia Maria Anelli†, Cristina Martelli†, Chiara Novielli, Fabrizia Lisso†, Alessia Lo Dico†, Anais Serati†, Irene Cetin, Luisa Ottobrini. *Università degli Studi di Milano, Milan, Italy.*
- W-072**     **Maternal Underweight and Obesity Are Associated with Placental Pathologies in Human Pregnancy.**  
Hailey Scott†, <sup>1</sup> David Gynspan, <sup>2</sup> Laura N Anderson, <sup>3</sup> Kristin L Connor\*. <sup>1</sup> *Carleton University, Ottawa, ON, Canada;* <sup>2</sup>*University of British Columbia, Vancouver, BC, Canada;* <sup>3</sup>*McMaster University, Hamilton, ON, Canada.*
- W-073**     **Gestational Diabetes Stratification According to Body Mass Index in a Multiracial Cohort.**  
Kevin Saiki†, Kelly Yamasato, Benny Beth Paula, Bartholomew Lisa Marguerite, Men-Jean Lee\*. *John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, United States.*
- W-074**     **How Do Maternal BMI and Fetal Membrane Inflammation Influence Infant Outcomes at Birth?**  
Eleanor Duffley†, <sup>1</sup> Marina White†, <sup>1</sup> David Gynspan\*, <sup>2</sup> Shannon Bainbridge\*, <sup>3</sup> Kristin Connor\*. <sup>1</sup> *Carleton University, Ottawa, ON, Canada;* <sup>2</sup>*Vernon Jubilee Hospital, Vernon, BC, Canada;* <sup>3</sup>*University of Ottawa, Ottawa, ON, Canada.*
- W-075**     **Opiate Use Postpartum in Subjects with a History of Bariatric Surgery by Type of Surgery.**  
Samantha R Lauhon†, Meredith Cruz, Katherine Allen, Kia Semons-Booker, Rachel Harrison. *Medical College of Wisconsin, Wauwatosa, WI, United States.*
- W-076**     **Psychological Therapies Used to Improve Lifestyle Behaviors in (Pre)Pregnant Women: A Systematic Review.**  
Melissa van der Windt†, <sup>1</sup> Sofie van Zundert†, <sup>1</sup> Sam Schoenmakers\*, <sup>1</sup> Pauline Jansen\*, <sup>1,2</sup> Lenie van Rossem\*, <sup>1</sup> Régine Steegers-Theunissen\*. <sup>1</sup> *Erasmus MC, Rotterdam, Netherlands;* <sup>2</sup>*Erasmus University Rotterdam, Rotterdam, Netherlands.*
- W-077**     **Numeracy Scores and Perinatal Outcomes among Women with Gestational and Pregestational Diabetes.**  
Jennifer Jacobson†, <sup>1</sup> Amy Godecker, <sup>1</sup> Jennifer Janik, <sup>1</sup> April Eddy, <sup>2</sup> Jacquelyn Adams\*. <sup>1</sup> *University of Wisconsin School of Medicine and Public Health, Madison, WI, United States;* <sup>2</sup>*Unity-Point Health Meriter Hospital, Madison, WI, United States.*
- W-078**     **Risk of Preeclampsia after Obese Women with Unexplained Infertility Conceive with Ovarian Stimulation Intrauterine Insemination (OS-IUI) Is Reduced by Immediate Pre-Pregnancy Weight Loss.**  
Robert A. Wild, <sup>1</sup> Rodney K Edwards, <sup>1</sup> David S Wrenn, <sup>2</sup> Y D Zhao, <sup>1</sup> Karl R Hansen. <sup>1</sup> *University of Oklahoma HSC, Oklahoma City, OK, United States;* <sup>2</sup>*Quest Diagnostics, Seacaucus, NJ, United States.*
- W-079**     **Evaluating Quality Metrics for Unexpected Complications in Newborns and Severe Maternal Mortality in Nulliparous Term Singleton Vertex Deliveries.**  
Carole A McBride, Erin A Morris, Marjorie C Meyer\*. *University of Vermont College of Medicine, Burlington, VT, United States.*
- W-080**     **Increased Maternal Morbidity in Women with Iron Deficiency Anemia Who Receive Intravenous Iron Infusion Therapy.**  
Martina S Burn†, Lisbet Lundsberg, Jennifer Culhane, Caitlin Partridge, Moeun Son\*. *Yale University, New Haven, CT, United States.*
- W-081**     **Circulating ACE2 Increases during Pregnancy and Is Associated with Preterm Preeclampsia.**  
Robin Shoemaker, Katherine Vignes, Hong Huang, Aarthi Srinivasan, Aric Schadler, Zachary Stanley, Cynthia Cockerham, Brittany McKinley, John Bauer, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*
- W-082**     **Maternal and Neonatal Morbidity Associated with TOLAC versus Elective Repeat Cesarean as a Function of VBAC Success Prediction.**  
Hayley Pierce†, Frank B. Williams†, Carole McBride†, Kelley McLean\*. *University of Vermont Medical Center, Burlington, VT, United States.*
- W-083**     **The Inhibitory Effect of Amniotic Fluid Contamination on Anticoagulation Pathway in the Maternal Plasma Measured by an Activated Protein C-Sensitivity Test.**  
Divyanu Jain†, <sup>1,2</sup> Tomoaki Oda\*, <sup>2</sup> Naoki Tamura\*, <sup>2</sup> David M Olson\*, <sup>1</sup> Naohiro Kanayama\*, <sup>2</sup> Hiroaki Itoh\*. <sup>2</sup> *University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada;* <sup>1</sup>*Hamamatsu University School of Medicine, Hamamatsu, Japan.*
- W-084**     **Longitudinal Metabolic Profiling in Pregnancy of Women with and without Pregestational Diabetes Who Develop Preeclampsia.**  
Kathryn J Gray, <sup>1</sup> Mengxi Yang†, <sup>2</sup> Liming Liang, <sup>2</sup> Richa Saxena\*, <sup>3</sup> *Brigham and Women's Hospital, Boston, MA, United States;* <sup>2</sup>*Harvard University, Boston, MA, United States;* <sup>3</sup>*Massachusetts General Hospital, Boston, MA, United States.*

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- W-085** **TOLAC Morbidity as a Function of VBAC Predicted Success and Labor Onset.**  
Hayley Pierce†, Frank B. Williams†, Michael DeSarno\*, Carole McBride\*, Kelley McLean\*. *University of Vermont Medical Center, Burlington, VT, United States.*
- W-086** **Characteristics of Preventable Severe Maternal Morbidity.**  
Ashley Shea†, <sup>1,2</sup>Michelle P Debbink, <sup>1,2</sup>Susan Nourse†, <sup>1,2</sup>Alexandra Kroes†, <sup>1</sup>Sophie Janes†, <sup>1</sup>Cara Heuser, <sup>1,2</sup>Michael W Varner, <sup>1,2</sup>Torri D Metz\*, <sup>1,2</sup>University of Utah Health, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States.
- W-087** **Delivery Outside of a Transplant Center Is Associated with Emergent Cesarean and Increased Maternal and Neonatal Morbidity in Kidney and Liver Transplant Recipients.**  
Ophelia Yin†, <sup>1</sup>Kathleen Chung†, <sup>1</sup>Aneesh Kallapur†, <sup>1</sup>Lisa Coscia\*, <sup>2</sup>Serban Constantinescu\*, <sup>3</sup>Michael Moritz\*, <sup>4</sup>Yalda Afshar\*. <sup>1</sup>University of California, Los Angeles, Los Angeles, CA, United States; <sup>2</sup>Gift of Life Institute, Philadelphia, PA, United States; <sup>3</sup>Temple University, Philadelphia, PA, United States; <sup>4</sup>Lehigh Valley Health Network, Morsani College of Medicine, Allentown, PA, United States.
- W-088** **Platelet Count on Admission to Labor and Delivery and Hemorrhage Risk.**  
Megan Trostle†, Iffath Hoskins, Ashley S Roman\*. *NYU Langone Health, New York, NY, United States.*
- W-089** **Perinatal Outcomes in Once versus Twice Weekly Antenatal Surveillance in A2 Gestational Diabetes Mellitus.**  
Devon O'Brien†, Danielle Calvo†, Patrick Hilden\*, Jonathan O'Brien\*, Richard Miller\*, Kathy Matthews\*. *Saint Barnabas Medical Center, Livingston, NJ, United States.*
- W-090** **Uterine Conservation with Placenta Accreta Spectrum.**  
Nicola C Perlman†, Michaela Farber, Jean Marie Carabuena, Daniela A Carusi\*. *Brigham and Women's Hospital, Boston, MA, United States.*
- W-091** **A Systematic Review of Prior Termination of Pregnancy as a Risk Factor for Cervical Health in Pregnant Women.**  
Julia J Brittain†, <sup>1</sup>Stacey E Wahl, <sup>2</sup>John W Cyrus, <sup>2</sup>Hope M Wolf, <sup>2</sup>Jerome F Strauss III, <sup>2</sup>Timothy P York\*. <sup>1</sup>University of Richmond, Richmond, VA, United States; <sup>2</sup>Virginia Commonwealth University, Richmond, VA, United States.
- W-092** **Discrepancies between Ultrasound Based Composite Biometry for Estimated Fetal Weight and Abdominal Circumference Alone in Predicting Large for Gestational Age Fetuses and Newborns.**  
Lina Fouad, Bernard Gonik\*. *Wayne State University, Detroit, MI, United States.*
- W-093** **Persistence of Maternal Group B Streptococcal Colonization after Intravenous Ampicillin Administration.**  
Melissa L Kozakiewicz†, <sup>1</sup>Sarah E White, <sup>1</sup>Mallory Alkis, <sup>2</sup>Rita Kaplon, <sup>1</sup>Brian C Brost. <sup>1</sup>Wake Forest University School of Medicine, Winston Salem, NC, United States; <sup>2</sup>Medical University of South Carolina, Charleston, SC, United States.
- W-094** **COVID-19 Ethnic and Racial Disparity in the State of Maryland. A Report from the Maryland Study Group.**  
Livia Cojocar†, <sup>1</sup>Irina Burd\*, <sup>2</sup>Ramya Reddy†, <sup>2</sup>Seung Hyunuk†, <sup>1</sup>Katelyn Uribe†, <sup>1</sup>Katherine Rajat†, <sup>1</sup>Autusa Pahlavan†, <sup>1</sup>Sifa Turan\*. <sup>1</sup>University of Maryland School of Medicine, Baltimore, MD, United States; <sup>2</sup>Johns Hopkins University, Baltimore, MD, United States.
- W-095** **Influence of Maternal Race/Ethnicity on Adverse Neonatal Outcomes in the Setting of Shoulder Dystocia.**  
Sarina Rebecca Chaiken†, Claire H Packer†, Bharti Garg\*, Aaron B Caughey\*. *Oregon Health & Science University, Portland, OR, United States.*
- ### DEVELOPMENTAL PROGRAMMING
- W-096** **Normalization of Circulating Adiponectin Levels in Obese Pregnant Mice Prevents Left Ventricle Mitochondrial Respiratory Dysfunction in Adult Offspring.**  
Jerad H Dumolt†\*, Owen R Vaughan, Kathryn Erickson, Theresa L Powell, Thomas Jansson. *University of Colorado Anschutz Medical Campus, Aurora, CO, United States.*
- W-097** **Maternal Resveratrol Intervention Prevents Offspring Cardiac Dysfunction in a Rat Model of Obese Pregnancy.**  
Nozomi Itani†, <sup>1</sup>Guadalupe L Rodriguez-Gonzalez†, <sup>2</sup>Elena Zambrano, <sup>2</sup>Peter W Nathanielsz, <sup>3</sup>Paul D Taylor\*. <sup>1</sup>King's College London, London, United Kingdom; <sup>2</sup>Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; <sup>3</sup>University of Wyoming, Laramie, WY, United States.
- W-098** **Long-Term Effects of a Placenta-Targeted Treatment during Hypoxic Pregnancies on Cardiac Capacity to Recover from Ischemia/Reperfusion Insult in Adult Offspring.**  
Natalia Hula†, <sup>1</sup>Floor Spaans, <sup>1</sup>Jennie Vu, <sup>1</sup>Anita Quon, <sup>1</sup>Raven Kirschenman, <sup>1</sup>Christy-Lynn M. Cooke, <sup>1</sup>Tom J. Phillips, <sup>2</sup>C. Patrick Case, <sup>3</sup>Sandra T. Davidge. <sup>1</sup>University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Cardiff University, Cardiff, United Kingdom; <sup>3</sup>University of Bristol, Bristol, United Kingdom.
- W-099** **The Role of HPA-Axis Genetics in the Relationship between Birthweight and Adult Disease.**  
Carol A Wang, <sup>1,2</sup>Wriwu N Martin†, <sup>1</sup>Stephen J Lye, <sup>3</sup>Rebecca M Reynolds, <sup>4</sup>Stephen G Matthews, <sup>3,5</sup>Carly E McLaughlin, <sup>6</sup>Roger Smith, <sup>1,2</sup>Craig E Pennell\*. <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>4</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>5</sup>University of Toronto, Toronto, ON, Canada; <sup>6</sup>Curtin University, Western Australia, Australia.
- W-100** **Associations of Gestational Hypertensive Disorders and Maternal Blood Pressure with Offspring Blood Pressure, and Early Markers of Atherosclerosis at the Age of 10.**  
Clarissa J. Wiertsema†, Vincent W.V. Jaddoe, Annemarie G.M.G.J. Mulders, Romy Gaillard. *Erasmus MC, Rotterdam, Netherlands.*
- W-101** **Sex-Specific Upregulation of Estrogen Receptor 2 and Insulin-Like Growth Factor 1 Receptor in the Left Ventricle of Fetal Sheep by Prenatal Testosterone Excess.**  
Adel Ghnenis†, <sup>1</sup>Vasanth Padmanabhan, <sup>1</sup>Arpita Vyas\*. <sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>California Northstate University, Elk Grove, CA, United States.
- W-102** **Chronobiological Effects of Gestational Hypoxia on the Placental-Cardiac Clock Axis.**  
Rachael C Crew†, <sup>1</sup>Kimberley C. W Wang, <sup>1</sup>Peter B Noble, <sup>1</sup>Peter J Mark, <sup>1</sup>Caitlin S Wyrwoll, <sup>1</sup>Dino A Giussani\*. <sup>1</sup>The University of Western Australia, Perth, Australia; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.

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- W-103**     **Transcriptomic Responses Are Sex-Dependent in the Skeletal Muscle and Liver in Offspring of Obese Mice.**  
Amy C. Kelly†, Jeannie Chan,<sup>2</sup> Theresa L Powell,<sup>1</sup> Laura A Cox,<sup>2</sup> Thomas Jansson\*. <sup>1</sup>University of Colorado Anschutz, Aurora, CO, United States; <sup>2</sup>Wake Forest, Winston-Salem, NC, United States.
- W-104**     **In Utero Exposure to D9-tetrahydrocannabinol Leads to Rapid Postnatal Catch-Up Growth and Hepatic Dysmetabolism.**  
Shelby Louise Oke†, <sup>1,2</sup> Kendrick Lee,<sup>1</sup> Rosie Papp,<sup>1</sup> Patti Kiser,<sup>1</sup> Steven Laviolette,<sup>1</sup> Daniel B Hardy\*. <sup>1,2</sup> Western University, London, ON, Canada; <sup>2</sup>Children's Health Foundation, London, ON, Canada.
- W-105**     **Fetal Hyperuricemia in Pregnancy Induces Severe Postnatal Sex-Specific Development Deficits in an Animal Model.**  
Benjamin P. Lüscher†, <sup>1,2</sup> Andreina Schoeberlein,<sup>1,2</sup> Daniel V Surbek\*, <sup>1,2</sup> Marc U Baumann\*. <sup>1,2</sup> Department of Obstetrics and Gynaecology, University Hospital of Bern, Bern, Switzerland; <sup>2</sup>Department of Clinical Research, University of Bern, Bern, Switzerland.
- W-106**     **Are There Sex-Specific Effects of Placental Gross Morphology on Early Childhood Growth of Term Newborns in a Low Risk Community Based Setting?**  
Sylvia Dygulski, <sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,3</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongsachai,<sup>1,2</sup> Jennifer S Feng†, <sup>1,2,4</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†, <sup>1,2,4</sup> Adwoa Nantwi,<sup>5</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*. <sup>1,3,2</sup> Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>Institute for Basic Research, Staten Island, NY, United States; <sup>4</sup>CUNY Hunter College, New York, NY, United States; <sup>5</sup>New York University College of Global Public Health, New York, NY, United States.
- W-107**     **Differential DNA Methylation Signatures Following Antenatal Corticosteroids in Human Neonatal Blood.**  
Bona Kim†, Aya Sasaki, Kellie Murphy, Stephen G Matthews\*. University of Toronto, Toronto, ON, Canada.
- FETUS**
- W-108**     **Enrichment and Absolute Quantification of Cell-Free Placenta DNA in Maternal Blood.**  
Samantha L Wilson†, <sup>1</sup> Shu Yi Shen,<sup>1</sup> Tim Triche Jr,<sup>2</sup> Daniel D De Carvalho,<sup>1</sup> Michael M Hoffman\*. <sup>1</sup>Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; <sup>2</sup>Van Andel Institute, Grand Rapids, MI, United States.
- W-109**     **Noninvasive Prenatal Genetic Testing after Uterus Transplant.**  
Jessica Ruth Walter†, Nawar Latif, Eileen Wang, Kathleen O'Neill\*. University of Pennsylvania, Philadelphia, PA, United States.
- W-110**     **Maternal Diabetes Alters Gene Expression in the Developing Mouse Embryonic Heart.**  
Rolanda Lister, Chisom Ezenekwe, Etoi Garrison, Scott Baldwin. Vanderbilt University Medical Center, Nashville, TN, United States.
- W-111**     **Vaginal Delivery Is Feasible and Safe in Giant Omphaloceles.**  
Nicole R Gavin†, Amanda C Mahle†, Eric B Jelin, Clark T Johnson, Angie C Jelin\*. The Johns Hopkins Hospital, Baltimore, MD, United States.
- W-112**     **A Systematic Review to Guide Future Efforts in the Determination of Genetic Causes of Pregnancy Loss.**  
Andrew Zaricor Carey†, Nathan R Blue†, Michael W Varner, Jessica M Page, Aaron R Quinlan, D Ware Branch, Robert M Silver, Tsegaselassie Workalemahu\*. University of Utah Health, Salt Lake City, UT, United States.
- W-113**     **Late Gestation Fetal Hyperglucagonemia Results in Lower Fetal Weight, Fetal Hypoaminoacidemia, and Decreased Uteroplacental Nutrient Uptake.**  
Sarah Cilvik, <sup>1</sup> Stephanie R. Wesolowski,<sup>2</sup> Russ V. Anthony,<sup>3</sup> Laura D. Brown,<sup>2</sup> Paul Rozance\*. <sup>1</sup>Wake Forest University Health Sciences, Winston-Salem, NC, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>Colorado State University College of Veterinary Medicine, Fort Collins, CO, United States.
- W-114**     **Gestational Diabetes Mellitus-Associated Changes in the Proteomic Profile of Extracellular Vesicles in the Fetal Circulating Reveal a Potential Role in Cell Metabolism.**  
Ormazabal Valeska\*, <sup>1</sup> Soumyalekshmi Nair†, <sup>2</sup> Andrew Lai†, <sup>2</sup> Katherin Scholz-Romero†, <sup>2</sup> Emilio Diaz\*, <sup>1</sup> Felipe Zuñiga\*, <sup>1</sup> McIntyre H. David\*, <sup>2</sup> Martha Lappas\*, <sup>3</sup> Salomon Carlos\*, <sup>2,1</sup> University of Concepcion, Concepcion, Chile; <sup>2</sup>The University of Queensland, Brisbane, Australia; <sup>3</sup>University of Melbourne, Melbourne, Australia.
- W-115**     **Metformin Has Direct Signaling and Metabolic Effects in Fetal Hepatocytes.**  
Amanda Jones, Michael Nash, Dong Wang, Paul Rozance, Laura Brown, Stephanie Wesolowski\*. University of Colorado Anschutz Medical Campus, Aurora, CO, United States.
- W-116**     **Association between Maternal and Cord Serum Lipid Profile Markers with Anthropometrical Newborn Outcomes.**  
Catherine Everest†, Jessica L Puranda†, Danilo F da Silva†, Sara C.S Souza†, Alexandra D Goudreau†, Velislava Tzaneva, Kristi B Adamo\*. University of Ottawa, Ottawa, ON, Canada.
- W-117**     **Long Noncoding RNA-XIST Augments miR-424 to Regulate MEK1-FGFR1 Pathway in Intrauterine Growth Restriction.**  
Lu Huang†, <sup>1</sup> Nanbert Zhong\*, <sup>2</sup> Wuxi Maternity and Child Healthcare Hospital, Wuxi, China; <sup>2</sup>New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States.
- W-118**     **Chronic Intrauterine Hypoxia Alters Mitochondrial Dynamics in the Fetal Guinea Pig Heart in a Sex Dependent Manner.**  
Loren P. Thompson, Hong Song. University of Maryland SOM, Baltimore, MD, United States.
- W-119**     **Mitochondrial Respiration and Citrate Synthase Activity Are Lower in the IUGR Fetal Sheep Heart.**  
Eileen I. Chang†, Jane E. Stremming, Leslie A. Knaub, Jane E. Reusch, Laura D. Brown\*. University of Colorado School of Medicine, Aurora, CO, United States.
- W-120**     **Novel Gasotransmitter Cardio-Protection in the Developing Heart: Comparative Roles of Hydrogen Sulphide and Carbon Monoxide.**  
Y Niu†, <sup>1</sup> Q. Lyu†, <sup>1,2</sup> R. M. Hess†, <sup>1</sup> S. G. Ford†, <sup>1</sup> T. A. Garrud†, <sup>1</sup> A. Iqbal†, <sup>1</sup> J. O. Louca†, <sup>1</sup> W. Tong†, <sup>1</sup> K. J. Botting†, <sup>1</sup> D. A. Giussani\*. <sup>1</sup> University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>The Fourth Military Medical University, Xi'an, China.

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- W-121** **Pyrroloquinoline Quinone (PQQ) Supplementation Negatively Alters Skeletal Muscle Gene Expression in Addition to Oxidative Stress/IUGR's Effects.**  
Allyson Wood†, <sup>1</sup> Lin Zhao, <sup>1</sup> Timothy Regnault\*, <sup>1,2,3</sup>  
<sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>Lawson Research Institute, London, ON, Canada; <sup>3</sup>Children's Health Research Institute, London, ON, Canada.
- W-122** **High Altitude Fetal Genetics and the Influence on Birthweight.**  
Sara L Hillman\*, <sup>1</sup> Sushil Bhandari, <sup>2</sup> Padma Dolma, <sup>3</sup> Mitali Mukerji, <sup>4</sup> Bhavana Prasher, <sup>4</sup> Hugh Montgomery, <sup>5</sup> David J Williams, <sup>6</sup> Aniket Bhattacharyya, <sup>7</sup> Gianpiero Cavalleri. <sup>2</sup> <sup>1</sup>Univ. College London Institute for Women's Health, London, United Kingdom; <sup>2</sup>Royal College Surgeons Ireland, Dublin, Ireland; <sup>3</sup>Sonam Norboo Memorial Hospital, Leh, Ladakh, India; <sup>4</sup>Institute for Genomics and Integrative Biology, Delhi, India; <sup>5</sup>Univ. College London Institute for Human Health, London, United Kingdom; <sup>6</sup>David Williams, London, United Kingdom; <sup>7</sup>Institute for Genomics and Integrative Biology, Delhi, United Kingdom.
- W-123** **The Cardiac Adenosinergic System Is Developmentally Regulated.**  
 Lowell Davis, Samantha Louey, Sonnet S Jonker\*, Oregon Health & Science University, Portland, OR, United States.
- W-124** **Antenatal Synthetic Glucocorticoid Exposure Modifies Blood-Brain Barrier Function after Birth.**  
Margaret E Eng†, <sup>1</sup> Alice Kostaki, <sup>1</sup> Stephen G Matthews\*, <sup>1,1,2</sup> <sup>1</sup>The University of Toronto, Toronto, ON, Canada; <sup>2</sup>Sinai Health System, Toronto, ON, Canada.
- W-125** **Targeting Reactive Astrocyte Polarity as a Strategy for Neuroprotection in Acute Perinatal White Matter Injury.**  
Amanda Brosius Lutz†, <sup>1</sup> Patricia Renz†, <sup>2</sup> Vera Tscherrig†, <sup>2</sup> Marialuigia Giovannini-Spinelli†, <sup>1</sup> Valerie Haesler, <sup>2</sup> Shane Liddelow\*, <sup>3</sup> Andreina Schoeberlein\*, <sup>2</sup> Daniel Surbek\*. <sup>1</sup> <sup>1</sup>University Hospital Insel, University of Bern, Bern, Switzerland; <sup>2</sup>University of Bern, Bern, Switzerland; <sup>3</sup>New York University, New York, NY, United States.
- GYNECOLOGIC ONCOLOGY**
- W-127** **Targeted Blockade of STAT-3 Signaling Inhibits Cell Proliferation of Uterine Leiomyosarcoma Cells.**  
Collin Sittler†, Minnie Malik, Joy Britten, Paul Driggers, William H Catherino\*. *Uniformed Services University of Health Sciences, Bethesda, MD, United States.*
- MATERNAL BIOLOGY**
- W-128** **First Trimester Maternal Cortisol Signatures in Small-for-Gestational Age Infants.**  
Chaelin Lee, <sup>1</sup> Seung Mi Lee, <sup>2</sup> Dong Jun Byun, <sup>1</sup> So Yeon Kim, <sup>2</sup> Hugh I Kim, <sup>3</sup> Do Yup Lee, <sup>4</sup> Young Mi Jung, <sup>2</sup> Chan-Wook Park, <sup>2</sup> Joong Shin Park, <sup>2</sup> Man-Ho Choi. <sup>1</sup> <sup>1</sup>KIST, Seoul, Korea, Republic of; <sup>2</sup>Seoul National University College of Medicine, Seoul, Korea, Republic of; <sup>3</sup>Korea University, Seoul, Korea, Republic of; <sup>4</sup>Seoul National University, Seoul, Korea, Republic of.
- W-129** **Differential Expression Changes in Human Decidua at Labor, Term versus Preterm - Role for Upstream Targets in the PG Pathway.**  
Kylie Hornaday†, Moss Bruton Joe†, Stephen Wood\*, David Anderson\*, Donna Slater\*. *University of Calgary, Calgary, AB, Canada.*
- W-130** **Attenuated Piezo1-Mediated Vasodilation in the Reduced Uteroplacental Perfusion Model of Preeclampsia in Rat.**  
 Danielle Marasa†, Susannah Chilton, Annie Glessner-Fischer, Nga Ling Ko\*. *The University of Vermont, Burlington, VT, United States.*
- W-131** **Pregnant Women Release Greater Levels of Small Extracellular Vesicles after an Acute Bout of Moderate-Intensity Physical Activity Compared to Non-Pregnant Women.**  
Shuhiba Mohammad†, <sup>1</sup> Kelly Ann Hutchinson†, <sup>1</sup> Danilo Fernandes da Silva†, <sup>1</sup> Dylan Burger\*, <sup>1,2</sup> Kristi B Adamo\*. <sup>1</sup> <sup>1</sup>University of Ottawa, Ottawa, ON, Canada; <sup>2</sup>Ottawa Hospital Research Institute, Ottawa, ON, Canada.
- W-132** **Suppression of Maternal Iron-Regulatory Hormone Hcpcidin Is Essential for Healthy Pregnancy and Is Mediated by Secreted Placental Proteins.**  
Veena Sangkhae, <sup>1</sup> Yoel Sadovsky, <sup>2</sup> Tomas Ganz, <sup>1</sup> Elizabeta Nemeth\*. <sup>1</sup> <sup>1</sup>UCLA, Los Angeles, CA, United States; <sup>2</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States.
- W-133** **Women Who Develop Placental Maternal Vascular Malperfusion Show Evidence of a Procoagulant Phenotype in Early Pregnancy.**  
Carole A McBride, Maria C Bravo, Kelley C McLean, Thomas Orfeo, Ira M Bernstein\*. *University of Vermont Larner College of Medicine, Burlington, VT, United States.*
- W-134** **Paternal Deficiency of Complement Component C1q Leads to Vascular Dysfunction in Apolipoprotein-E Female Knockouts: A Novel High-Risk Mouse Model of Preeclampsia.**  
Mary Gemmel†, Elizabeth Sutton, Marcia Gallaher, Robert W. Powers\*. *University of Pittsburgh, Pittsburgh, PA, United States.*
- W-135** **Blood Pressure and Hypertensive Disorders of Pregnancy at High Altitude: A Systematic Review and Meta-Analysis.**  
Imogen D Grant†, Dino A Giussani\*, Catherine E Aiken\*. *University of Cambridge, Cambridge, United Kingdom.*
- W-136** **Cardiovascular Effects of Extra Virgin Olive Oil (EVOO) in Healthy Reproductive-Aged Women: A Randomized Controlled Trial.**  
Erin A Morris, <sup>1</sup> Carole A McBride, <sup>1</sup> Megan Boyert†, <sup>1</sup> Lorinda Roberts, <sup>1</sup> Joan Skelly, <sup>1</sup> Maurizio Mandalà, <sup>2</sup> Ira M Bernstein\*. <sup>1</sup> <sup>1</sup>University of Vermont Larner College of Medicine, Burlington, VT, United States; <sup>2</sup>University of Calabria, Rende (CS), Italy.
- W-137** **Continuous Core Body Temperature and the Onset of Parturition in Humans.**  
Elise N Erickson, Kierstyn Tuel, Leslie Myatt, Leonardo Pereira. *Oregon Health and Science University, Portland, OR, United States.*
- W-138** **The Sodium-Dependent Phosphate Transporter Slc20a2 Protects the Placenta from Ectopic Calcification.**  
Ana Correia-Branco†, <sup>1</sup> Ciara Benson, <sup>2</sup> Nirmala Jayaraman†, <sup>1</sup> Olga Kashpur†, <sup>1</sup> Mary C. Wallingford\*, <sup>1</sup>  
<sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>University of Washington, Seattle, WA, United States.
- W-139** **Prolonged Hypoxia Inhibits HMSMC HIF1a-Related Contractility Expressions but Not Apoptosis, Possible Leading to Uterine Atony at Labor.**  
Yunshan Chen. *Guangzhou Women and Children's Medical Center, Guangzhou, China.*

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- W-140** **Impaired Endothelium-Dependent Vascular Function in Female Mice with a History of a Pregnancy Complicated by Dyslipidemia.**  
Tamara Sáez†,<sup>1,2</sup> Abbey Pagée†,<sup>1,2</sup> Raven Kirschenman,<sup>1,2</sup> Floor Spaans,<sup>1,2</sup> Sandra T Davidge\*,<sup>1,2</sup>  
<sup>1</sup>University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Women and Children's Health Research Institute, Edmonton, AB, Canada.
- W-141** **Role of Monocyte Chemoattractant Protein 1 in Mesenteric Artery Remodeling Postpartum in Mice.**  
Kirtika Prakash, Rebecca I Fairchild, Nicole M DeLance, Natalia I Gokina, Elizabeth A Bonney\*. *University of Vermont, Larner College of Medicine, Burlington, VT, United States.*
- W-142** **Relationship between Low-Dose Epidural Analgesia and Obstetric Laceration Location and Severity.**  
Gillian Horwitz†, Megan Trostlet†, Iffath Hoskins\*, Ashley S. Roman\*. *NYU Langone Health, New York, NY, United States.*
- W-143** **Are Regulatory T-cells Involved in Attaining an Ongoing Uncomplicated Pregnancy after Unexplained Recurrent Pregnancy Loss?**  
Juliette Krop†, Hanneke Kapsenberg\*, Carin van der Keur\*, Marie-Louise van der Hoorn\*, Frits Koning\*, Frans Claas\*, Sebastiaan Heidt\*, Michael Eikmans\*. *Leiden University Medical Center, Leiden, Netherlands.*
- W-144** **Preterm Birth in Chronic Toxoplasma Gondii Infection.**  
Maureen Edith Groer\*,<sup>1</sup> Adetola Louis-Jacques\*,<sup>1</sup> Samia Dutra†,<sup>1,2</sup> Ming Ji.<sup>1</sup> <sup>1</sup>University of South Florida, Tampa, FL, United States; <sup>2</sup>University of Tennessee, Knoxville, TN, United States.
- W-145** **CBS-Derived Endogenous H<sub>2</sub>S Contributes to Estradiol-Induced Pregnancy-Dependent Uterine Artery Relaxation via Activation of BK<sub>Ca</sub> Channels in Human Uterine Artery Smooth Muscle Cells.**  
Yan Li†,<sup>1</sup> Yihua Yang†,<sup>1</sup> Sam Zhang†,<sup>1</sup> Qianrong Qi†,<sup>1</sup> Jin Bai†,<sup>1</sup> Ronald R. Magness\*,<sup>2</sup> Naoto Hoshi\*,<sup>1</sup> Dongbao Chen\*. <sup>1</sup>University of California, Irvine, CA, United States; <sup>2</sup>University of South Florida, Tampa, FL, United States.
- W-146** **Factors Associated with Addition of Pharmacotherapy for Gestational Diabetes Mellitus Treatment.**  
Vishmayaa Saravanan,<sup>1</sup> Rachel Harrison,<sup>2</sup> Lauren Pavlik,<sup>1</sup> Anna Palatnik.<sup>1</sup> <sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>2</sup>Advocate-Aurora Medical Group, Chicago, IL, United States.
- PLACENTA**
- W-147** **The Developmental Mouse Placenta Proteome Identifies Dynamic Temporal Changes.**  
 Olga Kashpur,<sup>1</sup> Ariel Mei,<sup>1</sup> Shiori Kuraoka,<sup>2</sup> Hideyuki Higashi,<sup>2</sup> Sasha Singh,<sup>2</sup> Elena Aikawa,<sup>2</sup> Mary C. Wallingford\*,<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>Brigham and Women's Hospital, Boston, MA, United States.
- W-148** **Identification of Subtype-Specific Markers for Preeclampsia Using Placental Pathology and RNAseq.**  
Mariko Horii, Cuong To, Rebecca Adami, Kathy Zhang-Rutledge, Leah Lamale-Smith, Louise C Laurent\*, Mana Parast\*. *University of California, San Diego, La Jolla, CA, United States.*
- W-149** **Use of the Hypoxia-Inducible Factor (HIF)-2 $\alpha$  Inhibitor PT2385 in Placental Dysfunction: New Intervention Addressing Fetal Growth Restriction and Preeclampsia.**  
Arthur Colson†,<sup>1,2</sup> Isaline Lambert†,<sup>1</sup> Christophe L Depoix\*,<sup>1</sup> Corinne Hubinont\*,<sup>1,2</sup> Pierre Sonveaux\*,<sup>1</sup> Frédéric Debiève\*,<sup>1,2</sup> <sup>1</sup>Catholic University of Louvain, Brussels, Belgium; <sup>2</sup>Saint-Luc University Hospital, Brussels, Belgium.
- W-150** **Characterizing Sex-Specific Differences in Placental Epigenome Using DNase-Sequencing Data.**  
Yeon Mi Hwang†,<sup>1</sup> Alison G Paquette,<sup>1</sup> Paul Shannon,<sup>1</sup> Cory Funk,<sup>1</sup> Jocelynn Pearl,<sup>2</sup> Hanna Liao,<sup>2</sup> Yoel Sadovsky,<sup>3</sup> Leslie Myatt,<sup>4</sup> John Stamatoyannopoulos,<sup>2</sup> Louis Muglia,<sup>5</sup> Nathan D Price.<sup>1</sup> <sup>1</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>2</sup>Altius Institute for Biomedical Sciences, Seattle, WA, United States; <sup>3</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States; <sup>4</sup>Oregon Health Sciences University, Portland, OR, United States; <sup>5</sup>Cincinnati Children's Hospital, Cincinnati, OH, United States.
- W-151** **Reduced Placental Decorin Expression Is Associated with Pregnancies Affected by Fetal Growth Restriction Independently of Gestational Age at Delivery.**  
 Kasia Maksym†,<sup>1</sup> Hannah EJ Yong,<sup>2</sup> Anna L David,<sup>1</sup> Amanda N Sferuzzi-Perri,<sup>3</sup> Sara L Hillman\*,<sup>1</sup> <sup>1</sup>University College London Institute for Women's Health, London, United Kingdom; <sup>2</sup>Agency for Science, Technology and Research, Singapore, Singapore; <sup>3</sup>Cambridge University, Cambridge, United Kingdom.
- W-152** **Human Placenta and Trophoblasts Express a Novel Atypical Protein Kinase C- $\zeta$  Isoform.**  
Sumaiyah Zubair Shaha†, Khushali Patel†, Saba Saadat, Meghan Riddell\*. *University of Alberta, Edmonton, AB, Canada.*
- W-153** **Differential DNA Methylation between Infection Associated and Idiopathic Spontaneous Preterm Birth.**  
Heather M Brockway\*, Jones N Helen\*. *University of Florida, Gainesville, FL, United States.*
- W-154** **Rosiglitazone Restores Expression of HO1 in the Preeclamptic Placenta.**  
Brooke A Armistead†, Hamid-Reza Kohan-Ghadr\*, Sascha Drewlo\*. *Michigan State University, Grand Rapids, MI, United States.*
- W-155** **Lifelong Western Diet Exposure Impacts upon the Vasculature of Placental Labyrinth at Mid-Gestation in a Non-Obese Guinea Pig Model.**  
Takashi Hashimoto,<sup>1,2</sup> Flavien Delhaest†,<sup>3</sup> Karen Nygard,<sup>2</sup> Lanette J Friesen-Waldner,<sup>2</sup> Charles A McKenzie,<sup>2,4,5</sup> Barbra de Vrijer,<sup>2,4,5</sup> Bryan S Richardson,<sup>2,4,5</sup> Patti Kiser,<sup>2</sup> Timothy RH Regnault\*,<sup>2,4,5</sup> <sup>1</sup>Kagoshima City Hospital, Kagoshima City, Japan; <sup>2</sup>University of Western Ontario, London, ON, Canada; <sup>3</sup>Geneva University, Geneva, Switzerland; <sup>4</sup>Children's Health Research Institute, London, ON, Canada; <sup>5</sup>Lawson Health Research Institute, London, ON, Canada.
- W-156** **Expression Quantitative Trait Loci Analysis in the Human Placenta.**  
Clara Apicella†,<sup>1</sup> Camino SM Ruan†,<sup>1</sup> Géraldine Gascoin\*,<sup>2</sup> Francisco Miralles\*,<sup>1</sup> Celine Méhats\*,<sup>1</sup> Daniel Vaiman\*. <sup>1</sup>Institut Cochin, U1016 INSERM, Paris, France; <sup>2</sup>CHU Angers, Angers, France.
- W-157** **Long Noncoding RNA XIST Regulates Fetal Growth by Acting as a Molecular Augment of miR-424 to Modulate MEK1 and FGFR1 Expression.**  
Lu Huang. *The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi, China.*

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- W-158**     **Exposure to  $\Delta$ -9-Tetrahydrocannabinol Disrupts Mitochondrial Function and Inhibits Syncytialization in BeWo Cells.**  
O'Llenecia Walker, Harmeet Gurmt†, Linda L May, Reginald Ragost†, Mariah Lapierre†, Sandeep Raha\*. *McMaster University, Hamilton, ON, Canada.*
- W-159**     **Influence of Placental Matrix Metalloproteinase-9 Protein Expression on the Branching Architecture of Chorionic Blood Vessels of Human Placenta.**  
Sara Oraee†, Jayasri Basu\*, Yingyi Wu†, Diana Encalada†, Lara Molina†, Magdy Mikhail. *BronxCare Health System, Bronx, NY, United States.*
- W-160**     **Leptin, IGFBP3 and IGFBP7 Are Potential Serum Biomarkers of Placenta accreta Spectrum.**  
Bradley H. Sipe†, Ozlem Guzeloglu-Kayisli, Kellie Larsen, Xiaofang Guo, Asli Ozmen, Nihan Semerci, Charles J. Lockwood, Umit A. Kayisli\*. *University of South Florida Morsani College of Medicine, Tampa, FL, United States.*
- W-161**     **Study on Role of Hypoxia-Inducing Factor 1 $\alpha$  Mediated Aquaporin1 in Trophoblast Invasion.**  
Xiaoyan Sha†, Binsheng Wu, Kaimin Guo†, Junjie Bao, Huishu Liu\*. *Guangzhou Women & Children Medical Center, Guangzhou, China.*
- W-162**     **VEGFA and FGF2 Alter Protein Phosphorylation Profiles in Human Fetoplacental Endothelial Cells.**  
Chi Zhou,<sup>1,2</sup> Xin-wen Chang†,<sup>3</sup> Jing Zheng\*.<sup>1</sup> *University of Wisconsin-Madison, Madison, WI, United States;* <sup>2</sup>*University of Arizona, Tucson, AZ, United States;* <sup>3</sup>*Tonji University Hospital, Shanghai, China.*
- W-163**     **Is Extracellular Outflow of Transcription-Related Factor High-Mobility Group A1 Protein Involved in the Pathogenesis of Preeclampsia?**  
Yuka Uchikura, Keiichi Matsubara, Yuko Matsubara, Takashi Sugiyama. *Ehime University, Toon, Japan.*
- W-164**     **The Impact of Maternal Diabetes on Fetal-Placental Size at Birth and Umbilical Cord Oxygen Values.**  
Sheryl Choo†,<sup>1</sup> Barbra de Vrijer,<sup>1</sup> Hilary Brown,<sup>2</sup> Larry Stitt,<sup>1</sup> Timothy RH Regnault,<sup>1</sup> Bryan S. Richardson.<sup>1</sup> *<sup>1</sup>Western University, London, ON, Canada;* *<sup>2</sup>University of Toronto, London, ON, Canada.*
- W-165**     **Cardiovascular, Anti-Angiogenic, Metabolic and Mitochondrial Signatures of Preeclampsia in Hypoxic Pregnancy.**  
Wen Tong†,<sup>1,2</sup> Kirsty L. Brain,<sup>1</sup> Beth J Allison,<sup>1</sup> Kimberley J Botting,<sup>1</sup> Youguo Niu,<sup>1,2</sup> Sage G Ford,<sup>1</sup> Tess A Garrud†,<sup>1</sup> Peter F.B. Wooding,<sup>1,2</sup> Olga V Patey,<sup>1</sup> Qiang Lyu,<sup>1</sup> Lin Zhang,<sup>1</sup> Caroline J Shaw,<sup>1</sup> Katie A O'Brien†,<sup>1</sup> Alice P Sowton†,<sup>1</sup> Tereza Cindrova-Davies,<sup>1,2</sup> Hong W Yung,<sup>1,2</sup> Graham J Burton\*,<sup>1,2</sup> Andrew J Murray\*,<sup>1</sup> Dino A Giussani\*.<sup>1,2</sup> *<sup>1</sup>Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom;* *<sup>2</sup>Centre for Trophoblast Research, Cambridge, United Kingdom.*
- W-166**     **Long Chain Polyunsaturated Fatty Acids Are Predominantly Incorporated into Phospholipids in Uterine and Umbilical Circulations in Pregnant Sheep.**  
Stephanie S Chassen, Stefanie Raymond-Whish, Stephanie R Wesolowski, Paul J Rozance, Theresa L Powell\*. *University of Colorado, Aurora, CO, United States.*
- W-167**     **Pathologic Lesions in Placentas of Fetuses with Left Ventricular Outflow Tract Obstruction.**  
Rachel L. Leon, Kavita Sharma, Imran N. Mir, Lina F. Chalak\*. *University of Texas Southwestern Medical Center, Dallas, TX, United States.*
- W-168**     **Sexual Dimorphism in Cytotrophoblast and Syncytiotrophoblast Energetics.**  
Matthew Bucher†, Leslie Myatt\*. *Oregon Health & Science University, Portland, OR, United States.*
- W-169**     **Detangle the Anatomical Heterogeneity of the Human Placenta and Identify the Placental Lesions Using Multi-Contrast Magnetic Resonance Imaging.**  
Zhexian Sun†,<sup>1,2</sup> Wenjie Wu†,<sup>1,2</sup> Hui Wang,<sup>1,2</sup> Xiao Ma,<sup>1</sup> Hansong Gao,<sup>1,2</sup> Sicheng Wang,<sup>1,2</sup> Peinan Zhao,<sup>2</sup> Zichao Wen,<sup>2</sup> Robert McKinstry,<sup>2</sup> D. Michael Nelson,<sup>2</sup> Pamela Woodard,<sup>2</sup> Qing Wang,<sup>2</sup> Alison G Cahill,<sup>3</sup> Yong Wang.<sup>2</sup> *<sup>1</sup>Washington University in St. Louis, St. Louis, MO, United States;* *<sup>2</sup>Washington University in St. Louis, School of Medicine, St. Louis, MO, United States;* *<sup>3</sup>University of Texas at Austin, Austin, TX, United States.*
- W-170**     **Sexual Dimorphism in Placental IGF-II Expression in Hypoxia-Induced Fetal Growth Restriction.**  
Emad Elsamadicy†, Loren P. Thompson\*. *University of Maryland School of Medicine, Baltimore, MD, United States.*
- W-171**     **Urinary Cell-Free Transcriptomic Signature Potentially Provides a Modality for Non-Invasive Detection of Adverse Outcomes during Pregnancies.**  
Giorgia Del Vecchio†,<sup>1</sup> Shanthie Thamotharan\*,<sup>1</sup> Fang Wei\*,<sup>2</sup> Kyunghyun Sung\*,<sup>1</sup> Carla Janzen\*,<sup>1</sup> Sherin U Devaskar\*.<sup>1</sup> *<sup>1</sup>David Geffen School of Medicine at UCLA, Los Angeles, CA, United States;* *<sup>2</sup>University of California Los Angeles, Los Angeles, CA, United States.*
- W-172**     **X-Ray Fluorescence Microscopy Reveals Disruptions in Placental Element Homeostasis: An Indicator of Dysregulated Oxidative Stress Pathways?**  
Vladimira Fotevat†,<sup>1</sup> Roger Smith,<sup>1</sup> David Paterson,<sup>2</sup> Michael Jones,<sup>3</sup> Lee Dedman,<sup>4</sup> Kaushik Maiti.<sup>1</sup> *<sup>1</sup>Hunter Medical Research Institute, Newcastle, Australia;* *<sup>2</sup>Australian Synchrotron, Clayton, Australia;* *<sup>3</sup>Queensland University of Technology, Brisbane, Australia;* *<sup>4</sup>University of Newcastle, Newcastle, Australia.*
- W-173**     **Regulation of Insulin-Like Growth Factor 1 (IGF1) Signaling in the Guinea Pig Placenta Following Nanoparticle Delivery of Human IGF1 Is Dependent on Fetal Phenotype.**  
Rebecca L Wilson†,<sup>1</sup> Kendal Stephens,<sup>2</sup> Kristin Lampe,<sup>2</sup> Helen Jones\*.<sup>1</sup> *<sup>1</sup>University of Florida, College of Medicine, Gainesville, FL, United States;* *<sup>2</sup>Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, United States.*
- W-174**     **Evidence-Based View of Safety and Effectiveness of Prokineticin Receptors Antagonists during Pregnancy Prokineticin Receptors Antagonists during Pregnancy.**  
Deborah Reynaud†,<sup>1</sup> Frédéric Sergent\*,<sup>1</sup> Roland Abi Nahed†,<sup>1</sup> Wael Traboulsi†,<sup>2</sup> Constance Collet†,<sup>1</sup> Padma Murthi\*,<sup>3</sup> Nadia Alfaidy\*,<sup>1</sup> Mohamed Benharouga\*.<sup>1</sup> *<sup>1</sup>INSERM U1292, Grenoble, France;* *<sup>2</sup>Georgetown University, Georgetown, WA, United States;* *<sup>3</sup>Monash University, Victoria, Australia.*
- W-175**     **COVID-19 in Pregnancy Is Associated with Increased Natural Killer Cell and Macrophage Infiltration at the Maternal-Fetal Interface.**  
Lillian Juttukonda†,<sup>1</sup> Wachman Elisha\*,<sup>2</sup> Yoel Benarroch†,<sup>3</sup> Jeffery Boateng†,<sup>2</sup> Elizabeth Taglauer\*.<sup>1</sup> *<sup>1</sup>Boston Children's Hospital, Boston, MA, United States;* *<sup>2</sup>Boston Medical Center, Boston, MA, United States;* *<sup>3</sup>Boston University School of Medicine, Boston, MA, United States.*
- W-176**     **The Immune Checkpoint PD-1/PD-L1 Expression Differs in Mouse Placenta in Acute and Sub-Chronic Maternal Inflammation.**  
Yang Liu†, Jin Liu†, Anguo Liu†, Jun Lei\*, Irina Burd\*. *Johns Hopkins University, Baltimore, MD, United States.*

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- W-177** **IL-11 Is Regulated by TGF $\beta$  in the Human Amnion and Is Induced by Teratogenic Viruses.**  
Christina Megli, Pallavi Karunakaran, Azia Evans, Carolyn Coyne\*. *University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.*
- W-178** **Viral Infection Followed by Lipopolysaccharide Exposure Upregulates IP-10 and ITAC in Human First Trimester Decidual Cells: Implications for Preeclampsia Pathogenesis.**  
Bradley H. Sipe†, Xiaofang Guo, Ozlem Guzeloglu-Kayisli, Nihan Semerci, Asli Ozmen, Kellie Larsen, Frederick Schatz, Umit A. Kayisli, Charles J. Lockwood\*. *University of South Florida Morsani College of Medicine, Tampa, FL, United States.*
- W-179** **Role of Androgen in GBS Induced Innate Immune Response and Subsequent Neurobehavioral Impairments.**  
Seline Yasmine Vancolet†, Ayash Taghreed†, Bernard Robaire\*, Sébire Guillaume\*. *McGill University, Montreal, QC, Canada.*
- W-180** **Saturated Fatty Acids and Group B Streptococcus Synergize to Induce Proinflammatory Responses in Gestational Membranes.**  
Alison J Eastman†, Lisa M Rogers, David M Aronoff\*. *Vanderbilt University Medical Center, Nashville, TN, United States.*
- W-181** **Downregulation of WNT Signaling Is Critical for Extravillous Trophoblast Cell Differentiation.**  
Vinay Shukla†, Kaela M. Varberg, Marja Kuna, Anna Galligos, Khursheed Iqbal, Michael J. Soares\*. <sup>1,2</sup>*University of Kansas Medical Center, Kansas City, KS, United States;* <sup>2</sup>*Children's Mercy, Kansas City, MO, United States.*
- W-182** **The Feasibility of Isolation, Identification and Quantification of Mesenchymal Umbilical Cord Blood Stem Cells in the Delayed Cord-Clamping Era.**  
Emily Rose Smith\*, William Curtin\*, Kevin Yeagle†, Nurgul Carkaci-Salli\*, Serdar Ural\*. <sup>3</sup>*Medical College of Wisconsin, Milwaukee, WI, United States;* <sup>2</sup>*Penn State Health, Reading, PA, United States;* <sup>3</sup>*Penn State Health, Hershey, PA, United States;* <sup>4</sup>*Penn State University, Hershey, PA, United States.*
- PREECLAMPSIA**
- W-183** **The Putative Antihypertensive, Kynurenine, Activates BK<sub>Ca</sub> Channels Directly and Increases Ca<sup>2+</sup> Sparks.**  
Stephanie A Worton†, Harry AT Pritchard†, Susan L Greenwood\*, Mariam Alakrawi†, Adam Greenstein\*, Jenny E Myers\*. *University of Manchester, Manchester, United Kingdom.*
- W-184** **Progesterone Induced Blocking Factor Attenuates Hypertension, Placental and Endothelial Cell Mitochondrial Dysfunction and Reactive Oxygen Species in Response to sFlt-1 during Pregnancy.**  
Lorena M Amaral, Evangeline Deer†, Kyleigh Comley, Denise C Cornelius, Jalisa Jones, Tarek Ibrahim, Ramana Vaka, Michael Franks, Babbette LaMarca\*. *University of Mississippi Medical Center, Jackson, MS, United States.*
- W-185** **Aldosterone Levels in Pregnancy with Aspirin Prophylaxis for the Prevention of Pre-Eclampsia in High and Low Risk Women.**  
Katherine Vignes†, Robin Shoemaker, Hong Huang, Aarthi Srinivasan, Aric Shadler, Zachary Stanley†, Cynthia Cockerham, Brittany McKinley†, Erin MacLeod†, John Bauer\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*
- W-186** **Aspirin Is Associated with Changes in Serum Aldosterone in High Risk Pregnancies.**  
Katherine Vignes†, Robin Shoemaker, Hong Huang, Aarthi Srinivasan, Aric Shadler, Zachary Stanley†, Cynthia Cockerham, Brittany McKinley†, Erin MacLeod†, John Bauer\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*
- W-187** **Placental Hypoplasia and Maternal Organic Vascular Disorder in Pregnant Women with Hypertensive Disorders of Pregnancy.**  
Kazushi Watanabe, Akihiko Wakatsuki. *Aichi Medical University School of Medicine, Nagakute, Japan.*
- W-188** **Multiple Kinases Mediate TNF $\alpha$ - Induced Endothelial Damage in Uterine Artery Endothelial Monolayers; Could Complexity = Opportunity?**  
Rachel L Dahn†, Luca Clemente\*, Amanda C Ampey\*, Jason A Austin\*, Ian M Bird\*. *University of Wisconsin, Madison, WI, United States.*
- W-189** **Finding the Missing Connection to Preeclampsia GWAS.**  
Jaeyong Choi,<sup>1</sup> Seung Mi Lee,<sup>2</sup> Hee Jin Ham,<sup>2</sup> Young Mi Jung,<sup>2</sup> Chan-Wook Park,<sup>2</sup> Jong Kwan Jun,<sup>2</sup> Jong-Il Kim,<sup>2</sup> Joong Shin Park.<sup>2</sup> <sup>1</sup>*Medical Research Center, Seoul National University, Seoul, Korea, Republic of;* <sup>2</sup>*Seoul National University College of Medicine, Seoul, Korea, Republic of.*
- W-190** **Uterine Artery Endothelial Cell Proliferation during Pregnancy Can Be Elevated by Exogenous and Endogenous Catecholamines.**  
Ronald R. Magness,<sup>1</sup> Maja Okuka,<sup>1</sup> Omar S Jobe.<sup>2</sup> <sup>1</sup>*University of South Florida, Tampa, FL, United States;* <sup>2</sup>*University of Wisconsin-Madison, Madison, WI, United States.*
- W-191** **Galectin-7: A Novel Placental-Released Driver of Preeclampsia.**  
Ellen Menkhorst†, Wei Zhou†, Leilani Santost†, Teresa So,<sup>1</sup> Sarah Delforce,<sup>2</sup> Argyro Syngelaki,<sup>3</sup> Kristy Pringle,<sup>2</sup> Kypros Nicolaides,<sup>3</sup> Yves St-Pierre,<sup>4</sup> Eva Dimitriadis\*. <sup>1</sup>*The University of Melbourne, Melbourne, Australia;* <sup>2</sup>*Hunter Institute of Medical Research, Newcastle, Australia;* <sup>3</sup>*King's College Hospital, London, United Kingdom;* <sup>4</sup>*Institut National de la Recherche Scientifique, Laval, QC, Canada.*
- W-192** **Peripheral Blood Monocytes Subsets Are Dysregulated in Women with Reproductive Failure.**  
Lujain AlSubki†, Nayoung Sung†, Shahrukh Syed†, Giovanni Jubiz, Xiuhua Yang, Wen-Juan Wang, Qiaohua He, Gloria Deutche, Svetlana Dambaeva, Alice Gilman-Sachs\*, Kenneth Beaman\*, Joanne Kwak-Kim\*. *Rosalind Franklin University of Medicine and Science, Vernon Hills, IL, United States.*
- W-193** **Biologic Differences between Preterm and Term Preeclampsia Revealed by Transcriptomic Analyses.**  
Olesya Plazyo, Ashley Hesson, Langen Elizabeth, Joseph Kirma, Santhi Ganesh, Johann Gudjonsson. *University of Michigan, Ann Arbor, MI, United States.*
- W-194** **Associations between Circulating sFlt1 and PlGF and Preeclampsia with Severe Maternal Complications, or Eclampsia.**  
Roxanne Hastie,<sup>1</sup> Lina Bergman,<sup>2</sup> Susan Walker,<sup>1</sup> Tu'uhevaha Kaitu'u-Lino,<sup>1</sup> Natalie J Hannan,<sup>1</sup> Fiona Brownfoot,<sup>1</sup> Alesia Harper,<sup>1</sup> Ping Cannon,<sup>1</sup> Cathy A Cluver,<sup>3</sup> Stephen Tong.<sup>1</sup> <sup>1</sup>*University of Melbourne, Heidelberg, Australia;* <sup>2</sup>*Uppsala University, Uppsala, Sweden;* <sup>3</sup>*Stellenbosch University, Cape Town, South Africa.*

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- W-195** **ACOG Guidelines Identify More At Risk Pregnancies in Bolivia.**  
Litzzi Lazo-Vega,<sup>1</sup> Lilian Toledo-Jaldin,<sup>1</sup> Alison Larrea,<sup>2</sup> Colleen G Julian,<sup>3</sup> Lorna G Moore\*,<sup>3</sup> <sup>1</sup>*Hospital Materno Infantil, La Paz, Bolivia, Plurinational State of*; <sup>2</sup>*University of Colorado Denver, La Paz, Bolivia, Plurinational State of*; <sup>3</sup>*University of Colorado Denver, Aurora, CO, United States.*
- W-196** **Clinical Outcome after First Trimester Pre-Eclampsia Screening-Directed High Dose Aspirin Therapy in High Risk Patients.**  
Nicole Rose Gavin†, Jena Miller\*, CiCi McShane†, Mara Rosner\*, Ahmet Baschat\*. *The Johns Hopkins Hospital, Baltimore, MD, United States.*
- W-197** **Investigation of Premature Cellular Senescence in Pre-Eclampsia and Intrauterine Growth Restriction.**  
Samprikta Manna†, Fergus P McCarthy\*, Colm J McElwain†, Marta Giralt Martin†, Cathal McCarthy\*. *University College Cork, Cork, Ireland.*
- W-198** **Using Machine Learning Tools to Classify and Predict Preeclampsia in Asymptomatic Women.**  
Jianhong Zhang\*,<sup>1</sup> Melanie Audette,<sup>1,2</sup> Nir Melamed,<sup>3</sup> Stephen J. Lye,<sup>1,2</sup> John C. Kingdom,<sup>1,2</sup> Kelsey McLaughlin,<sup>1,2</sup> <sup>1</sup>*Sinai Health System, Toronto, ON, Canada*; <sup>2</sup>*University of Toronto, Toronto, ON, Canada*; <sup>3</sup>*Sunnybrook Health Sciences, Toronto, ON, Canada.*
- W-199** **Fast Kit for Eclampsia.**  
Marcello Bargione, Simona Lunardi, Pietro Alimondi, Maria Chiara Di Liberto, Walter Alio, Claudia Amato, Francesco Forlani, Federica Cusimano, Clara Ferrara, Jessica Presti, Margherita Giunta, Roberta Vaccaro, Maria Antonietta Coppola, Rosaria Amato, Daniele Francesco Lo Gerfo. *ARNAS Civico, Palermo, Italy.*
- REPRODUCTIVE ENDOCRINOLOGY**
- W-200** **Analysis of Inflammatory Signaling in Reprometabolic Syndrome Induced by Acute Hyperinsulinemia and Hyperlipidemia in Normal Weight Women.**  
Andrew Tannous†,<sup>1</sup> Andrew P Bradford\*,<sup>1</sup> Katherine N Kuhn,<sup>1</sup> Angela Fought,<sup>1</sup> Irene Schauer\*,<sup>2,1</sup> Nanette Santoro\*.<sup>1</sup> <sup>1</sup>*University of Colorado School of Medicine, Aurora, CO, United States*; <sup>2</sup>*Rocky Mountain Regional Veterans Affairs Medical Center, Aurora, CO, United States.*
- W-201** **Dydrogesterone Supplementation in Cycles Triggered with Lone GnRH Agonist for Final Oocyte Maturation Resulted in an Acceptable Pregnancy Rate.**  
Myriam Safrai†, Shmuel Hertsberg†, Assaf Ben Meir\*, Benjamin Reubino†, Tal Imbar\*, Talya Mordechai-Daniel\*, Alexander Simon\*. *Hadassah Medical Center, Jerusalem, Israel.*
- W-202** **The Correlation between Human Papillomavirus Infection on Sperm Motility and Morphology among Infertile Men.**  
Sareh Abdollahifard†\*,<sup>1</sup> Iman Arbab Kazem Zadeh\*,<sup>2</sup> <sup>1</sup>*Jahrom University of Medical Sciences, Jahrom, Iran, Jahrom, Iran, Islamic Republic of*; <sup>2</sup>*University of Kassel, Kassel, Germany, Kassel, Germany.*
- W-203** **Anti-Mullerian Hormone and Time to Dominant Follicle in Intrauterine Insemination Cycles.**  
Victoria W Fitz†, Stylianos Vagios†, Irene Souter\*, Caitlin Sacha†, Karissa Hammer†, Charles Bormann, Kaitlyn James. *Massachusetts General Hospital, Boston, MA, United States.*
- W-204** **The Effect of Medical Treatment Including Dienogest after Surgical Removal of Ovarian Endometrioma on In Vitro Fertilization (IVF) Outcome.**  
Sejin Kim, Han Soo Jin, Kim Sung Woo, Kim Hoon, Ku Seung-Yup, Suh Chang Suk, Kim Seok Hyun. *Seoul National University Hospital, Seoul, Korea, Republic of.*
- W-205** **Intramuscular Progesterone for Luteal Phase Support May Not Improve Live Birth Rate Following Frozen Embryo Transfer (FET) in Patients with Polycystic Ovarian Syndrome (PCOS).**  
Daniel Miranian†, Colby Foster†, Emily Kobernik, Erin Inman†, Micaela Stevenson†, Samantha B Schon, Molly B Moravek\*. *University of Michigan, Ann Arbor, MI, United States.*
- W-206** **Development and Validation of a Noninvasive High-Resolution Imaging System for Uterine Peristalsis in Nonpregnant Women.**  
Sicheng Wang†, Zichao Wen, Stephanie Pizzella, Kelsey Anderson, Yiqi Lin, Wenjie Wu, Qing Wang, Valerie Ratts, Yong Wang. *Washington University in St. Louis, St. Louis, MO, United States.*
- W-207** **Estradiol Level during Ovulation Induction and Intrauterine Insemination with Letrozole Does Not Correlate with Pregnancy Outcome.**  
Erika P New†,<sup>1</sup> Shayne Plosker\*,<sup>2,1</sup> Kate Devine,<sup>3</sup> Samad Jahandideh,<sup>3</sup> Anthony N Imudia,<sup>2,1</sup> <sup>1</sup>*University of South Florida, Tampa, FL, United States*; <sup>2</sup>*Shady Grove Fertility Center Tampa Bay, Tampa, FL, United States*; <sup>3</sup>*Shady Grove Fertility Reproductive Science Center, Rockville, MD, United States.*
- W-208** **Drug-Drug Interactions In-Silico Analysis in Female Treatments Reveals Drugs with a High Number of Unconsidered Interactions and Potential Side Effects.**  
Ismael Henarejos-Castillo†,<sup>1,2</sup> Pablo Garcia-Acerot†,<sup>1,2</sup> Patricia Sebastian-Leon,<sup>1</sup> Alejandro Aleman,<sup>1</sup> Antonio Parraga-Leo†,<sup>1,2</sup> Patricia Diaz-Gimeno\*.<sup>1</sup> <sup>1</sup>*IVI Foundation / IIS La Fe, Valencia, Spain*; <sup>2</sup>*Universidad de Valencia, Valencia, Spain.*
- W-209** **A Call to Action: The Need for Genetic Carrier Screening Guidelines.**  
Sonia Patel, Lauren Grimm, Caroline Peschansky, Sarah Dynia, Safina Usmani, Jawaria Amir, Kayla Vitale, Royi Lynn, Erica Loudon, Angeline Beltsos, Roohi Jeelani. *Vios Fertility Institute, Chicago, IL, United States.*
- W-210** **Parenterally-Delivered Human Mesenchymal Stem Cells Are Effective in Restoring Fertility in a Chemotherapy Induced Premature Ovarian Insufficiency Mouse Model.**  
Hang-soo Park,<sup>1</sup> Rishi Man Chugh,<sup>2</sup> Esra Cetin,<sup>1</sup> Hiba Siblini,<sup>1</sup> Amro Elsharoud,<sup>3</sup> Mara Ulin,<sup>3</sup> Sahar Esfandiyari,<sup>3</sup> Ayman Al-Hendy.<sup>1</sup> <sup>1</sup>*University of Chicago, Chicago, IL, United States*; <sup>2</sup>*University of Kansas Medical Center, Kansas City, KS, United States*; <sup>3</sup>*University of Illinois at Chicago, Chicago, IL, United States.*
- W-211** **Testosterone Independent Biomarkers May Improve Prediction Models of Polycystic Ovary Syndrome.**  
KyEra V. Actkins†,<sup>1</sup> Lea K. Davis\*,<sup>2</sup> <sup>1</sup>*Meharry Medical College, Nashville, TN, United States*; <sup>2</sup>*Vanderbilt University Medical Center, Nashville, TN, United States.*
- W-212** **Impact of Gestational Metformin-Exposure on Fetal Anthropometric Measures in PCOS.**  
Carol Nader†,<sup>1</sup> Rachel Isaacs†,<sup>1</sup> Vasantha Padmanabhan,<sup>2</sup> Jean Claude Veille,<sup>1</sup> Arpita Vyas\*,<sup>1</sup> <sup>1</sup>*California Northstate University, Elk Grove, CA, United States*; <sup>2</sup>*University of Michigan, Ann Arbor, MI, United States.*

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- W-213** **Increased Prevalence of Positive Depression Screen during COVID19 in PCOS Patients at an Academically-Affiliated Institution.**  
Elizabeth Kravitz†, Liubin Yang†, Janet Bruno-Gaston†, Amy Schutt\*. *Baylor College of Medicine, Houston, TX, United States.*
- W-214** **Effect of Caloric Restriction on Reproductive Parameters on the Age Wistar Rats.**  
Pablo López de Jesús†, <sup>1,2</sup> Israel Enrique Crisanto López†, <sup>3</sup> Saúl Rodríguez Flores†, <sup>4</sup> Edith Arenas Ríos\*, <sup>1</sup> Isabel Arrieta Cruz\*, <sup>5</sup> Marcela Arteaga Silva\*, <sup>1</sup> Juan Carlos Flores Alonso\*. <sup>6</sup> *Universidad Autónoma Metropolitana, Mexico, Mexico; <sup>2</sup>Instituto Mexicano del Seguro Social, Atlixco, Puebla, Mexico; <sup>3</sup>Benemérita Universidad Autónoma de Puebla, Puebla, Mexico; <sup>4</sup>Benemérita Universidad Autónoma de Puebla, Mexico, Mexico; <sup>5</sup>Instituto Nacional de Geriatria, Mexico, Mexico; <sup>6</sup>Instituto Mexicano del Seguro Social, Mexico, Mexico.*
- W-215** **Outcomes of IVF Cycles Using Testicular and Zymot Obtained Sperm from Men with High Sperm DNA Fragmentation.**  
Nicole D Ulrich†, Min Xu, James Dupree, Samantha Schon\*. *University of Michigan, Ann Arbor, MI, United States.*
- W-216** **Caloric Restriction Effects on Female Wistar Rats Reproductive Health.**  
Israel Enrique Crisanto López\*, <sup>1</sup> Pablo López de Jesús\*, <sup>2</sup> Isabel Arrieta Cruz\*, <sup>3</sup> Juan Carlos Flores Alonso\*. <sup>4</sup> *Benemérita Universidad Autónoma de Puebla, Puebla, Mexico; <sup>2</sup>Universidad Autónoma Metropolitana, Iztapalapa, Ciudad de México, Mexico; <sup>3</sup>Instituto Nacional de Geriatria, Ciudad de México, Mexico; <sup>4</sup>Laboratorio de Biología de la Reproducción, Centro de Investigación Biomédica de Oriente-IMSS, Puebla, Mexico.*
- REPRODUCTIVE BIOLOGY**
- W-217** **Exposure to Synthetic Glucocorticoids Modifies the Sperm MicroRNA Profile: Implications for Intergenerational Transmission.**  
Christopher Casciaro†, <sup>1</sup> Hiroataka Hamada†, <sup>1</sup> Alisa Kostaki, <sup>1</sup> Stephen Matthews\*. <sup>1,2,3</sup> *University of Toronto, Toronto, ON, Canada; <sup>2</sup>Univeristy of Toronto, Toronto, ON, Canada; <sup>3</sup>Sinai Health System, Toronto, ON, Canada.*
- W-218** **Developing a Genetically Modified Marmoset Model of Neurodevelopmental Disease.**  
Joshua T Brennan, <sup>1</sup> Jessica Izzi, <sup>1</sup> Yan-Ling Feng, <sup>1</sup> Ping Xia, <sup>1</sup> Xindong Song, <sup>1</sup> Jacqueline Maher, <sup>1</sup> Bhuchitra Singh, <sup>1</sup> Shanshan Zhu, <sup>1</sup> Christopher Ross, <sup>1</sup> John Davis, <sup>2</sup> James C Harris, <sup>1</sup> Xiaojin Wang, <sup>1</sup> James H Segars\*. <sup>1</sup> *Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>2</sup>University of Illinois, Chicago, IL, United States.*
- W-219** **Effects of Road Salt Components on Zebrafish Embryo Development and Viability.**  
Denis Aydin Seli†, Hugh Taylor\*. *Yale School of Medicine, New Haven, CT, United States.*
- W-220** **Umbilical Cord-Derived Mesenchymal Stem Cells Modulate Endometrial Stromal Cell Migration and Invasion.**  
Anat Chemerin†, Qingshi Zhao, Daniel Cho, Natak Douglas\*, Yahaira Naaldijk, Pranela Rameshwar\*, Sara Morelli\*. *Rutgers New Jersey Medical School, Newark, NJ, United States.*
- W-221** **Transcriptional Regulation Analysis from a Systems Biology Perspective Reveals New Ways in Menstrual Cycle Regulation and Disease.**  
Antonio Parraga-Leo†, <sup>1,2</sup> Patricia Sebastian-Leon, <sup>2</sup> Almudena Devesa-Peiro†, <sup>1,2</sup> José Remohí, <sup>1,3</sup> Patricia Diaz-Gimeno\*. <sup>2</sup> *University of Valencia, Valencia, Spain; <sup>1</sup>IVI Foundation - Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain; <sup>3</sup>IVI-RMA IVI Valencia, Valencia, Spain.*
- W-222** **Spontaneous Early Embryonic Loss in Wild-Type Mice Is Associated with Dysregulated Uterine and Serum Lipid Profiles.**  
Sydney L Lane†, William B Schoolcraft, Mandy G Katz-Jaffe\*. *Colorado Center for Reproductive Medicine, Lone Tree, CO, United States.*
- W-223** **Structural and Functional Reorganization of Secretory Organelles in Decidualized Endometrial Stromal Cells.**  
Marco Dalla Torre, Tiziana Anelli, Paola Panina-Bordignon. *Vita-Salute San Raffaele University, Milano, Italy.*
- W-224** **Endometrium >13mm Negatively Impacts Pregnancy Rates Following Transfer of a Single Euploid Embryo.**  
Tia Y Brodeur†, <sup>1,2</sup> Katelyn Tessier, <sup>1</sup> April Batcheller\*. <sup>3</sup> *University of Minnesota, Minneapolis, MN, United States; <sup>2</sup>University of Vermont, Burlington, VT, United States; <sup>3</sup>Colorado Center for Reproductive Medicine, Minneapolis, MN, United States.*
- W-225** **Risk Factors for the Delayed Diagnosis of Interstitial Ectopic Pregnancies.**  
Christana O Ajewole†, Ann Doherty†, Joseph Politch, Alexis Gadsont†, Yeon Woo Lee†, Neha Khemani†, Christina LeBedis, Wendy Kuohung\*. *Boston University School of Medicine, Boston, MA, United States.*
- W-226** **Plasma Enriched in Stem Cell Secreted Factors Improves Ovarian Function in a Mouse Model of Advanced Maternal Aged.**  
Maria Marchant†, <sup>1</sup> Anna Buiguest, <sup>2</sup> Jessica Martinez, <sup>1</sup> Antonio Pellicer\*, <sup>3</sup> Sonia Herraiz\*. <sup>2</sup> *IVI Foundation-University of Valencia, Valencia, Spain; <sup>1</sup>IVI Foundation-IIS la Fe, Valencia, Spain; <sup>3</sup>IVIRMA, Rome, Italy.*
- W-227** **Generation of a Maternal Haploinsufficient *Akap13* Allele in Mice via Oocyte-Specific Cre-Lox Recombination Does Not Impair Fertility.**  
Joshua T Brennan, <sup>1</sup> Jia Huang, <sup>2</sup> Kamaria C Cayton Vaught, <sup>1</sup> Carter M Owen, <sup>1</sup> Kimberlyn M Baig-Ward, <sup>1</sup> Hisashi Koide, <sup>3</sup> James H Segars\*. <sup>1</sup> *Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>2</sup>The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; <sup>3</sup>Chiba University Graduate School of Medicine, Chiba, Japan.*
- W-228** **Anti Mullerian Hormone (AMH) Is Not Increased in Women with Spontaneous Conceptions at Highly Advanced Reproductive Age 43-47y.**  
Keren Rotshenker-Olshinka, Jennia Michaeli, Naama Srebniak, Arnon Samueloff, Sophie Magen, Rivka Farkash, Talia Eldar-Geva. *Shaare Zedek Medical Center, Jerusalem, Israel.*
- W-229** **Dysfunctional Multidrug Resistance Transporter-1 (MDR1) Associated with Premature Diminished Ovarian Reserve and Metabolic Syndrome.**  
Dalileh Nabit†, <sup>1</sup> Zijiang Zhang†, <sup>2</sup> Lynae Maria Brayboy\*. <sup>3,4,5</sup> *Technische Universität Dresden, Dresden, Germany, Germany; <sup>2</sup>University of Arkansas for Medical Sciences, Little Rock, AR, United States; <sup>3</sup>Clue by Biowink, Berlin, Germany, Germany; <sup>4</sup>Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany; <sup>5</sup>Alpert Medical School of Brown University, Providence, RI, United States.*

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- W-230**     **Single Cell RNAseq Analysis Identifies Previously Unacknowledged Populations and Across-Cycle Transcriptomic Changes in Immune Cells in Healthy Human Endometrium.**  
Wanxin Wang†, Juan Irwin, Linda Giudice\*. *University of California, San Francisco, San Francisco, CA, United States.*
- W-231**     **Fibroblasts of the Bovine Corpus Luteum Release Prostaglandin F2α into the Microenvironment in Response to Pro-Inflammatory Cytokines.**  
Corrine F Monaco†, John S. Davis\*. *University of Nebraska Medical Center, Omaha, NE, United States.*
- W-232**     **Physical Activity Throughout Gestation Differentially Regulates CD206 Expression in Term Placenta.**  
Alexandra D Goudreau†, Catherine Everest†, Velislava Tzaneva\*, Kristi B Adamo\*. *University of Ottawa, Ottawa, ON, Canada.*
- W-233**     **Vascular-Tissue Trafficking of Decidual Innate Lymphoid Cells.**  
Jessica Vazquez†, Payton Lindner, Yan Li, Aleksandar K Stanic\*. *University of Wisconsin-Madison, Madison, WI, United States.*
- W-234**     **One-Sided Chronic Intervillositis of Unknown Etiology in Dizygotic Twins: A Description of 3 Cases.**  
Manon Bos,<sup>1</sup> Lotte van der Meer\*,<sup>1</sup> Juliette Krop,<sup>1</sup> Kyra Dijkstra,<sup>1</sup> Kitty Bloemenkamp,<sup>2</sup> Emily Cornish,<sup>3</sup> Peter Nikkels,<sup>2</sup> Marie-Louise van der Hooft.<sup>1</sup> *<sup>1</sup>Leiden University Medical Center, Leiden, Netherlands;* *<sup>2</sup>University Medical Center Utrecht, Utrecht, Netherlands;* *<sup>3</sup>University College London, London, United Kingdom.*
- W-235**     **Inhibition of MLL1 and HDAC Activity Reverses Reprogrammed Inflammatory Components Induced by Developmental Exposure to an Endocrine Disruptor (Diethylstilbesterol) in Myometrial Stem Cells.**  
Mohamed Ali†,<sup>1</sup> Hoda ElKafas†,<sup>2</sup> Nahed Ismail\*,<sup>2</sup> Ayman Al-Hendy\*,<sup>3</sup> Qiwei Yang\*,<sup>3</sup> *<sup>1</sup>Ain Shams University, Cairo, Egypt;* *<sup>2</sup>University of Illinois at Chicago, Chicago, IL, United States;* *<sup>3</sup>University of Chicago, Chicago, IL, United States.*
- W-236**     **Using Live Imaging and Cell Cycle Indicator Embryonic Stem Cells to Predict Dose-Dependent Suppression of Leading Indicators of Growth by PFOA and DEP.**  
Daniel A Rappolee\*, Mohammed Abdulhasan\*, Ximena Ruden\*, Douglas M Ruden\*, Elizabeth E Puscheck\*. *Wayne State University, Detroit, MI, United States.*
- W-237**     **Towards the Development of the Bioengineered Ovary: Comparative Proteomic Analysis of Different Extracellular Matrix Hydrogels.**  
Hannes Campo†,<sup>1,2</sup> Emilio Francés-Herrero†,<sup>2,3</sup> Sara López-Martínez†,<sup>2</sup> Amparo Faus,<sup>2</sup> Antonio Pellicer\*,<sup>4</sup> Irene Cervelló\*,<sup>2</sup> *<sup>1</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States;* *<sup>2</sup>IVI Foundation-IIS La Fe, Valencia, Spain;* *<sup>3</sup>Universitat de València, Valencia, Spain;* *<sup>4</sup>IVIRMA-Rome, Rome, Italy.*
- W-238**     **Microenvironment or Microstructure: Potential Role of Acellular Lesions in the Pathogenesis of Endometriosis.**  
Masoumeh Majidi Zolbin†,<sup>1</sup> Ashkan Azimzadeh Fardkhatoni,<sup>1</sup> Roxana Sahmani,<sup>1</sup> Ameneh Haghgoo,<sup>2</sup> Ali Mohebbi,<sup>1</sup> Abdol-Mohammad Kajbafzadeh.<sup>1</sup> *<sup>1</sup>Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of;* *<sup>2</sup>Nikan Hospital, Tehran, Iran, Islamic Republic of.*
- W-239**     **OnPrognosis after Unexplained Recurrent Pregnancy Losses (RPL): a Systematic Review and External Validation of Clinical Prediction Models.**  
Angelos Youssef†. *LUMC, Leiden, Netherlands.*
- W-240**     **Placental Gross Pathology in a Population Based Case-Control Study of Autism Spectrum Disorder.**  
Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Mehrin Jan,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulska,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Jillamika Pongsachai,<sup>1,2</sup> Michael Joyce,<sup>1</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*,<sup>1,6,2</sup> *<sup>1</sup>Placental Analytics, New Rochelle, NY, United States;* *<sup>2</sup>NYPBMH, Brooklyn, NY, United States;* *<sup>3</sup>CUNY Hunter College, New York, NY, United States;* *<sup>4</sup>NYU CGPH, New York, NY, United States;* *<sup>5</sup>Institute of Basic Research, Staten Island, NY, United States;* *<sup>6</sup>Institute for Basic Research, Staten Island, NY, United States.*
- W-241**     **Gestational Diabetes Mellitus and Placental DNA Methylation in the Rhode Island Child and Health Study.**  
Corina Lesseur†,<sup>1</sup> Amber Burt†,<sup>2</sup> Jia Chen\*,<sup>1</sup> Carmen J Marsit\*,<sup>2</sup> *<sup>1</sup>Icahn School of Medicine at Mount Sinai, New York, NY, United States;* *<sup>2</sup>Emory University, Atlanta, GA, United States.*
- W-242**     **Fetal Growth Restriction and Longer Term Outcomes. Are We Causing Harm?**  
Roshan Selvaratnam†,<sup>1,2</sup> Euan M Wallace\*,<sup>1,2</sup> Rory Wolfe\*,<sup>3</sup> Peter J Anderson\*,<sup>4</sup> Mary-Ann Davey\*,<sup>1,2</sup> *<sup>1</sup>The Ritchie Centre, Monash University, Melbourne, Australia;* *<sup>2</sup>Safer Care Victoria, Melbourne, Australia;* *<sup>3</sup>Monash University, School of Public Health and Preventative Medicine, Australia;* *<sup>4</sup>Monash University, Melbourne, Australia.*
- W-243**     **Association of Phthalate and DINCH Urinary Metabolite Concentrations during Pregnancy with Gestational Age at Birth and Birth Weight.**  
Victoria Fruh,<sup>1</sup> Emma V Preston,<sup>1</sup> Marlee R Quinn,<sup>1</sup> Michele R Hacker,<sup>2</sup> Blair J Wylie,<sup>2</sup> Karen O'Brien,<sup>2</sup> Tamarra James-Todd\*,<sup>1</sup> Shruthi Mahalingaiah\*,<sup>1</sup> *<sup>1</sup>Harvard University, Boston, MA, United States;* *<sup>2</sup>Beth Israel Deaconess Medical Center, Boston, MA, United States.*
- W-244**     **Placental Histology of Acute and Chronic Inflammation in a Population Based Case Control Study of Autism.**  
Jillamika Pongsachai,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulska,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Michael Joyce,<sup>1</sup> Jennifer S Feng†,<sup>1,2,3</sup> Serena Chen,<sup>1,2</sup> Beata Dygulska,<sup>2</sup> Christine Chen,<sup>1,2</sup> Carolyn Salafia\*,<sup>1,2,5</sup> *<sup>1</sup>Placental Analytics, New Rochelle, NY, United States;* *<sup>2</sup>NYPBMH, Brooklyn, NY, United States;* *<sup>3</sup>CUNY Hunter College, New York, NY, United States;* *<sup>4</sup>NYU CGPH, New York, NY, United States;* *<sup>5</sup>Institute of Basic Research, Staten Island, NY, United States.*
- W-245**     **Maternal Microchimeric Cells Predict Early Life Respiratory Infections in Children.**  
Christopher Urbschat†, Steven Schepanski, Kristin Thiele, Agnes Wiczorek, Marie Albrecht, Boris Fehse, Anke Diemert, Petra Arck\*. *University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*
- W-246**     **Does Placental Acute or Chronic Inflammation Impact Placental Efficiency in Term Newborns in a Low Risk Community Setting?**  
Sadia F Chowdhury†,<sup>1,2,3</sup> Ruchit Shah,<sup>4,1</sup> Michael Joyce†,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongsachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Adwoa Nantwi†,<sup>5</sup> Sylvia Dygulska,<sup>1,2</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*,<sup>1,2,4</sup> *<sup>1</sup>Placental Analytics, New Rochelle, NY, United States;* *<sup>2</sup>NYPBMH, Brooklyn, NY, United States;* *<sup>3</sup>CUNY Hunter College, New York, NY, United States;* *<sup>4</sup>Institute for Basic Research, Staten Island, NY, United States;* *<sup>5</sup>NYU CGPH, New York, NY, United States.*

### EPIDEMIOLOGY

- W-239**     **OnPrognosis after Unexplained Recurrent Pregnancy Losses (RPL): a Systematic Review and External Validation of Clinical Prediction Models.**  
Angelos Youssef†. *LUMC, Leiden, Netherlands.*

## Wednesday, July 7, 2021 - Poster Session I - Back Bay Conference and Exhibition Center

- W-247 Does Placental Histopathology Impact Fetal Placental Weight Ratio in Term Well Newborns?**  
Adwoa Nantwi†, Sylvia Dygulska,<sup>2,3</sup> Hannah Bromberg,<sup>2,3</sup> Ruchit Shah,<sup>2,4</sup> Michael Joyce,<sup>2</sup> Mehrin Jan,<sup>2,3</sup> Serena Chen,<sup>2,3</sup> Christine Chen,<sup>2,3</sup> Jillamika Pongsachai,<sup>2,3</sup> Jennifer S Feng†,<sup>2,3,5</sup> Joan Krickellas,<sup>2,3</sup> Sadia F Chowdhury†,<sup>2,3,5</sup> Beata Dygulska,<sup>3</sup> Carolyn Salafia\*,<sup>2,3,6</sup>  
<sup>1</sup>NYU CGPH, New York, NY, United States; <sup>2</sup>Placental Analytics, New Rochelle, NY, United States; <sup>3</sup>NYPBMH, Brooklyn, NY, United States; <sup>4</sup>Institute for Basic Research, Staten Island, NY, United States; <sup>5</sup>CUNY Hunter College, New York, NY, United States; <sup>6</sup>Institute of Basic Research, Staten Island, NY, United States.

### POPULATION HEALTH

- W-248 The Association between Vitamin D Levels and Insulin Resistance.**  
 Roxana Guerra,<sup>1</sup> Cassandra Charles,<sup>2</sup> Fadi Yacoub,<sup>2</sup> Daniella Alviar,<sup>2</sup> Courtney Chiu,<sup>2</sup> Serin Seckin†,<sup>2</sup> Ozgul Muneyyirci-Delale.<sup>2</sup> <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>SUNY Downstate Health Sciences University, Brooklyn, NY, United States.
- W-249 Interpregnancy Interval and Adverse Pregnancy Outcome-Analysis on US Birth Data.**  
Vanessa Graf†, Khadija Haleem†, W Spencer McClelland,<sup>2</sup> Teresa Cheon,<sup>2</sup> M. Teresa Benedetto,<sup>2</sup> Yuzuru Anzai\*.<sup>2</sup> <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>Northwell Health, New York, NY, United States.
- W-250 Preliminary Results of the Shen Zhen Gong in Pregnancy Study: A Randomized Control Trial to Evaluate Maternal and Fetal Responses to Exercise.**  
Clarissa L Velayo\*,<sup>1</sup> Sherri Ann L Suplido\*,<sup>2</sup> Alvin R Sy\*,<sup>2</sup> Kiyoe Funamoto\*,<sup>3</sup> Yoshitaka Kimura\*,<sup>3</sup> <sup>1</sup>University of the Philippines, Manila, Philippines; <sup>2</sup>Philippine General Hospital, Manila, Philippines; <sup>3</sup>Tohoku University Graduate School of Medicine, Sendai, Japan.
- W-251 Emergency Department Utilization for Miscarriage Management: Trends from the Nationwide Emergency Department Sample (NEDS) from 2006 - 2018.**  
Amanda R Schwartz†, Jiang Li, Min Xu, Duyhoang Dinh, Daniel Miranian, Leah Mitchell Solomon, Erica E Marsh\*. *University of Michigan, Ann Arbor, MI, United States.*

### WOMEN'S HEALTH DISPARITIES AND INEQUITIES

- W-252 Comparing Vaginal Birth after Cesarean Section Success Rate Calculators with and without Race and Ethnicity at Mount Sinai Hospital.**  
Ayisha Brielle Buckley†, Tonia Ogundipe†, Stephanie Sestito†, Jacqueline Roigt†, Mitchell Rosenberg†, Chelsea DeBolt†, Rachel Meislin†, Angela Bianco\*, Vieira Luciana\*. *Icahn School of Medicine at Mount Sinai Hospital, Manhattan, NY, United States.*
- W-253 Comorbidities in Pregnancy, Refugee Status, and Their Effects on Birth Outcome.**  
Swathi Somisetty†, Ralph Mendez†, Pooja Doehman\*,<sup>1,2</sup> <sup>1</sup>Creighton School of Medicine, Phoenix, AZ, United States; <sup>2</sup>Department of Obstetrics & Gynecology, Phoenix, AZ, United States.
- W-254 Nudging Women with a Vulnerable Health Status to Encourage Adequate Pregnancy Preparation.**  
Sharissa M Smith†, Rianne M.J.J. Van der Kleij,<sup>2</sup> Babette Baist†, Maartje H.N. Schermer\*, Régine P.M. Steegers-Theunissen\*, Hafez Ismaili M'hamdi†.  
<sup>1</sup>Erasmus Medical Center, Rotterdam, Netherlands; <sup>2</sup>Leiden University Medical Center, Leiden, Netherlands.

### COVID-19

- W-255 Association between Maternal SARS-CoV-2 Infection during Pregnancy and Adverse Pregnancy Outcomes.**  
Darios Getahun\*,<sup>1</sup> Lurvey D Lawrence,<sup>2</sup> David Braun,<sup>3</sup> Sacks A David,<sup>4</sup> Alex Fong,<sup>5</sup> Neha Trivedi,<sup>6</sup> Jiaxiao Shi,<sup>1</sup> Vicki Y Chiu,<sup>1</sup> Morgan R Peltier,<sup>7</sup> Michael J Fassett.<sup>8</sup>  
<sup>1</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente Southern California; Keck School of Medicine, University of Southern California, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Irvine Medical Center, Irvine, CA, United States; <sup>6</sup>Kaiser Permanente San Diego Medical Center, San Diego, CA, United States; <sup>7</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>8</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States.
- W-256 Progesterone Suppresses SARS-CoV-2 Viral Load, Cytotoxicity and Inflammatory Response in a Lung Cell Line.**  
Matthew B Dacanay†, Miranda Li†, H Huang\*, Tsung-Yen Wu, T-Y Hsiang, M Gale, Jr.\*, K Adams Waldorf\*. *University of Washington, Seattle, WA, United States.*
- W-257 SARS CoV2 Nonstructural Proteins Modulate Autophagic Flux and Lipid Droplet Biogenesis in Placental Trophoblasts.**  
 Deepak Kumar†, Indira U. Mysorekar\*. *Washington University SOM, St. Louis, MO, United States.*
- W-258 The Early COVID-19 Era Negatively Impacted Symptoms, Stress, and Access to Care of Endometriosis Patients.**  
Paola M Ramos-Echevarria†, Denisse M Soto-Soto†,<sup>2</sup> Annelyn Torres-Reverón,<sup>3</sup> Caroline B Appleyard,<sup>1</sup> Tala Akkawi†, Barbara D Barros-Cartagena,<sup>1</sup> Veronica López-Rodríguez,<sup>1</sup> Eida M Castro-Figueroa,<sup>1</sup> Idhaliz Flores\*,<sup>1</sup> <sup>1</sup>Ponce Health Sciences University - Ponce Research Institute, Ponce, PR, United States; <sup>2</sup>Centro Médico Episcopal San Lucas, Ponce, PR, Ponce, PR, United States; <sup>3</sup>DHR Health Institute for Research and Development, Edinburg, TX, United States.
- W-259 The Impact of the COVID-19 Lockdowns on the Global Rates of Preterm Deliveries - A Systematic Review.**  
Rani Haj Yahya†, Stacey Peart, Jeanie Cheong, Brett Manley, Clare Whitehead. *The Royal Women's Hospital, Melbourne, Australia.*
- W-260 E-Health Modalities for Gynecologic Care during the COVID-19 Pandemic Are Well Accepted by Women of Reproductive Age.**  
Ariana Alvarado†, Paola Ramos,<sup>2</sup> Carlos Sierra,<sup>2</sup> Madeline Zapata,<sup>2</sup> Denisse Soto,<sup>3</sup> Idhaliz Flores\*,<sup>2</sup>  
<sup>1</sup>Pontifical Catholic University of Puerto Rico, Ponce, PR, United States; <sup>2</sup>Ponce Health Sciences University, Ponce, PR, United States; <sup>3</sup>Centro Médico Episcopal San Lucas, Ponce, PR, United States.
- W-261 Impact of COVID-19 Pandemic on Mental Health of Patients Seeking Infertility Evaluation.**  
Alvin To†, Liubin Yang†, Janet Bruno-Gaston†, Schutt Amy\*. *Baylor College of Medicine, Houston, TX, United States.*

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**Thursday, July 8, 2021 - Poster Session II - Back Bay Conference and Exhibition Center**


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**W-262**      **Disparities in SARS-CoV-2 Infection among Pregnant Women in a Large Integrated Healthcare System.**  
Michael J Fassett,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiaxiao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Darios Getahun.<sup>7</sup> <sup>1</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente West Los Angeles Medical Center; Keck School of Medicine, Los Angeles, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.

**W-263**      **Contraceptive Access for US Women in the Era of COVID19.**  
Lynae Maria Brayboy,<sup>1,2,3</sup> Rachel Michelt,<sup>4</sup> Amanda Shea,<sup>1</sup> Virginia Vitzthum\*,<sup>4,1,5</sup> <sup>1</sup>Clue by Biowink, Berlin, Germany, Germany; <sup>2</sup>Charité-Universitätsmedizin Berlin, corporate member of Freie Universi, Berlin, Germany, Germany; <sup>3</sup>Alpert Medical School of Brown University Providence, RI USA, Providence, RI, United States; <sup>4</sup>Indiana University, Bloomington, IN, United States; <sup>5</sup>Kinsey Institute, Bloomington, IN, United States.

2:45 PM - 4:15 PM

Poster

**BASIC PARTURITION**

- T-001**      **Development and Validation of a Quantitative Assay for Neurosteroids and Steroid Hormones in Pregnancy.**  
Gabriella Mayne†, <sup>1</sup>Erik De Bloois†, <sup>2</sup>Dana Dabelea, <sup>2</sup>K. Joseph Hurt, <sup>3</sup>Uwe Christians\*. <sup>1</sup>University of Colorado, Denver, CO, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>University of Colorado School of Medicine, Aurora, CO, United States.
- T-002**      **Preeclampsia Is Associated with Increased Levels of Intrinsically Disordered Protein - Prostate Associated Gene (PAGE)-4.**  
Asli Ozmen†, Millena Levin, Alexa Taylor, Duygu Mutluay, Xiaofang Guo†, Nihan Semerci†, Ozlem Guzeloglu Kayisli, Frederick Schatz, Charles Lockwood, Umit Kayisli. *University of South Florida, Morsani College of Medicine, Tampa, FL, United States.*
- T-003**      **Gardnerella vaginalis, but Not Lactobacillus crispatus, Disrupts Cervicovaginal Epithelial Cell Function and Immune Response through TLR2 Activation.**  
Lauren Anton, Amy G Brown, Kristin Gerson, Michal Elovitz\*. *University of Pennsylvania, Philadelphia, PA, United States.*
- T-004**      **Is There a Threshold of Inflammation Needed to Trigger Preterm Labor?**  
M Cappelletti†, <sup>1</sup>P Presicce†, <sup>1</sup>F Ma†, <sup>1</sup>P Sentharamaikannan†, <sup>2</sup>L Miller\*, <sup>3</sup>M Pellegrini\*, <sup>4</sup>A Jobe\*, <sup>2</sup>S Divanovic\*, <sup>2</sup>SS Way\*, <sup>2</sup>C Chougnet\*, <sup>2</sup>S Kallapur\*. <sup>1</sup>UCLA, Los Angeles, CA, United States; <sup>2</sup>Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States; <sup>3</sup>UCD, Davis, CA, United States; <sup>4</sup>University of California Los Angeles, Los Angeles, CA, United States.
- T-005**      **Immune Regulation in the Cervicovaginal Environment in Women with High Risk of Delivering Preterm.**  
Amirah Mohd Zaki†, <sup>1</sup>Alicia Hadingham, <sup>1</sup>Flavia Flaviani, <sup>1,2</sup>Deena Gibbons\*, <sup>1</sup>Yasmin Haque, <sup>1</sup>Jia Dai Mi, <sup>1</sup>Debbie Finucane, <sup>1</sup>Giorgia DallaValle, <sup>1</sup>Mansoor Saqi\*, <sup>2</sup>Rachel M. Tribe\*. <sup>1</sup>King's College London, London, United Kingdom; <sup>2</sup>Guy's and St. Thomas' NHS Foundation Trust and King's College London, London, United Kingdom.
- T-006**      **Full Length Sequencing of Cervicovaginal Microbiota Associated with Spontaneous Preterm Birth.**  
Neha Satish Kulkarni†, Megan Cavanagh†, Emmanuel Amabebe†, Dilly OC Anumba\*. *University of Sheffield, Sheffield, United Kingdom.*
- T-007**      **Administration of Statins Preceding the Maternal Exposure to Lipopolysaccharide Results in Anti-Inflammatory Effects in Fetal Brain.**  
Egle Bytautiene Prewit, <sup>1</sup>Mulampurath Achuthan Pillai Sureshkumar, <sup>1</sup>Tia Pearcy, <sup>1</sup>Mauricio F. La Rosa, <sup>2,3</sup>Maged Costantine. <sup>4</sup>UT Health San Antonio, San Antonio, TX, United States; <sup>2</sup>UTMB, Galveston, TX, United States; <sup>3</sup>Universidad Peruana Cayetano Heredia, Lima, Peru; <sup>4</sup>The Ohio State University, Columbus, OH, United States.

## Thursday, July 8, 2021 - Poster Session II - Back Bay Conference and Exhibition Center

- T-008** **Increased Pro-Inflammatory Signaling of Stem Cells Correlating with the Phenotype of Uterine Fibroids.**  
Hoda ElHossiny Elkafas†,<sup>1,2</sup> Mohamed Ahmed ALI†,<sup>1,3</sup> Lauren Prusinski Fernung†,<sup>4</sup> Sribalashubashini Muralimanoharan†,<sup>5</sup> Hailian Shen†,<sup>5</sup> Thomas G. Boyer\*,<sup>5</sup> Nahed Ismail\*,<sup>1</sup> Ayman Al-Hendy\*,<sup>1</sup> Qiwei Yang\*,<sup>5</sup> <sup>1</sup>University of Illinois at Chicago, Chicago, IL, United States; <sup>2</sup>Egyptian Drug Authority (EDA) formally (NODCAR), Cairo, Egypt; <sup>3</sup>Faculty of Pharmacy, Ain Shams University, Cairo, Egypt; <sup>4</sup>Augusta University, Augusta, GA, United States; <sup>5</sup>University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; <sup>6</sup>University of Texas Health Science Center at San Antonio, Chicago, IL, United States.
- T-009** **In Utero Exposure to *Ralstonia Insidiosa* Occurs during Normal Pregnancy and May Promote Tolerance.**  
Sonam Verma†,<sup>1</sup> Rachel B Silverstein,<sup>1</sup> Elaine Parker,<sup>1</sup> Lindsay A Parnell,<sup>1</sup> Chetan S Joshi,<sup>1</sup> Matthew J Wargo,<sup>2</sup> Indira U. Mysorekar\*,<sup>1</sup> <sup>1</sup>Washington University SOM, St. Louis, MO, United States; <sup>2</sup>University of Vermont Larner College of Medicine, Burlington, VT, United States.
- T-010** ***Gardnerella Vaginalis* Promotes Features of Epithelial-Mesenchymal Transition in the Cervicovaginal Space: Novel Pathways Underlying Premature Cervical Remodeling in Spontaneous Preterm Birth.**  
Kristin D Gerson†,<sup>1</sup> Yusra Gimie, Lauren Anton, Michal A Elowitz\*, *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*
- T-011** **Myometrial-Derived CXCL12 Promotes and the CXCR4 Antagonist AMD3100 Prevents LPS Induced Preterm Labor by Regulating Macrophage Migration, Polarization and Function in Mice.**  
Ramanaiah Mamillapalli, Lijuan Zhang, Shutaro Habata, Taylor S Hugh\*. *Yale University School of Medicine, New Haven, CT, United States.*
- T-012** **Metabolic Profiling of *Gardnerella vaginalis*: A Vaginal Dysbiosis and Preterm Birth-Associated Bacteria.**  
Georgia R May†, Emmanuel Amabebe†, Dilly O Anumba\*, Steven Reynolds\*. *University of Sheffield, Sheffield, United Kingdom.*
- T-013** **Macrophage Density Is Increased in the Endocervix in Women at Term Compared to Preterm.**  
Sandra E Reznik,<sup>1</sup> Rachel Scalest†,<sup>2</sup> Alison Yong†,<sup>1</sup> Gregory Dickinson†,<sup>3</sup> Pe'er Dar,<sup>3</sup> Steven M Yellon\*,<sup>2</sup> <sup>1</sup>St. John's University, Queens, NY, United States; <sup>2</sup>Loma Linda University, Loma Linda, CA, United States; <sup>3</sup>Albert Einstein College of Medicine, Bronx, NY, United States.
- T-014** **Differential Vaginal *Lactobacillus* Species Metabolism of Glucose, L- and D-lactate by <sup>13</sup>C-Nuclear Magnetic Resonance Spectroscopy.**  
Emmanuel Amabebe†, Dilly Anumba\*, Steven Reynolds\*. *University of Sheffield, Sheffield, United Kingdom.*
- T-015** **Decreased Levels of Triggering Receptor Expressed on Myeloid Cells-Like (TREM-like) Transcript-1 (TLT-1) Are Present in Cord Blood from Premature Infants, and Deficiency in Mice Promotes the In-Utero Inflammatory Response to Maternal Systemic Lipopolysaccharide (LPS) Exposure.**  
Paola E Pena Garcia†,<sup>1</sup> Jessica Morales-Ortiz,<sup>1</sup> Barry A Finette,<sup>2</sup> Anthony V Washington,<sup>1</sup> Elizabeth A Bonney\*,<sup>2</sup> <sup>1</sup>University of Puerto Rico-Rio Piedras, San Juan, PR, United States; <sup>2</sup>University of Vermont, Burlington, VT, United States.
- T-016** **3D Imaging of Epithelial-Mesenchymal Transition (EMT) Facilitated Amnion Epithelial Cells Migration Through Extracellular Matrix.**  
Enkhtuya Radnaa, Lauren Richardson, Ramkumar Menon\*. *University of Texas Medical Branch at Galveston, Galveston, TX, United States.*
- T-017** **Alarmin- and Endotoxin-Induced Intra-Amniotic Inflammation Induce Cervical Shortening without Altering the Cervico-Vaginal Microbiome.**  
Jose Galaz†,<sup>1</sup> Roberto Romero\*,<sup>2</sup> Andrew D Winters,<sup>1</sup> Kevin R Theis\*,<sup>1</sup> Nardhy Gomez-Lopez\*,<sup>1</sup> <sup>1</sup>Wayne State University SOM, Detroit, MI, United States; <sup>2</sup>Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, United States.
- T-018** ***Ureaplasma parvum* Induces Cervical Epithelial and Stromal Cell Inflammation and May Propagate via Exosomes.**  
Ourlad Alzeus Gaddi Tantengco†,<sup>1,2</sup> Richard B Pyles,<sup>3</sup> Kathleen Vincent,<sup>2</sup> Paul Mark B Medina,<sup>1</sup> Ramkumar Menon\*,<sup>2</sup> <sup>1</sup>University of the Philippines Manila, Manila, Philippines; <sup>2</sup>The University of Texas Medical Branch at Galveston, Galveston, TX, United States; <sup>3</sup>The University of Texas Medical Branch, Galveston, Galveston, TX, United States.
- T-019** **Quantification of Cervical Microstructure Change in Normal Pregnancies Using Diffusion Basis Spectrum Imaging.**  
Hansong Gao, Wenjie Wu, Zhexian Sun, Sicheng Wang, Zichao Wen, Qing Wang, Pamela K Woodard, Yong Wang. *Washington University School of Medicine, St. Louis, MO, United States.*
- T-020** **Sulforaphane Abrogates ROS Mediated Nrf2 Decrease in Mechanically Stretched Primary Amnion Cells.**  
Justin G Padron†, Chelsea Saito-Reis, Claire E Kendall-Wright\*. *Chaminade University of Honolulu, Honolulu, HI, United States.*
- T-021** **Determining the Role of Elastic Fibers on Cervical Contractility *In Vivo* and *In Vitro*.**  
Cassandra K. Conway†, Kristin S. Miller\*. *Tulane University, New Orleans, LA, United States.*
- T-022** **Polarized Light Imaging of the Pregnant Cervix.**  
Jessica Ramella-Roman\*, Ilyas Saytashev†, Sudipta Saha†. *Florida International University, Miami, FL, United States.*
- T-023** **Methods to Quantify the Genetic Architecture of Cervical Length.**  
Hope M Wolff†,<sup>1</sup> Roberto Romero\*,<sup>2</sup> Jerome F Strauss, III\*,<sup>1</sup> Sonia S Hassan\*,<sup>3</sup> Shawn J Latendresse\*,<sup>4</sup> Bradley T Webb\*,<sup>1</sup> Aaron R Wolen\*,<sup>5</sup> Adi L Tarca\*,<sup>3</sup> Timothy P York\*,<sup>1</sup> <sup>1</sup>Virginia Commonwealth University School of Medicine, Richmond, VA, United States; <sup>2</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Rockville, MD, United States; <sup>3</sup>Wayne State University School of Medicine, Detroit, MI, United States; <sup>4</sup>Baylor University, Waco, TX, United States; <sup>5</sup>The University of Tennessee Health Science Center, Memphis, TN, United States.
- T-024** **Oxytocin Receptor Is Degraded via the Ubiquitin-Proteasome System Following Prolonged Agonist Exposure.**  
Kevin Prifti, Manasi Malik†, Sarah K England, Antonina I Frolova\*. *Washington University School of Medicine in St. Louis, St. Louis, MO, United States.*
- T-025** **A Patient-Specific Multi-Scale, Multi-Physics Simulation of Whole Uterine Contraction.**  
Yiqi Lin†,<sup>1</sup> Jazmin Aguado-Sierra,<sup>2</sup> Zhexian Sun,<sup>1</sup> Constantine Butakoff,<sup>3</sup> Sicheng Wang,<sup>1</sup> Mariano Vazquez,<sup>2</sup> Yong Wang.<sup>1</sup> <sup>1</sup>Washington University, St. Louis, MO, United States; <sup>2</sup>Barcelona Supercomputing Center, Barcelona, Spain; <sup>3</sup>Elem Biotech, S.L., Barcelona, Spain.

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- T-026**      **Contraction Synchronization Predicts the Onset of "True" Labor.**  
 Ponnilla S Marinescu†,<sup>1</sup> Roger C Young,<sup>2</sup> David Adair,<sup>3</sup> Braxton Hern,<sup>3</sup> Eva K Pressman,<sup>1</sup> Neil S Seligman\*.<sup>1</sup> <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>PreTeL, Inc., Chattanooga, TN, United States; <sup>3</sup>University of Tennessee College of Medicine, Chattanooga, TN, United States.
- T-027**      **Somatostatin Receptor Type 2 Expression in Pregnant and Labouring Human, Non-Human Primate, and Mouse Myometrium.**  
 Oksana Shynlova,<sup>1</sup> Adam Boros-Rausch†,<sup>2</sup> Anna Dorogin,<sup>2</sup> Tsung-Yen Wu,<sup>3</sup> Kristina Adams Waldorf,<sup>3</sup> Stephen Lye\*.<sup>1</sup> <sup>1</sup>Sinai Health System, Toronto, ON, Canada; <sup>2</sup>SHS, Toronto, ON, Canada; <sup>3</sup>U of W, Seattle, WA, United States.
- T-028**      **BK<sub>Ca</sub> Channels Are Involved in Both Spontaneous and LPS-Stimulated Uterine Contractions in Pregnant Mice.**  
 Junjie Bao,<sup>1,2</sup> Xiaofeng Ma,<sup>1</sup> Monali Wakle-Prabakaran,<sup>1</sup> Ronald McCarthy,<sup>1</sup> Sarah K England\*.<sup>1</sup> <sup>1</sup>Washington University School of Medicine, St. Louis, MO, United States; <sup>2</sup>Guangzhou Women & Children's Medical Center, Guangzhou Medical University, Guangzhou, China.
- T-029**      **The Anisotropic Mechanical Properties of Human Uterus.**  
 Shuyang Fang,<sup>1</sup> James McLean,<sup>1</sup> Joy Vink,<sup>2</sup> Christine Hendon,<sup>1</sup> Kristin Myers.<sup>1</sup> <sup>1</sup>Columbia University, New York, NY, United States; <sup>2</sup>Columbia University Medical Center, New York, NY, United States.
- T-030**      **Genome-Wide Changes Accompanying Myometrial Contraction and Labor: Integrated Analysis of ChIP-seq and RNA-seq Data Reveals Critical Steroid-Target Genes.**  
 Ariel J Dotts†, Ping Yin\*, William A Grobman\*, Serdar E Bulun\*. *Northwestern University, Chicago, IL, United States.*
- T-031**      **Uterine Electromyography: Novel Uterine Bioelectrical Signaling Patterns Lead to Advances in Understanding of Uterine Contractile Activity.**  
 Ponnilla S Marinescu†,<sup>1</sup> Lauren A Miller,<sup>2</sup> Roger C Young,<sup>3</sup> Braxton Hern,<sup>4</sup> Eva K Pressman,<sup>1</sup> Neil S Seligman\*.<sup>1</sup> <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>St. Luke's Clinic, Boise, ID, United States; <sup>3</sup>PreTeL, Inc., Chattanooga, TN, United States; <sup>4</sup>University of Tennessee College of Medicine, Chattanooga, TN, United States.
- T-032**      **HIF-1α, but Not HIF-2α, May Modulate Myometrial Contraction via Upregulating Oxytocin Receptor during Labor.**  
 Bolun Wen†,<sup>1</sup> Xueya Qian,<sup>1</sup> Lele Wang,<sup>1</sup> Xiaodi Wang†,<sup>1</sup> Junjie Bao,<sup>1</sup> Binsheng Wu†,<sup>1</sup> Wenfeng Deng†,<sup>1</sup> Fan Yang†,<sup>2</sup> Lina Chen†,<sup>2</sup> Huishu Liu\*.<sup>1</sup> <sup>1</sup>Guangzhou Women & Children Medical Center, Guangzhou Medical University, Guangzhou, China; <sup>2</sup>Guangzhou Women & Children Medical Center, South China University of Technology, Guangzhou, China.
- T-033**      **HIF1α Activation Modulates Autophagy during Labor by Inhibiting RAB7B, Enlarging Mitochondrial Fuel Oxidation.**  
 Xiaodi Wang†, Bolun Wen†, Junjie Bao†, Huishu Liu\*, Lele Wang†. *Guangzhou Women & Children Medical Center, Guangzhou, China.*
- GYNECOLOGY**
- T-034**      **Neuropathic Pain Marker Expression in Endometriosis Patients with Chronic Pelvic Pain.**  
 Ian Waldman†, Emily R Disler, Ankrish Milne, Kha U Dam, Nicholas W Ng, Xinjie Chen, Marian Damian Cruz, Maya Seshan, Bradley J Quade, Raymond M Anchan\*. *Brigham & Women's Hospital, Harvard Medical School, Boston, MA, United States.*
- T-035**      **Patient-Derived Xenograft Murine Model for Precision Medicine in Endometriosis.**  
 Valerie Flores†, Cagdas Sahin, Hugh S Taylor\*. *Yale School of Medicine, New Haven, CT, United States.*
- T-036**      **Neuropeptide S Receptor 1 Is a Novel Non-Hormonal Treatment Target in Endometriosis.**  
 Thomas T Tapmeier,<sup>1</sup> Nilufer Rahmioglu,<sup>1</sup> Jianghai Lin,<sup>2</sup> Maik Obendorf,<sup>3</sup> Bianca de Leo,<sup>3</sup> Grant Montgomery,<sup>4</sup> Udo Oppermann,<sup>1</sup> Stephen Kennedy,<sup>1</sup> Thomas Zollner,<sup>3</sup> Christian M Becker,<sup>1</sup> Joseph Kemnitz,<sup>5</sup> Jeffrey Rogers,<sup>6</sup> Krina T Zondervan\*.<sup>1</sup> <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Jinan University, Guangzhou, China; <sup>3</sup>Bayer AG, Berlin, Germany; <sup>4</sup>University of Queensland, Brisbane, Australia; <sup>5</sup>University of Wisconsin, Madison, WI, United States; <sup>6</sup>Baylor College of Medicine, Houston, TX, United States.
- T-037**      **Immune Dysfunction in the Menstrual Effluent of Women with Endometriosis: Implications for Disease Pathogenesis.**  
 Jessica E. Miller†,<sup>1</sup> Harshavardhan Lingegowda†,<sup>1</sup> Danielle Sissett†,<sup>1</sup> Christine N Metz,<sup>2</sup> Peter K Gregerson,<sup>2</sup> Madhuri Koti,<sup>1</sup> Chandrakant Tayade\*.<sup>1</sup> <sup>1</sup>Queen's University, Kingston, ON, Canada; <sup>2</sup>Northwell Health, Manhasset, NY, United States.
- T-038**      **The Effect of Peritoneal Fluid-Derived Exosomes from Endometriosis Patients on Mesothelial Cells.**  
 Kayla Y Li†,<sup>1</sup> Kavita S Subramaniam,<sup>1</sup> Hannah M Nazri,<sup>1</sup> Thomas T Tapmeier,<sup>1</sup> Krina T Zondervan,<sup>1,2</sup> Christian M Becker\*.<sup>1</sup> <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Wellcome Centre for Human Genetics, Oxford, United Kingdom.
- T-039**      **Collagen I Triggers Directional Migration, Invasion and Matrix Remodeling of Stroma Cells in a 3D Spheroid Model of Endometriosis.**  
 Stejskalova Anna, Fincke Victoria†, Sebastian D. Schäfer, Ludwig Kiesel, Martin Götte\*. *Muenster University Hospital, Muenster, Germany.*
- T-040**      **Artificial Intelligence for Diagnosis and Quantification of Adenomyosis: Can Robots Assist?**  
 Joseph Huang\*,<sup>1</sup> Yan-Ru Su,<sup>2</sup> Chun-Yen Huang,<sup>1</sup> Yu-Chun Yu,<sup>1</sup> Yi-Wu Chiang,<sup>3</sup> Nari Kay.<sup>1</sup> <sup>1</sup>E-Da Hospital, Kaohsiung, Taiwan; <sup>2</sup>National Sun Yat-Sen University, Kaohsiung, Taiwan; <sup>3</sup>E-National Sun Yat-Sen University, Kaohsiung, Taiwan.
- T-041**      **Immortalization of Murine Uterine Stromal and Epithelial Cell Lines for Endometriosis Research.**  
 Danielle Peterse†, Samuel Garrard†, Victor Fattori†, Aram Ghalali†, Michael Rogers\*. *Boston Childrens Hospital / Harvard Medical School, Boston, MA, United States.*
- T-042**      **Endometriosispathoetiology: The Role of microRNAs in the Dysregulation of Endometrial Function.**  
 Bhuchitra Singh†,<sup>1</sup> Jiahui Zhang†,<sup>2</sup> Isabelle Baptista†,<sup>3</sup> Ping Xia,<sup>1</sup> James Segars\*.<sup>1</sup> <sup>1</sup>Johns Hopkins University School Of Medicine, Baltimore, MD, United States; <sup>2</sup>Renaissance School of Medicine, Stony Brook, NY, United States; <sup>3</sup>Johns Hopkins University, Baltimore, MD, United States.

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- T-043**     **The G Protein-Coupled Receptor 55 (GPR55) as Putative Target for the Treatment of Endometriosis.**  
Frank Sacher\*,<sup>1</sup> Gernot Langer\*,<sup>1</sup> Bernd Bojahr\*,<sup>2</sup> Martin Fritsch\*,<sup>1</sup> René Wenzl\*,<sup>3</sup> Thomas M Zollner\*,<sup>1</sup> Maik Obendorf\*,<sup>1</sup> Jens Nagel\*,<sup>1</sup> <sup>1</sup>Bayer AG, Berlin, Germany; <sup>2</sup>MIC, Berlin, Germany; <sup>3</sup>Medical University of Vienna, Vienna, Austria.
- T-044**     **Simvastatin Suppresses Wnt/β-catenin Pathway in Human Leiomyoma Cells.**  
Malak El Sabeh†, Subbroto Kumar Saha†, Sadia Afrin†, Mostafa Borahay\*. *Johns Hopkins University, Baltimore, MD, United States.*
- T-045**     **Integrative Cistrome and Transcriptome Analysis Identifies Tryptophan-Kynurenine-AHR Pathway as a Novel Regulator of Leiomyoma Growth.**  
Azna Zuberi†. *Northwestern University, Feinberg School of Medicine, Chicago, IL, United States.*
- T-046**     **Tissue Factor Pathway Inhibitor 2 Expression in Uterine Fibroids.**  
Papri Sarkar†, Xiaofang Guo, Ozlem Guzeloglu-Kayisli, Asli Ozmen, Alexa Taylor, Erika New, Anthony Imudia, Charles Lockwood, Umit Kayisli\*. *University of South Florida, Tampa, FL, United States.*
- T-047**     **Glucocorticoids Repress Vitamin D Receptor Expression in Human Uterine Fibroid Cells: Implications for the Benefit of Vitamin D.**  
Erin Silva†,<sup>1</sup> Tanya Glenn†,<sup>1</sup> Pablo Suarez†,<sup>1</sup> Natalie A DeWitt†,<sup>1</sup> Andrew Nguyen†,<sup>1</sup> Andreanna Burman†,<sup>1</sup> James Segars,<sup>2</sup> Shannon Whirlledge\*.<sup>1</sup> <sup>1</sup>Yale University, New Haven, CT, United States; <sup>2</sup>Johns Hopkins School of Medicine, Baltimore, MD, United States.
- T-048**     **The Effect of Isolation Method on the Phenotype of Uterine Fibroid Cells in Culture.**  
Tanya L Glenn†, Erin Silva†, Pablo Suarez, Clare Flannery, Shannon Whirlledge\*. *Yale University, New Haven, CT, United States.*
- T-049**     **Correlation of Methylation Status and Gene Expression Shows Epigenetics Involvement in Key Biological Processes of Uterine Leiomyoma Development.**  
María Cristina Carbajo-García†,<sup>1,2</sup> Ana Corachán†,<sup>1,2</sup> Elena Juárez-Barber†,<sup>2</sup> Javier Monleón\*,<sup>3</sup> Vicente Payá\*,<sup>3</sup> Alexandra Trelis†,<sup>3</sup> Alicia Quiñero\*,<sup>2</sup> Antonio Pellicer\*,<sup>1,4</sup> Hortensia Ferrero\*,<sup>2</sup> <sup>1</sup>University of Valencia, Valencia, Spain; <sup>2</sup>IVI Foundation, Health Research Institute la Fe, Valencia, Spain; <sup>3</sup>La Fe University and Polytechnic Hospital, Valencia, Spain; <sup>4</sup>IVIRMA Rome, Rome, Italy.
- T-050**     **Obesity-Related Leptin and Ghrelin Alterations on Leiomyoma Growth.**  
Lauren Reschke†, Sadia Afrin†, Malak El Sabeh†. *Johns Hopkins University, School of Medicine, Baltimore, MD, United States.*
- T-051**     **Evaluating the Inhibitory Effect of Elagolix & Relugolix on Leiomyoma Growth in 2D Cell Culture.**  
Danielle Wright, Joy Britten-Webb, Minnie Malik, William Catherino. *Uniformed Services University of Health Sciences, Bethesda, MD, United States.*
- T-052**     **Single Cell Transcriptomes from Uterine Fibroids and Fibroid-Free Myometrium Elucidate Myometrial Tumorigenesis.**  
Wanxin Wang†,<sup>1</sup> Aymara Mas,<sup>2</sup> Patricia Escorcía,<sup>2</sup> Javier Monleón,<sup>3</sup> Stephen Quake\*,<sup>1,4</sup> Carlos Simon\*,<sup>5,2,6</sup> <sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>Igenomix Foundation, Valencia, Spain; <sup>3</sup>Hospital Universitario La Fe, Valencia, Spain; <sup>4</sup>Chan Zuckerberg Biohub, San Francisco, CA, United States; <sup>5</sup>Harvard University, Boston, MA, United States; <sup>6</sup>Valencia University, Valencia, Spain.
- T-053**     **Nintedanib Alters Hippo Signaling in Uterine Fibroid Cells Leading to Decreased Levels of Connective Tissue Growth Factor and Cyclin D1, and Reduced Fibroid Cell Proliferation.**  
Md Soriful Islam, Ha Vi S Nguyen, Jacqueline Y Maher, Joshua T Brennan, James H Segars\*. *Johns Hopkins University, School of Medicine, Baltimore, MD, United States.*
- T-054**     **Impact of Vilaprisan on Well-Being in Mice in the Induced Menstrual Bleeding Model.**  
Oliver M Fischer, Frank Sacher, Jens Nagel, Thomas M Zollner. *Bayer AG, Berlin, Germany.*
- T-055**     **Complementary and Alternative Medicine Use among Women with Symptomatic Uterine Fibroids.**  
Elia Marina Rubio†, Joan Hilton, Vanessa Jacoby\*. *University of California San Francisco, San Francisco, CA, United States.*
- T-056**     **Increased FK506-Binding Protein 51 (FKBP51) Promotes Uterine Fibroid Cell Differentiation Leading to Extracellular Matrix Abundance.**  
Erika P New†,<sup>1</sup> Xiaofang Guo,<sup>1</sup> Nihan Semerci,<sup>1</sup> Ozlem Guzeloglu-Kayisli,<sup>1</sup> Anthony N Imudia,<sup>1,2</sup> Charles J Lockwood,<sup>1</sup> Umit A Kayisli\*,<sup>1</sup> <sup>1</sup>University of South Florida, Tampa, FL, United States; <sup>2</sup>Shady Grove Fertility Center Tampa Bay, Tampa, FL, United States.
- T-057**     **Effect of Selective Progesterone Receptor Modulator (SPRM), Ulipristal Acetate (UPA) on Uterine and Fibroid Volume Measured by Unbiased Stereology and MRI.**  
Hilary Critchley\*,<sup>1</sup> Kaiming Yin,<sup>1</sup> Lucy Whitaker,<sup>1</sup> Suzanne McLenachan,<sup>2</sup> Jane Walker,<sup>2</sup> Graham McKillop,<sup>2</sup> Neil Roberts.<sup>1</sup> <sup>1</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>2</sup>Royal Infirmary, Edinburgh, United Kingdom.
- T-058**     **Differential Hypoxic Response in Uterine Leiomyoma & Myometrial Cells.**  
Mariko Miyashita-Ishiwata†, Malak El Sabeh†, Sadia Afrin†, Mostafa Borahay. *The Johns Hopkins University, Baltimore, MD, United States.*
- T-059**     **Insights Into Primary Ovarian Insufficiency Through Clinical and Biochemical Data at the NIH.**  
Jamie Merkison, Ninet Sinaii, Veronica Gomez-Lobo, Jacqueline Maher\*. *National Institutes of Health, Bethesda, MD, United States.*
- T-060**     **Advancing Equity and Diversity in Gynecologic Biobanking.**  
Pablo Suarez, Shannon Whirlledge\*. *Yale University, New Haven, CT, United States.*

### CLINICAL PERINATOLOGY

- T-061**     **Ultrasonographic Anterior Uterocervical Angle (UCA) and Prediction of Preterm Birth: A Patient Level Meta-Analysis.**  
Erica K Nicasio†,<sup>1</sup> Zi-Qi Liew†,<sup>1</sup> Jesse Llop,<sup>1</sup> Tiffany Meit†,<sup>1</sup> Lynch Tara,<sup>2</sup> Neil S Seligman\*. <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>Albany Medical Center, Albany, NY, United States.

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- T-062 Multi-Omic, Longitudinal Profile of Third-Trimester Pregnancies Identifies a Molecular Switch That Predicts the Onset of Labor.**  
Ina Stelzer<sup>1</sup>, Mohammad Sajjad Ghaemi,<sup>1,2</sup> Xiaoyuan Han,<sup>1,3</sup> Kazuo Ando,<sup>1</sup> Julien Hedou,<sup>1</sup> Dorien Feyaerts,<sup>1</sup> Laura Peterson,<sup>1</sup> Edward Ganio,<sup>1</sup> Amy Tsai,<sup>1</sup> Eileen Tsai,<sup>1</sup> Kristen Rumer,<sup>1</sup> Natalie Stanley,<sup>1</sup> Ramin Fallazadeh,<sup>1</sup> Martin Becker,<sup>1</sup> Anthony Culos,<sup>1</sup> Dyani Gaudilliere,<sup>1</sup> Ronald Wong,<sup>1</sup> Virginia Winn,<sup>1</sup> Gary Shaw,<sup>1</sup> Michael Snyder,<sup>1</sup> David Stevenson,<sup>1</sup> Kevin Contrepois,<sup>1</sup> Martin Angst\*,<sup>1</sup> Nima Aghaepour\*,<sup>1</sup> Brice Gaudilliere\*.<sup>1</sup>  
<sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>National Research Council Canada, Toronto, ON, Canada; <sup>3</sup>University of the Pacific, San Francisco, CA, United States.
- T-063 Increased Rates of Cesarean and Operative Vaginal Delivery with Extended Second Stage Pushing.**  
Derek Lee†, Lisa Duong†, Michael G Ross\*. Harbor-UCLA Medical Center, Torrance, CA, United States.
- T-064 Investigating Differential Effects of Interpregnancy Interval on Pregnancy Complications by Country Developmental Status.**  
Caitlyn E Flint†, Jasmine M De Giovanni,<sup>1</sup> Jason Phung,<sup>1,2,3</sup> Craig E Pennell\*.<sup>1,2,3</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>John Hunter Hospital, New South Wales, Australia.
- T-065 Predictors of Breastfeeding among Women Admitted with Preterm Pre-Labor Rupture of Membranes.**  
Carmen Maria Avram†, Alice Darling†, Melissa Montoya†, Jennifer Gilner\*, Sarah Wheeler\*, Sarah Dotters-Katz\*. Duke University, Durham, NC, United States.
- T-066 Antibiotic Use during Pregnancy and Preterm Birth.**  
Brittani Steinberg†, Yanzhi Wang\*,<sup>1</sup> Laura Meints\*.<sup>2</sup> <sup>1</sup>University of Illinois College of Medicine, Peoria, IL, United States; <sup>2</sup>St. Francis Medical Center, OSF HealthCare System, Peoria, IL, United States.
- T-067 ECM-Associated Long Non-Coding RNAs Inc-ADAM9 and Inc-PCDH10 Regulate Cell Adhesion Pathway in Premature Rupture of Fetal Membrane.**  
Guixian Wang,<sup>1</sup> Dongxia Hou,<sup>1</sup> Xiaoyan Dong,<sup>2</sup> Nanbert Zhong\*.<sup>2</sup> <sup>1</sup>Inner Mongolia Maternal and Children's Hospital, Hohhot, China; <sup>2</sup>New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States.
- T-068 Early Childhood Growth after Term NICU Admission by Admission Diagnosis.**  
Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dyguski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongasachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*.<sup>1,2,5</sup>  
<sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute for Basic Research, Staten Island, NY, United States.
- T-069 Investigating the Neuro-Regenerative Potential of MicroRNAs in Wharton's Jelly-Derived Small Extracellular Vesicles (sEV) for Perinatal White Matter Injury Outcomes.**  
Vera Tscherrig†,<sup>1,2,3</sup> Sophie Cottagnoud†,<sup>1,2</sup> Valérie Haesler,<sup>1,2</sup> Patricia Renz†,<sup>1,2,3</sup> Daniel Surbek,<sup>1,2</sup> Andreina Schoeberlein,<sup>1,2</sup> Marianne Jörgger-Messeri.<sup>1,2</sup>  
<sup>1</sup>Department of Obstetrics and Feto-maternal Medicine, University Women's Hospital, Inselspital, Bern University Hospital, Bern, Switzerland; <sup>2</sup>Prenatal Medicine, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland; <sup>3</sup>Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland.
- T-070 Diabetic Environment Affects the Proteomic Profile of Different Populations of Extracellular Vesicles Originating from Placental Cells.**  
Carlos Palma†, Andrew Lai,<sup>1</sup> David McIntyre,<sup>1</sup> Carlos Salomon.<sup>1,2</sup> <sup>1</sup>The University of Queensland, Brisbane, Australia; <sup>2</sup>University of Concepción, Concepcion, Chile.
- T-071 Immediate Pre-Pregnancy Weight Loss Improves Highly Atherogenic Dyslipidemia Throughout Pregnancy.**  
Robert A. Wild,<sup>1</sup> Rodney K Edwards,<sup>1</sup> David S Wrenn,<sup>2</sup> Daniel Y Zhao,<sup>1</sup> Karl R Hansen.<sup>1</sup> <sup>1</sup>University of Oklahoma HSC, Oklahoma City, OK, United States; <sup>2</sup>Quest Diagnostics, Seacaucus, NJ, United States.
- T-072 Investigating a Role for the NLRP3 Inflammasome in the Pathophysiology of Gestational Diabetes Mellitus.**  
Colm J McElwain†, Samprika Manna,<sup>1</sup> Fergus P McCarthy\*,<sup>2</sup> Cathal M McCarthy\*.<sup>1</sup> <sup>1</sup>University College Cork, Cork, Ireland; <sup>2</sup>Cork University Maternity Hospital, Cork, Ireland.
- T-073 Does Preconception Bariatric Surgery Detrimentially Influence Maternal Nutritional Status during Pregnancy, Fetal Growth and Birth Weight?**  
Katinka Snoek,<sup>1</sup> Régine Steegers-Theunissen\*,<sup>1</sup> Nadia van de Woestijne†,<sup>1</sup> Sten Willemsen\*,<sup>1</sup> René Klaassen†,<sup>2</sup> Sam Schoenmakers\*.<sup>1</sup> <sup>1</sup>Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Maasstad Hospital, Rotterdam, Netherlands.
- T-074 Impact of Western/USA Diet on Maternal Metabolism in Vervet Pregnancy.**  
Sarah Therese Shepard†, Jeffrey Denney\*, Kylie Kavanagh\*, Mathew Jorgensen\*, Brian Brost\*. Wake Forest Medical School, Winston-Salem, NC, United States.
- T-075 Diabetes Distress Scores and Perinatal Outcomes among Women with Gestational and Pregestational Diabetes.**  
Jennifer Jacobson†,<sup>1</sup> Amy Godecker,<sup>1</sup> Jennifer Janik,<sup>1</sup> April Eddy,<sup>2</sup> Jacquelyn Adams\*.<sup>1</sup> <sup>1</sup>University of Wisconsin School of Medicine and Public Health, Madison, WI, United States; <sup>2</sup>Unity-Point Health Meriter Hospital, Madison, WI, United States.
- T-076 The Relationships between BMI and Patient-Provider Weight Goals during Pregnancy.**  
Hannah Dugoni†,<sup>1</sup> Shelby Alsupt†,<sup>1</sup> Katherine Elder\*,<sup>1</sup> Olivia Doyle†,<sup>2</sup> Kristen Mackiewicz Seghete\*,<sup>2</sup> Alice Graham\*.<sup>2</sup> <sup>1</sup>Pacific University, Hillsboro, OR, United States; <sup>2</sup>Oregon Health & Science University, Portland, OR, United States.
- T-077 Enhanced Fatty Acid Binding Protein (FABP)-4 Secretion in Placental Villi with Gestational Diabetes Mellitus: Implication for Impaired Glucose Homeostasis.**  
Anthony M Kendle†, Nihan Semerci, Asli Ozmen, Xiaofang Guo, Ali Wells†, Ozlem Guzeloglu-Kayisli\*, Umit Kayisli\*, Charles J Lockwood\*. The University of South Florida, Tampa, FL, United States.

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- T-078 Neonatal Outcomes in Preterm Trial of Labor in Women without a Prior Vaginal Delivery.**  
Sunitha C Suresh†, Annie Dude\*. *University of Chicago, Chicago, IL, United States.*
- T-079 First Trimester Subchorionic Hemorrhage and Impact on Pregnancy Outcomes in the IVF Population.**  
Avanthi Sai Ajjara†, Liubin Yang†, William Gibbons\*. *Baylor College of Medicine, Houston, TX, United States.*
- T-080 History of Infant Respiratory Morbidity Predictive of Neonatal Respiratory Morbidity in Subsequent Pregnancy.**  
Naima Ross†, Sunitha Suresh†, Ann Dude\*. *University of Chicago, Chicago, IL, United States.*
- T-081 Identification of Bacterial Metabolic Abundances in the Gut Microbiome of Preterm Infants.**  
Anujit Sarkar\*,<sup>1</sup> Ji Youn Yoo†,<sup>1</sup> Jean Lim†,<sup>1,2</sup> Samia Dutra†,<sup>1</sup> Larry Dishaw\*,<sup>1</sup> Bradley Kane\*,<sup>1</sup> Maureen Groer Edith Groer\*,<sup>1</sup> Elizabeth Miller\*.<sup>1</sup> *University of South Florida, Tampa, FL, United States;* <sup>2</sup>*University of Tennessee, Knoxville, TN, United States.*
- T-082 Trends in Postpartum Venous Thromboembolism and Chemical Thromboprophylaxis among Insured U.S. Patients.**  
Ann M Brunot†,<sup>1,2</sup> Amanda A Allshouse,<sup>1</sup> Brett D Einerson,<sup>1,2</sup> Heather M Campbell,<sup>1</sup> D Ware Branch,<sup>1,2</sup> Robert M Silver,<sup>1,2</sup> Torri D Metz\*,<sup>1,2</sup> <sup>1</sup>*University of Utah Health, Salt Lake City, UT, United States;* <sup>2</sup>*Intermountain Healthcare, Murray, UT, United States.*
- T-083 Is Antenatal Vaginal Bleeding in Placenta accreta Spectrum a Harbinger of Adverse Outcomes?**  
Lihong Mo†, Nandini R Nittur†, Zahabiya H Chithiwala†, Herman L Hedriana\*. *UC Davis, Sacramento, CA, United States.*
- T-084 Umbilical Artery Abnormalities in Women with OUD: Is a Revision of Cutoffs Appropriate?**  
Brittany McKinley†, Calvin Lee Ward, Katia Vela†, Aarthi Srinivasan, Erin MacLeod†, Zachary Stanley†, Brooke Andrews†, Katherine Vignes†, Cynthia Cockerham†, Leon Su, Arnold Stromberg\*, John O'Brien\*. *University of Kentucky College of Medicine, Lexington, KY, United States.*
- T-085 Paternal Smoking Is Associated with an Increased Risk of Pregnancy Loss in a Dose-Dependent Manner: A Systematic Review and Meta-Analysis.**  
Nadia A. du Fossé†, Marie-Louise P. van der Hoon\*, Nina H. Buisman†, Jan M.M. van Lith\*, Saskia le Cessie\*, Eileen E.L.O. Lashley\*. *Leiden University Medical Center, Leiden, Netherlands.*
- T-086 Association of Abnormal Doppler Evaluation in Suspected Fetal Growth Restriction Near Term with Placental Pathology.**  
William M Curtin\*,<sup>1</sup> Kristi L Haedrich,<sup>2</sup> Emily O'Brien,<sup>1</sup> Laura H Brubaker,<sup>1</sup> Niamh A Condon,<sup>3</sup> Serdar H Ural,<sup>1</sup> Jaimie L Maines,<sup>1</sup> Karmaine A Millington.<sup>4</sup> <sup>1</sup>*Penn State Health, Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, PA, United States;* <sup>2</sup>*Penn State Health Milton S. Hershey Medical Center, Hershey, PA, United States;* <sup>3</sup>*University of Florida Health, Jacksonville, FL, United States;* <sup>4</sup>*Northwell Health/Long Island Jewish Medical Center and Donald and Barbara Zucker School of Medicine at Hofstra University, New Hyde Park, NY, United States.*
- T-087 Isolated Fetal Neural Tube Defects Associate with Increased Risk of Placental Pathology.**  
Marina White†,<sup>1</sup> David Grynspan,<sup>2</sup> Tim Van Mieghem,<sup>3</sup> Kristin L Connor\*.<sup>1</sup> <sup>1</sup>*Carleton University, Ottawa, ON, Canada;* <sup>2</sup>*University of British Columbia, Vancouver, BC, Canada;* <sup>3</sup>*Mount Sinai Hospital, Toronto, ON, Canada.*
- T-088 Factors Associated with Delivery within Seven Days of Presentation with Self-Limited Suspected Placental Abruption.**  
Rachel Anne Newman†,<sup>1</sup> Joshua Makhoul†,<sup>2</sup> Jenny Chang\*,<sup>2</sup> Dana Senderoff†,<sup>2</sup> B. Adam Crosland†,<sup>2</sup> Emily Seet\*,<sup>3</sup> Kenneth Chan\*.<sup>3</sup> <sup>1</sup>*Cedars Sinai Medical Center, Los Angeles, CA, United States;* <sup>2</sup>*University of California, Irvine, Orange, CA, United States;* <sup>3</sup>*Long Beach Memorial Medical Center, Long Beach, CA, United States.*
- T-089 Pregnancy and Delivery Outcomes in Solid Organ Transplant Recipients: A Modern Cohort.**  
Jenny Yang Mei†, Ophelia Yin†, Yalda Afshar\*. *UCLA, Los Angeles, CA, United States.*
- T-090 Advanced Maternal Age and Obstetric Outcome.**  
Anna Maria Marconi\*. *University of Milano, Milano, Italy.*
- T-091 The Effects of Maternal In Utero Poly-Drug Exposure on Neonatal Abstinence Syndrome Outcomes.**  
Brooke Charlton Andrews†, Erin L. Macleod†, Zachary D. Stanley†, Brittany M McKinley†, Katia V Vela†, Katherine Vignes†, Cynthia Cockerham, Leon Su†, Arnold J Stromberg\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*
- T-092 Performance of Urinalysis as a Screen for Urinary Tract Infection in Symptomatic Patients Presenting to Triage.**  
Amanda M Wang†, Sara Jacobs, George Saade, Antonio F Saad. *University of Texas Medical Branch, Galveston, TX, United States.*
- T-093 Changes in the Antepartum Population Over Time.**  
Anna J Rujan†, Ashley Hesson\*, Deborah R Berman\*. *University of Michigan, Ann Arbor, MI, United States.*
- T-094 Racial and Ethnic Disparities in Mode of Delivery during Labor Induction.**  
Christina Ackerman†,<sup>1</sup> Masaru Negi,<sup>2</sup> Uma Reddy,<sup>1</sup> Lisbet Lundsberg,<sup>1</sup> Audrey Merriam,<sup>1</sup> Jessica Greenberg,<sup>1</sup> Sarah Meller,<sup>1</sup> Anna Sfakianaki\*.<sup>3</sup> <sup>1</sup>*Yale New Haven Hospital, New Haven, CT, United States;* <sup>2</sup>*UCLA, Los Angeles, CA, United States;* <sup>3</sup>*University of Miami, Miami, FL, United States.*
- T-095 A Characterization of a Modern Antepartum Inpatient Unit.**  
Anna J Rujan†, Ashley Hesson\*, Deborah R Berman\*. *University of Michigan, Ann Arbor, MI, United States.*

### DEVELOPMENTAL PROGRAMMING

- T-096 A History of Spontaneous Preterm Birth Does Not Increase Cardiovascular Risk among Women in the Fifth Decade of Life.**  
Laura E Janssen†,<sup>1</sup> Marjon A de Boer\*,<sup>1</sup> Eline C.E von Königslöw†,<sup>1</sup> Martijn A Oudijk\*,<sup>2</sup> Christianne J.M de Groot\*,<sup>1,2</sup> <sup>1</sup>*Amsterdam UMC, VU Medical Center, Amsterdam, Netherlands;* <sup>2</sup>*Amsterdam UMC, Amsterdam Medical Center, Amsterdam, Netherlands.*
- T-097 Transcriptomic and Epigenomic Sex Dimorphisms in Endothelial Cells Converge in a Prediction Model of Vascular Aging in Fetal Growth Restriction.**  
Bernardo J. Krause\*,<sup>1</sup> Estefania Peñaloza†,<sup>1</sup> Titia Lely\*,<sup>2</sup> Fieke Terstappen\*.<sup>2</sup> <sup>1</sup>*Universidad de O'Higgins, Rancagua, Chile;* <sup>2</sup>*University Medical Center Utrecht, Utrecht, Netherlands.*

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- T-098 Exploring the Fetal-Placental Vascular Axis in Growth-Restricted Pregnancy: New Perspectives on an Established Model.**  
Rachael C Crew†, <sup>1</sup>Yutthapong Tongpob,<sup>1,2</sup> Nikhilesh Bappoo,<sup>3</sup> Georgia Khinsoe,<sup>3</sup> Barry Doyle,<sup>3</sup> Caitlin S Wyrwoll\*. <sup>1</sup>The University of Western Australia, Perth, Australia; <sup>2</sup>Naresuan University, Bangkok, Thailand; <sup>3</sup>Harry Perkins Institute of Medical Research, Perth, Australia.
- T-099 Diastolic Left Ventricular Dysfunction on the Fifth Decade of Life in Women That Had a Spontaneous Preterm Birth: A Prospective Study.**  
Laura E Janssen†, <sup>1</sup>Marjon A Boer\*, <sup>1</sup>Elaine C.E von Königslöw†, <sup>1</sup>Elisa Dal Canto†, <sup>2</sup>Martijn A Oudijk\*, <sup>3</sup>Walter J Paulus\*, <sup>2</sup>Christianne J.M de Groot\*, <sup>1,3</sup> <sup>1</sup>Amsterdam UMC, VU Medical Center, Amsterdam, Netherlands; <sup>2</sup>Amsterdam Vascular Sciences, Amsterdam, Netherlands; <sup>3</sup>Amsterdam UMC, Amsterdam Medical Center, Amsterdam, Netherlands.
- T-100 Oxidative Stress Mediates the Potentiation of Adipogenesis by Exposure to the Bisphenol A (BPA) Analogue, BPS.**  
Radha Dutt Singh†, Anna Mikolajczak†, Sarah Easson†, Liam Connor†, Jennifer Thompson\*. University of Calgary, Calgary, Alberta, Canada, Calgary, AB, Canada.
- T-101 Maternal Food Restriction Programs Neonatal Cerebrovascular, Neurobehavioral and Glucocorticoid Responses to Mild Hypoxic-Ischemic Injury.**  
Naomi Franco†, Lara M Durrant, Coleen Doan, Alejandra Beltran†, William J Pearce\*. Loma Linda University, Loma Linda, CA, United States.
- T-102 Specific Changes in 3rd Trimester Maternal Fatty Acids Correlate with Maternal % Body Fat, Cytokines, and Neonatal Adiposity.**  
Stephanie Pierce, <sup>1</sup>David Fields, <sup>1</sup>Martin-Paul Agbaga, <sup>2</sup>Ravindu Gunatilake, <sup>3</sup>Jacob Friedman, <sup>1</sup>Dean Myers\*. <sup>1</sup>Univ. of Oklahoma HSC, OKC, OK, United States; <sup>2</sup>D. McGee Eye Inst., OKC, OK, United States; <sup>3</sup>Valley Perinatal, Phoenix, AZ, United States.
- T-103 Maternal Western-Style Diet Drives Glycolytic Programming in Hematopoietic Stem and Progenitor Cells and Underlies a Pro-Fibrotic Liver Response in Non-Human Primate Offspring.**  
Michael J Nash†, <sup>1</sup>Evgenia Dobrinskikh, <sup>1</sup>Taylor Soderborg†, <sup>1</sup>Oleg Varlamov, <sup>2</sup>Diana Takahashi, <sup>2</sup>Richard Stouffer, <sup>2</sup>Kjersti Aagaard, <sup>3</sup>Carrie McCurdy, <sup>4</sup>Maureen Gannon, <sup>5</sup>Eric Pietras, <sup>1</sup>Stephanie Wesolowski\*, <sup>1</sup>Jacob Friedman\*. <sup>1</sup>University of Colorado, Anschutz, Aurora, CO, United States; <sup>2</sup>Oregon Health & Science University, Beaverton, OR, United States; <sup>3</sup>Baylor College of Medicine, Houston, TX, United States; <sup>4</sup>University of Oregon, Eugene, OR, United States; <sup>5</sup>Vanderbilt University, Nashville, TN, United States; <sup>6</sup>University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States.
- T-104 Sex-Specific Differences in Immune Dysregulation Following Exposure to Maternal Inflammation.**  
Jin Liu†, Yang Liu†, Anguo Liu†, Jun Lei\*, Irina Burd\*. Johns Hopkins University, Baltimore, MD, United States.
- T-105 A Life Course Approach to the Relationship between Fetal Growth and HPA-Axis Function.**  
Wriyu M Martin†, <sup>1,2</sup>Carol A Wang, <sup>1,3</sup>Stephen J Lye, <sup>4</sup>Rebecca M Reynolds, <sup>5</sup>Stephen G Matthews, <sup>4,6</sup>Carly E McLaughlin, <sup>7</sup>Roger Smith, <sup>1,3</sup>Craig E Pennell\*. <sup>1,3</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter New England Local Health District, New South Wales, Australia; <sup>3</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>4</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>5</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>6</sup>University of Toronto, Toronto, ON, Canada; <sup>7</sup>Curtin University, Western Australia, Australia.
- T-106 Longitudinal Assessment of Exosomal SVATs in Preterm, Preeclampsia, and Gestational Diabetes Mellitus.**  
Nanbert Zhong\*, <sup>1</sup>Jing Pan†, <sup>2</sup>Yong Wang\*, <sup>3</sup>Weina Ju†. <sup>1</sup>New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States; <sup>2</sup>Southern Medical University, Guangzhou, China; <sup>3</sup>Washington University School of Medicine, St. Louis, MO, United States.
- T-107 Integrated Multi-Omics Analyses of High-Risk Myometrial Progenitor/Stem Cells: Implication for Uterine Fibroid Pathogenesis.**  
Qiwei Yang, Ayman Al-Hendy. University of Chicago, Chicago, IL, United States.
- FETUS**
- T-108 The Impact of Anticoagulation Use on Cell-Free DNA Metrics for Women without Autoimmune Disease.**  
H MacKinnon†, T Kolarova†, J Hedge†, E Vinopal†, S Delaney\*, C Lockwood\*, R Shree\*. University of Washington Medical Center, Seattle, WA, United States.
- T-109 Fetal Membrane Cells and Their Exosomes A Gateway for Drug Transportation during Pregnancy.**  
Ananth Kumar Kammala, Lauren Richardson, Enkhtuya Radnaa. The University of Texas Medical Branch, Galveston, TX, United States.
- T-110 Copy Number Changes and Fetal Malformations in Stillborn Fetuses.**  
Tsegaselassie Workalemahu, <sup>1</sup>Susan Dalton†, <sup>1</sup>Amanda Allshouse, <sup>1</sup>Jessica M Page, <sup>1,2</sup>Robert M Silver\*. <sup>1</sup>University of Utah, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States.
- T-111 Maternal Exposure to an Environmentally Relevant Mixture of Per- and Polyfluoroalkyl Substances (PFAS) Leads to Adverse Pregnancy Outcomes in a New Zealand White Rabbit Model.**  
Christine E Crute†, Samantha Hall†, Chelsea Landon, Angela Garner, Susan K. Murphy, Liping Feng\*. Duke University, Durham, NC, United States.
- T-112 Maternal Serum Micro RNA Correlates with Amniotic Fluid Micro RNA in Pregnancies Complicated by TTTS: A Potential Prenatal Marker for TTTS.**  
Chloe Nielsen†, <sup>1</sup>Henry Galan\*, <sup>1</sup>Hilary Hoffman\*, <sup>2</sup>Bettina Cuneo\*, <sup>1</sup>Shelley Miyamoto\*, <sup>2</sup>Carmen Sucharov\*. <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>Childrens Hospital Colorado, Aurora, CO, United States.
- T-113 A Two-Week Insulin Infusion in IUGR Fetal Sheep at 70% Gestation Increases Myoblast Proliferation but Not Total Myofibers.**  
Eileen I. Chang†, <sup>1</sup>Byron Hetrick, <sup>2</sup>Stephanie R. Wesolowski, <sup>1</sup>Paul J. Rozance, <sup>1</sup>Carrie E. McCurdy, <sup>2</sup>Laura D. Brown\*. <sup>1</sup>University of Colorado School of Medicine, Aurora, CO, United States; <sup>2</sup>University of Oregon, Eugene, OR, United States.

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- T-114**     **Late Gestation Fetal Hyperglucagonemia Lowers Pancreas Weight, Beta- and Alpha-Cell Proliferation, Islet Area, and Basal Insulin Concentrations.**  
Sarah N Cilvik,<sup>1</sup> Brit Boehmer,<sup>2</sup> Stephanie R Wesolowski,<sup>2</sup> Laura D Brown,<sup>2</sup> Paul J Rozance\*,<sup>2</sup> <sup>1</sup>Wake Forest University Health Sciences, Winston-Salem, NC, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States.
- T-115**     **Sheep-Specific IGF-1 Promotes Anabolic Growth in Fetal Sheep.**  
Jane Stremming,<sup>1</sup> Alicia White†,<sup>1</sup> Pamela A Doerner Barbour,<sup>1</sup> Eileen I Chang†,<sup>1</sup> Stephanie R Wesolowski,<sup>1</sup> Matt Seefeldt,<sup>1</sup> Byron Hetrick,<sup>2</sup> Carrie E McCurdy,<sup>2</sup> Paul J Rozance,<sup>1</sup> Laura D Brown\*,<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University of Oregon, Eugene, OR, United States.
- T-116**     **Associations of Maternal Bisphenol Urine Concentrations during Pregnancy with Neonatal Metabolomic Profiles.**  
Sophia Maria Blaauwendraad†,<sup>1</sup> Ellis Voerman,<sup>1</sup> Leonardo Trasande,<sup>2</sup> Kurunthachalam Kannan,<sup>2</sup> Susana Santos,<sup>1</sup> George Ruijter,<sup>1</sup> Chalana Sol,<sup>1</sup> Linda Marchioro,<sup>3</sup> Engy Shokry,<sup>3</sup> Berthold Koletzko,<sup>4</sup> Vincent Jaddoe,<sup>1</sup> Romy Gaillard.<sup>1</sup> <sup>1</sup>Erasmus Medical Center, Rotterdam, Netherlands; <sup>2</sup>New York University School of Medicine, New York, NY, United States; <sup>3</sup>Dr. von Hauners Children's Hospital, LMU München, Munich, Germany; <sup>4</sup>Dr. von Hauners Children's Hospital, LMU München, Munich, Netherlands.
- T-117**     **Fetal Growth Restriction: Isolated Abdominal Circumference and Perinatal Outcomes.**  
Maria Andrikopoulou†,<sup>1</sup> Natalie Bello,<sup>1</sup> Shai Bejerano,<sup>1</sup> Karin Fuchs,<sup>1</sup> Russell Miller,<sup>1</sup> Eliza Miller,<sup>1</sup> David M Haas,<sup>2</sup> William Grobman,<sup>3</sup> Brian M Mercer,<sup>4</sup> Samuel Parry,<sup>5</sup> Robert M Silver,<sup>6</sup> Ronald Wapner,<sup>1</sup> Deborah Wing,<sup>7</sup> George R Saade,<sup>8</sup> Uma Reddy,<sup>9</sup> Hyagriv Simhan,<sup>10</sup> Corette Parker,<sup>11</sup> Cynthia Gyamfi-Bannerman\*. <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>Indiana University School of Medicine, Indianapolis, IN, United States; <sup>3</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States; <sup>4</sup>MetroHealth Medical Cent Case Western Reserve University, Cleveland, OH, United States; <sup>5</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>6</sup>University of Utah, Salt Lake City, UT, United States; <sup>7</sup>University of California, Irvine, CA, United States; <sup>8</sup>University of Texas Medical Branch, Galveston, TX, United States; <sup>9</sup>Yale School of Medicine, New Haven, CT, United States; <sup>10</sup>University of Pittsburgh, Pittsburgh, PA, United States; <sup>11</sup>RTI International, Research Triangle Park, NC, United States.
- T-118**     **Intrauterine Hypoxia (HPX) Dysregulates Mitochondrial Respiratory Complex Expression and Activity in Fetal Guinea Pig (GP) Hearts.**  
Hong Song, Loren P. Thompson\*. Univ. of Maryland SOM, Baltimore, MD, United States.
- T-119**     **Translatable In Vivo Fetal Cardiac Geometry and Function in Adverse Pregnancy: A Comparison between Human Fetal Growth Restriction and Progressive Hypoxic Pregnancy in Sheep.**  
Olga V Patey†,<sup>1,2</sup> Kimberley L Botting,<sup>1</sup> Youguo Niu,<sup>1</sup> Lin Zhang,<sup>1</sup> Sage G Ford,<sup>1</sup> Wen Tong,<sup>1</sup> Conrado M Coutinho,<sup>2</sup> Basky Thilaganathan,<sup>2</sup> Dino A Giussani\*. <sup>1</sup>University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>St. George's University NHS Foundation Trust, London, United Kingdom.
- T-120**     **Modelling Obstructive Sleep Apnoea in Pregnancy: Direct Effects of Intermittent Hypoxia on Embryonic Cardiac Function and Growth.**  
Anna L K Cochrane†, Youguo Niu, Sage G Ford, Dino A Giussani\*. University of Cambridge, Cambridge, United Kingdom.
- T-121**     **Impaired Expression of Mechanosensing Genes in Human Pregnancy Complicated by Fetal Growth Restriction and Chronic Hypoxia.**  
German Arenas,<sup>1</sup> Estefania Peñaloza†,<sup>2</sup> Dino A Giussani\*,<sup>3,3,3</sup> Bernardo J Krause\*,<sup>2</sup> <sup>1</sup>Pontificia Univ. Catholica de Chile, Santiago, Chile; <sup>2</sup>Universidad de O'Higgins, Rancagua, Chile; <sup>3</sup>University of Cambridge, Cambridge, United Kingdom.
- T-122**     **Dexamethasone Reduces 11β-Hydroxysteroid Dehydrogenase 1 Expression the Fetal Lung Following Hypoxic Pregnancy.**  
Mitchell C Lock,<sup>1</sup> Kimberley J Botting,<sup>2</sup> Youguo Niu,<sup>2</sup> Sage G Ford,<sup>2</sup> Sandra Orgeig,<sup>1</sup> Dino A Giussani,<sup>2</sup> Janna L Morrison\*. <sup>1</sup>University of South Australia, Adelaide, Australia; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.
- T-123**     **Neuroinflammatory Pathways Investigated Using Neural Stem Cells.**  
Keith A Kwan Cheung†, Pevindu H Abeysinghe†, James M Bassett†, Murray D Mitchell\*. Queensland University of Technology, Brisbane, Australia.
- T-124**     **IFNγ-Producing Gamma/Delta T Cells in the Fetal Brain Following Intrauterine Inflammation: Possible Mechanism of Fetal Neuronal Injury.**  
Emma L Lewis†, Natalia Tulina, Michal A Elovitz\*. University of Pennsylvania, Philadelphia, PA, United States.
- GYNECOLOGIC ONCOLOGY**
- T-125**     **Ovarian Cancer Heterogeneity and Their Association with Differential Secretion of Extracellular Vesicles in Response to Hypoxia.**  
Nihar Godbole†,<sup>1</sup> Sharma Shayna†,<sup>1</sup> Priyakshi Kalita-de Croft†,<sup>1</sup> Carlos Salomon\*,<sup>1,2</sup> <sup>1</sup>The University of Queensland, Brisbane, Australia; <sup>2</sup>University of Concepcion, Concepcion, Chile.
- T-126**     **Applicability of Pre-Operative Patient Reported Duke Activity Scale Index (DASI) in Prediction of Postoperative Complications in Gynaecological Oncology.**  
Lusine Sevinyan†,<sup>1</sup> Anil Tailor,<sup>1</sup> Pradeep Prabhu,<sup>1</sup> Peter Williams,<sup>2</sup> Thumuluru Kavitha Madhuri,<sup>1,3</sup> <sup>1</sup>Royal Surrey Hospital NHS Foundation Trust, Guildford, United Kingdom; <sup>2</sup>University of Surrey, Guildford, United Kingdom; <sup>3</sup>University of Brighton, Brighton, United Kingdom.
- MATERNAL BIOLOGY**
- T-127**     **Placental Endocrine Function and Insulin-Like Growth Factor-2 (Igf2) Are Important Determinants of Maternal Metabolic State and Fetal Growth in Obese Mouse Pregnancies.**  
Samantha C Lean, Esteban Salazar Petres, Edina Gulacsi, Amanda N Sferruzzi-Perri\*. University of Cambridge, Cambridge, United Kingdom.
- T-128**     **Maternal Aging Impacts Vascular Adaptations to Pregnancy.**  
Mazhar Pasha, Raven Kirschenman, Amy Wooldridge, Floor Spaans, Sandra Davidge, Christy-Lynn Cooke. University of Alberta, Edmonton, AB, Canada.

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- T-129**      **Circulating Placental Alkaline Phosphatase Correlates with Systemic Changes in Cardiovascular Function.**  
Maria Cristina Bravo, Carole McBride, Kathleen Brummel-Ziedins, Ira Bernstein. *University of Vermont, Colchester, VT, United States.*
- T-130**      **Progesterone Withdrawal Induces Eosinophilic Inflammation in the Process of Mouse Cervical Ripening.**  
Yosuke Sugita, Yoshimitsu Kuwabara\*, Shigeru Matsuda, Yumiko Oishi, Toshiyuki Takeshita. *Nippon Medical School, Tokyo, Japan.*
- T-131**      **Maternal Obesity during Pregnancy Induces Oxidative Stress and Mitochondria Functional Alterations in Sheep Maternal Liver.**  
 Luis F Grilo†, João D Martins†, Mariana S Diniz†, Carolina Tocantins†, Chiara H Cavallaro†, Inês Baldeiras, Teresa Cunha-Oliveira, Stephen Ford\*, Peter W Nathanielsz\*, Paulo J Oliveira\*, Susana P. Pereira†.<sup>1</sup> *University of Coimbra, Coimbra, Portugal;* <sup>2</sup>*Coimbra University Hospital, Coimbra, Portugal;* <sup>3</sup>*University of Wyoming, Laramie, WY, United States;* <sup>4</sup>*University of Porto, Porto, Portugal.*
- T-132**      **Uterine Artery Adaptations to Pregnancy Are Impaired by Advanced Maternal Age.**  
Amy L Woolldridge†, Mazhar Pasha†, Raven Kirschenman, Floor Spaans, Sandra T Davidge, Christy-Lynn M Cooke\*. *University of Alberta, Edmonton, AB, Canada.*
- T-133**      **L-Citrulline Supplementation during Pregnancy Improves Maternal Vascular Dysfunction in a Preeclampsia-Like Mouse Model.**  
Mary Gemmelt†, Elizabeth Sutton, Marcia Gallaher, Robert W. Powers\*.<sup>1</sup> *University of Pittsburgh, Pittsburgh, PA, United States;* <sup>2</sup>*Woman's Hospital, Baton Rouge, LA, United States.*
- T-134**      **Alterations in Inorganic Phosphate and Calcium Maternal Excretion Associated with Gestational Age and Parity.**  
Ana Correia-Branco†, Monica Rincon, Leonardo Pereira, Mary C Wallingford. <sup>1</sup>*Tufts Medical Center, Boston, MA, United States;* <sup>2</sup>*Oregon Health Science Center, Oregon, OR, United States.*
- T-135**      **Predictors of Vaginal Delivery in Patients with Cardiac Disease.**  
Nicole Rose Gavin†, Jerome Federspiel†, Theresa Boyer†, Kristin Darwin†, Alexia Debrosse†, Anum Minhas†, Arthur Vaught\*.<sup>1</sup> *The Johns Hopkins Hospital, Baltimore, MD, United States;* <sup>2</sup>*Duke University School of Medicine, Baltimore, NC, United States.*
- T-136**      **Effect of Long-Term Storage and Pre-Pregnancy BMI on Lipid Parameters in Stored Maternal Plasma Samples.**  
Theresa Boyer, Nada Elsayed, Kimberly Jones-Beatty, Irina Burd\*. *Johns Hopkins University, Baltimore, MD, United States.*
- T-137**      **Myocardial Bridge in Pregnancy: Beyond a 'Normal Anatomic Variant'.**  
Noor Joudi†, Imee Datoc, Stephanie Leonard, Christine Lee, Ingela Schnittger, Abha Khandelwal, Katherine Bianco\*. *Stanford University Hospital and Clinics, Stanford, CA, United States.*
- T-138**      **Nursing Modifies the Immune Profile in Postpartum Mice.**  
Pauline DiGianvittorio†, Marlena Tyldesley†, Kirtika Prakash†, Elizabeth A Bonney\*. *University of Vermont, Lamer College of Medicine, Burlington, VT, United States.*
- T-139**      **Differential Shedding of Endothelial Cell Proteins during the Peripartum Period.**  
Maria Cristina Bravo, Ira Bernstein, Kelley McLean, Thomas Orfeo, Kathleen Brummel-Ziedins. *University of Vermont, Colchester, VT, United States.*
- T-140**      **Long Term Patient Follow-Up of Cardiac Disease in Pregnancy: Multidisciplinary Teams Tether At-Risk Patients to the System.**  
Sarah E Miller†, Danielle Panelli, Elizabeth Sherwin, Christine Lee, Hayley Miller†, Alisha Tolani†, Alana O'Mara†, Abha Khandelwal, Ylaly Katherine Bianco\*. *Stanford University, Stanford, CA, United States.*
- T-141**      **Relationship between Fetal Position and Obstetric Laceration Location and Severity.**  
Gillian Horwitz†, Megan Trostle†, Iffath Hoskins\*, Ashley S. Roman\*. *NYU Langone Health, New York, NY, United States.*
- T-142**      **Innate Lymphoid Cell Subsets Are Uniquely Distributed within the Maternal-Fetal Interface.**  
Stephen A McCartney, Nicholas Maurice, Marie Frutoso, Florian Mair, Shree S Raj, Martin Pricic\*.<sup>1</sup> *University of Washington, Seattle, WA, United States;* <sup>2</sup>*Fred Hutchinson Cancer Research Center, Seattle, WA, United States.*
- T-143**      **Migraine and Adverse Pregnancy Outcomes: The nuMoM2b Study.**  
Eliza C Miller, Sarah E. Vollbracht, Cynthia Gyamfi-Bannerman, Whitney Booker, Leslie Moroz, Marianna S. Yigrakh, Lisa D. Levine, David M. Haas, William A. Grobman, Mary D'Alton, Ronald Wapner, Natalie A. Bello\*.<sup>1</sup> *Columbia University, New York, NY, United States;* <sup>2</sup>*University of Pennsylvania, Philadelphia, PA, United States;* <sup>3</sup>*Indiana University, Indianapolis, IN, United States;* <sup>4</sup>*Northwestern University, Chicago, IL, United States.*
- T-144**      **Periconceptional Maternal Renin-Angiotensin-Aldosterone System Activation and the Association with Maternal Telomere Length: The Rotterdam Periconception Cohort.**  
Damiat Aoulad Fares†, Rosalieke E Wiegelt, Alex J Eggink\*, Joyce B.J. Van Meurs\*, Jan A.H. Danser\*, Eric A.P. Steegers\*, Régine P.M. Steegers-Theunissen\*. *Erasmus Medical Center, Rotterdam, Netherlands.*
- PLACENTA**
- T-145**      **Corpus Luteum Contribution and the Maternal Renin-Angiotensin-Aldosterone System as Underlying Mechanism to (Utero) Placental Vascular Development Throughout Pregnancy: The Rotterdam Periconception Cohort.**  
Rosalieke E Wiegelt, A.H. Jan Danser\*, Maud J.H. Karsten†, Igna F Reijnders†, L van Rossem\*, Sten P Willemsen\*, Annemarie G.M.G.J. Mulders\*, Eric A.P. Steegers\*, Régine P.M. Steegers-Theunissen\*. *Erasmus Medical Center, Rotterdam, Netherlands.*
- T-146**      **CDKN1C Is a Conserved Regulator of Trophoblast Cell Development.**  
Regan L Scott†, Khursheed Iqbal, Kaela M Varberg, Marija Kuna, Keisuke Kozai, Michael J Soares\*.<sup>1,2</sup> *University of Kansas Medical Center, Kansas City, KS, United States;* <sup>2</sup>*Children's Mercy Research Institute, Kansas City, MO, United States.*
- T-147**      **Global DNA Methylation Differences in Chorionic Villi from Euploid Miscarriages.**  
Winifred Mak\*, Jawon Song. <sup>1</sup>*Dell Medical School, UT Austin, Austin, TX, United States;* <sup>2</sup>*TACC, Austin, TX, United States.*

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- T-148 Mapping Enhancer - Transcription Factor Interactions in Human Placenta Development and Trophoblast Differentiation.**  
David Owen, Xuan Huang, Anusha Nagari, Tulip Nandu, W. Lee Kraus\*. *UT Southwestern Medical Center, Dallas, TX, United States.*
- T-149 Placental Pathology Associated with Liveborn and Stillbirth Infants in Women with and without Antiphospholipid Antibodies.**  
Jhenette Lauder†, <sup>1,2</sup> Jessica Page\*, <sup>1,2</sup> Amanda Allshouse\*, <sup>1</sup> Uma Reddy\*, <sup>3</sup> Robert Goldenberg\*, <sup>4</sup> Halit Pinar\*, <sup>5</sup> Silver Robert\*, <sup>1</sup> Ware Branch\*. <sup>1,2</sup> *University of Utah Health, SLC, UT, United States;* <sup>2</sup> *Intermountain Health Center, SLC, UT, United States;* <sup>3</sup> *Yale University, New Haven, CT, United States;* <sup>4</sup> *Columbia University, NYC, NY, United States;* <sup>5</sup> *Brown University, Providence, RI, United States.*
- T-150 Novel Mechanisms of Disrupted Placental Development: Lipid Mediators and Inflammatory Signaling.**  
Yuliya Fakhr†, <sup>1,2</sup> Kirsten Webster†, <sup>1,2</sup> Denise G Hemmings\*. <sup>1,2,3</sup> *Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada;* <sup>2</sup> *Women and Children's Health Research Institute, Edmonton, AB, Canada;* <sup>3</sup> *Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada.*
- T-151 AMPK Signaling Stimulates Mitophagy in Human Trophoblast Cell Line via Pathways Mediated by PINK1/PARKIN and FUNDC1.**  
Bin Wu, <sup>1</sup> Seyedeh Alaie†, <sup>2</sup> Yun Chen, <sup>3</sup> Guoyang Luo, <sup>2</sup> Haijun Gao\*. <sup>2</sup> *Central Hospital Affiliated to Shandong First Medical University, Jinan, China;* <sup>2</sup> *Howard University, Washington, DC, United States;* <sup>3</sup> *Rocket Pharmaceuticals, Inc., Cranbury, NJ, United States.*
- T-152 Placental Pathology Associated with Liveborn and Stillbirth Infants in Women with and without an Inherited Thrombophilia.**  
Jhenette Lauder†, <sup>1,2</sup> Jessica Page\*, <sup>1,2</sup> Amanda Allshouse\*, <sup>1</sup> Robert Goldenberg\*, <sup>3</sup> Carol Hogue\*, <sup>4</sup> Halit Pinar\*, <sup>5</sup> Ware Branch\*, <sup>1,2</sup> Robert Silver\*. <sup>1</sup> *University of Utah Health, SLC, UT, United States;* <sup>2</sup> *Intermountain Health Center, SLC, UT, United States;* <sup>3</sup> *Columbia University, NYC, NY, United States;* <sup>4</sup> *Emory University, Atlanta, GA, United States;* <sup>5</sup> *Brown University, Providence, RI, United States.*
- T-153 A Mechanistic Framework for Cytotrophoblast to Extravillous Trophoblast Differentiation.**  
Sonia C. DaSilva-Arnold, Stacy Zamudio, Abdulla Al-Khan, Nicholas P. Illsley\*. *Hackensack University Medical Center, Hackensack, NJ, United States.*
- T-154 Transcriptomic Analysis of Human Placenta Reveals a Distinct Gene Expression Pattern Associated with Dysregulated Apoptosis and Autophagy Leading to Preterm Birth.**  
Khondoker Mehedi Akram\*, Neha S Kulkarni†, Dilichukwu O Anumba\*. *University of Sheffield, Sheffield, United Kingdom.*
- T-155 Myostatin Increases Human Trophoblast Cell Invasion by Up-Regulating N-Cadherin via SMAD2/3-SMAD4 Signaling.**  
Faten Fa Ahmed†, Christian Klausen\*, Hua Zhu\*, Peter Leung\*. *UBC, Vancouver, BC, Canada.*
- T-156 Hypoxia Activates NOTCH1 Signaling to Promote HTR-8/SVneo Trophoblast Cell Migration but Not Invasion.**  
Barry E Perlman†, <sup>1</sup> Natak Douglas. <sup>2</sup> *Rutgers-NJMS, Newark, NJ, United States;* <sup>2</sup> *Rutgers, New Jersey Medical School, Newark, NJ, United States.*
- T-157 Do DNA Methylation Changes Contribute to the Phenotypic Differences between Human Cyto- and Extravillous Trophoblast?**  
Sonia C. DaSilva-Arnold, <sup>1</sup> Marthia Salas, <sup>2</sup> Stacy Zamudio, <sup>1</sup> Abdulla Al-Khan, <sup>1</sup> Benjamin Tycko, <sup>2</sup> Nicholas P. Illsley\*. <sup>1</sup> *Hackensack University Medical Center, Hackensack, NJ, United States;* <sup>2</sup> *Hackensack Meridian Health Center for Discovery and Innovation, Nutley, NJ, United States.*
- T-158 Palmitic Acid Impedes Extravillous Trophoblast Activity by Increasing MRP1 Expression and Function.**  
Yunali V Ashar†, Qiu-Xu Teng†, John ND Wurple, Zhe-Sheng Chen, Sandra E Reznik\*. *St. John's University, Queens, NY, United States.*
- T-159 Decorin-Induced MicroRNAs in Trophoblast Functions: Roles in Preeclampsia.**  
Chidambra D Halari†, Maria Sbirnac, Jasmine Sidhu, Pinki Nandi, Peeyush K Lala\*. *University of Western Ontario, London, ON, Canada.*
- T-160 Trophoblast-Derived Soluble Fms-Like Tyrosine Kinase-1 Production Is Modulated by pICln in Preeclampsia.**  
Yuko Matsubara, <sup>1</sup> Keiichi Matsubara\*, <sup>2</sup> Yuka Uchikura, <sup>1</sup> Katsuko Takagi, <sup>1</sup> Takashi Sugiyama. <sup>1</sup> *Ehime Univ. SOM, Toon, Ehime, Japan;* <sup>2</sup> *Ehime University Graduate SOM, Toon, Ehime, Japan.*
- T-161 Sustained Hypoxemia Activates Nutrient Shuttles between the Placenta and Fetus.**  
Amanda Jones, <sup>1</sup> Ashebo Betelhem, <sup>1</sup> Ramon Lorca, <sup>1</sup> Colleen Julian, <sup>1</sup> Lorna Moore, <sup>1</sup> Brown Laura, <sup>1</sup> Paul Rozance, <sup>1</sup> Sean Limesand, <sup>2</sup> Stephanie Wesolowski\*. <sup>1</sup> *University of Colorado, Aurora, CO, United States;* <sup>2</sup> *University of Arizona, Tucson, AZ, United States.*
- T-162 Interaction of Inorganic Phosphate and Unfolded Protein Response (UPR) in Placenta.**  
Ana C Correia-Branco†, <sup>1</sup> Olga C Kashpur†, <sup>1</sup> Ciara C Benson, <sup>2</sup> Nirmala Jayaraman, <sup>1</sup> Sasha A Singh, <sup>3</sup> Mark C Blaser, <sup>2</sup> Hideyuki Higashi, <sup>4</sup> Shiori Kuraoka, <sup>4</sup> Elena Aikawa, <sup>3</sup> Eugene W Hinderer III, <sup>5</sup> Mary C Wallingford\*. <sup>1</sup> *Tufts Medical Center, Boston, MA, United States;* <sup>2</sup> *Global Alliance to Prevent Prematurity and Stillbirth, Seattle, WA, United States;* <sup>3</sup> *Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States;* <sup>4</sup> *Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States;* <sup>5</sup> *Clinical and Translational Science Institute-CTSI, Boston, MA, United States.*
- T-163 Chromosomal Microarray (CMA) and Fetal Growth in Stillborn Fetuses.**  
Susan Dalton†\*, <sup>1</sup> Tsegaselassie Workalemahu, <sup>1</sup> Amanda A. Allshouse, <sup>1</sup> Jessica M. Page, <sup>2</sup> Silver M. Robert\*. <sup>1</sup> *University of Utah, Salt Lake City, UT, United States;* <sup>2</sup> *Intermountain Healthcare, Salt Lake City, UT, United States.*
- T-164 Localization and Kinetics of the Transferrin-Dependent Iron Transport Machinery in the Mouse Placenta.**  
Chang Cao†, Mark D Fleming. *Boston Children's Hospital, Boston, MA, United States.*
- T-165 Maternal Inositol Supplementation during Pregnancy Impacts Placental Metabolic Pathways in an Obese Murine Model.**  
Lidia Di Cerbo†, <sup>1,2</sup> Ahmed R Hamed\*, <sup>2</sup> Daniela Menichini†, <sup>1</sup> Francesca Ferrarini\*, <sup>1</sup> Baha M Sibai\*, <sup>2</sup> Fabio Facchinetti\*, <sup>1</sup> Sean Blackwell\*, <sup>2</sup> Monica Longo\*. <sup>1</sup> *University of Modena and Reggio Emilia, Modena, Italy;* <sup>2</sup> *McGovern Medical School at The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, United States.*

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- T-166**      **Dynamic Activation and Regulation of the Placental Unfolded Protein Response in Pregnancy.**  
Arren Simpson†, <sup>1</sup> Kyathanahalli Chandrashekar†, <sup>1</sup> Andrew D Kane, <sup>2</sup> Wen Tong, <sup>2</sup> Pancharatnam Jeyasuria, <sup>1</sup> Dino A Giussani, <sup>2</sup> Jennifer C Condon\*. <sup>1</sup>Wayne State University, Detroit, MI, United States; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.
- T-167**      **Placental DAGLbeta Regulates 2-arachidonoylglycerol Levels and Is Involved in Pregnancy Induced Inflammation.**  
Natascha Berger†, <sup>1</sup> Thomas Bärnthaler\*, <sup>2</sup> Jürgen Gindlhuber†, <sup>1</sup> Nermeen Girgis\*, <sup>2</sup> Tom van der Wel\*, <sup>3</sup> Birgit Hirschmugl\*, <sup>1</sup> Ruth Birner-Gruenberger\*, <sup>1</sup> Mario van der Stelt\*, <sup>3</sup> Robert Zimmermann\*, <sup>2</sup> Christian Wadsack\*. <sup>1</sup>Medical University of Graz, Graz, Austria; <sup>2</sup>University of Graz, Graz, Austria; <sup>3</sup>Leiden University, Leiden, Netherlands.
- T-168**      **Circulating microRNA Signatures Associated to Gestation Events Along the Same Healthy Human Pregnancy.**  
Erika Chavira-Suárez, <sup>1,2</sup> Alma Lilia Hernández-Olvera†, <sup>1</sup> Mariana Flores-Torrest†, <sup>2</sup> Karen Celaya-Cruz†, <sup>1</sup> Sofia Gitler†, <sup>1</sup> Juan Carlos de la Cerda-Ángeles\*, <sup>3</sup> Nidia Carolina Espinosa-Maldonado\*, <sup>1</sup> Carlos Fabián Flores-Jasso\*, <sup>2</sup> Humberto Gutiérrez\*, <sup>2</sup> Felipe Vadillo-Ortega\*. <sup>1,2,4</sup> Universidad Nacional Autónoma de México, CDMX, Mexico; <sup>2</sup>Instituto Nacional de Medicina Genómica, CDMX, Mexico; <sup>3</sup>Secretaría de Salud de la Ciudad de México, CDMX, Mexico; <sup>4</sup>University of Michigan School of Public Health, Ann Arbor, MI, United States.
- T-169**      **Using iHumanPlacenta Network and Longitudinal Metabolomics Data to Identify Metabolic Signatures Associated with Preterm Birth.**  
Priyanka Baloni, <sup>1</sup> Nagendra Monangi, <sup>2</sup> Alison Paquette, <sup>3</sup> Gina Huynh, <sup>1</sup> Heather Brockway, <sup>4</sup> Yoel Sadovsky, <sup>5</sup> Louis J Muglia, <sup>6</sup> Jones Jones\*, <sup>4</sup> Nathan D Price\*. <sup>1</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>2</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; <sup>3</sup>Seattle Children's Research Institute, Seattle, WA, United States; <sup>4</sup>University of Florida College of Medicine, Gainesville, FL, United States; <sup>5</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States; <sup>6</sup>Burroughs Wellcome Fund, Research Triangle Park, NC, United States.
- T-170**      **Early Gestation T2\*-Based BOLD Effect in Human Placenta.**  
Ruiming Chen†, <sup>1</sup> Ante Zhu, <sup>2,1</sup> Jitka Starekova, <sup>1</sup> Daniel Seiter†, <sup>1</sup> Kevin M Johnson, <sup>1</sup> Sean B Fain, <sup>1</sup> Scott B Reeder, <sup>1</sup> Dinesh M Shah, <sup>1</sup> Oliver Wieben, <sup>1</sup> Diego Hernando. <sup>1</sup>University of Wisconsin - Madison, Madison, WI, United States; <sup>2</sup>GE Research, Niskayuna, NY, United States.
- T-171**      **Maternal Doxycycline Treatment Causes Murine Fetal Cardiac Dysfunction Associated with Altered Placental Morphology and Endothelin-1 Expression.**  
Yuliya Fakhrt†, <sup>1,2</sup> Saba Saadat†, <sup>1,2</sup> Lisa K Hornberger, <sup>2,3</sup> Denise G Hemmings\*, <sup>1,2</sup> Luke Eckersley\*. <sup>2,3</sup>Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Women and Children's Health Research Institute (WCHR), University of Alberta, Edmonton, AB, Canada; <sup>3</sup>Department of Pediatrics, University of Alberta, Edmonton, AB, Canada.
- T-172**      **Zika Virus (Zikv) Infection at Early or Mid-Gestation Results in Persistent Uterine and Lymph Node Infection and Adverse Fetal CNS Pathology by Term Gestation in the Olive Baboon (*Papio anubis*).**  
Sunam G Dockins†, Darlene N Reuter, Marta E Maxted, Krista R Singleton, Molly E Dubois, James F Papin, Dean A Myers\*. OUHSC, Oklahoma City, OK, United States.
- T-173**      **A Dual Role for Progesterone in Mediating Influenza A Virus H1N1-Associated Injury in the Lung, but Protecting the Placenta.**  
Miranda Li†, H Huang\*, A Li†, K Adams Waldorf\*. University of Washington, Seattle, WA, United States.
- T-174**      **Maternal Administration of siRNA-SAA Alleviates Preterm Birth through PD-1/PD-L1 Signaling in a Mouse Model of Sub-Chronic Maternal Inflammation.**  
Yang Liu, Jin Liu†, Anguo Liu†, Irina Burd\*, Jun Lei\*. Johns Hopkins University, Baltimore, MD, United States.
- T-175**      **Functional Properties of Cytotoxic T Cells in Placenta after Sub-Chronic Inflammation Exposure.**  
Jin Liu†, Yang Liu†, Anguo Liu†, Irina Burd\*. -Johns Hopkins University, -Baltimore, MD, United States.
- T-176**      **Protective Effect of IL-1Ra on GBS-Induced Chorioamnionitis and Neurobehavioural Impairment of the Progeny.**  
Taghreed A. Ayash†. McGill University Health Center, Montréal, QC, Canada.
- T-177**      **Chronic Intervillositis Series with 2 Cases of Recurrence: Successful Rescue Treatment.**  
Kateri Lévesque, Dorothee Dal Soglio, Michele David, Evelyne Rey\*, Line Leduc\*. CHU Ste-Justine, Montréal, QC, Canada.
- T-178**      **Direct Induction of Trophoblast Stem Cells from Human Fibroblasts.**  
Moriyah Naama Shacham†, <sup>1</sup> Valery Zayat, <sup>2</sup> Shulamit Sebban, <sup>1</sup> Ahmed Radwan, <sup>1</sup> Rachel Lasry, <sup>1</sup> Ofra Sabag, <sup>1</sup> Silvina Epsztejn-Litman, <sup>3,4</sup> Michal Novoselsky Persky, <sup>5</sup> Kirill Makedonski, <sup>1</sup> Dana Orzech, <sup>1</sup> Noy Dery, <sup>1</sup> Debra Goldman-Wohl, <sup>5</sup> Howard Cedar, <sup>1</sup> Simcha Yagel, <sup>5</sup> Rachel Eiges, <sup>3</sup> Yosef Buganim\*. <sup>1</sup>The Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; <sup>2</sup>Mossakowski Medical Research Centre, Warsaw, Poland; <sup>3</sup>Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; <sup>4</sup>The Hebrew University School of Medicine, Jerusalem, Israel; <sup>5</sup>The Magda and Richard Hoffman Laboratory of Human Placental Research, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.
- T-179**      **Mitochondrial Citrate Carrier Regulates Syncytiotrophoblast Differentiation.**  
Sarah Wernimont\*, <sup>1</sup> Adam Rauckhorst, <sup>2</sup> Crawford Peter, <sup>1</sup> Eric Taylor. <sup>2</sup>University of Minnesota, Minneapolis, MN, United States; <sup>2</sup>University of Iowa, Iowa City, IA, United States.

### PRECLAMPسيا

- T-180**      **Methylation Analysis in Pathological Placental Samples.**  
Camino Sm Ruan†, Clara Apicella†, Francisco Miralles\*, Celine Mehats\*, Daniel Vaiman\*. *Institut Cochin, Paris, France.*
- T-181**      **A Potential Role for Calcitonin Gene Related Peptide in Regulating Mitochondrial Function in Endothelial Cells.**  
Akansha Mishra, Vipin Alukkal Vidyadharan, Chandra Yallampalli, Madhu Chauhan\*. Baylor College of Medicine, Houston, TX, United States.
- T-182**      **Peripheral Blood Mononuclear Cells (PBMCs) Induce Endothelial Dysfunction in Human Umbilical Vein Endothelial Cells (HUVECs) via Proinflammatory Cytokines.**  
Aishwarya Rengarajan†, Jason Austin, Amanda Mauro†, Derek Boeldt. University of Wisconsin Madison, Madison, WI, United States.

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- T-183 Aspirin Resistance Measurements during Pregnancy: The Effect of Aspirin on Platelet Function Compared to Placebo.**  
Anadeijda J.e.m.c. Landman†, <sup>1</sup>Jeske M. bij de Wegt, <sup>1</sup>Johanna I.p. de Vries, <sup>1</sup>Abel Thijs, <sup>1</sup>Ankie M. Harmsze, <sup>2</sup>Martijn A. Oudijk, <sup>3</sup>Christianne J.m. de Groot, <sup>1</sup>Marjon A. de Boer\*. <sup>1</sup>Amsterdam UMC - VUmc, Amsterdam, Netherlands; <sup>2</sup>St Antonius Hospital, Nieuwegein, Netherlands; <sup>3</sup>Amsterdam UMC - AMC, Amsterdam, Netherlands.
- T-184 Evaluation of the Nutraceutical Conjugated Linoleic Acid for Prevention of Monolayer Breakdown in PE.**  
Amanda Mauro†, Derek Boeldt\*. *University of Wisconsin-Madison, Madison, WI, United States.*
- T-185 Syncytiotrophoblast-Enriched Extracellular Vesicles from Normal and Preeclamptic Pregnancies Have a Different Impact on Nitrativ Stress in Human Umbilical Vein Endothelial Cells.**  
Roberto Esteban Villalobos-Labra†, Floor Spaans, Tamara Sáez, Anita Quon, Christy-Lynn Cooke, Sandra T Davidge. *University of Alberta, Edmonton, AB, Canada.*
- T-186 Src Inhibition Offers Monolayer Support in an In Vitro Model of Endothelial Dysfunction.**  
Amanda Mauro†, Derek Boeldt\*. *University of Wisconsin-Madison, Madison, WI, United States.*
- T-187 Hydroxychloroquine Effect on Healthy and Activated Fetoplacental Endothelial Cells *In Vitro*.**  
Maja Gajic†, Christian Wadsack\*, Mila Cervar-Zivkovic\*, Karoline Mayer-Pickel\*. *Medical University of Graz, Graz, Austria.*
- T-188 Syncytiotrophoblast Derived Extracellular Vesicles Aberrantly Express HLA DR in Preeclampsia.**  
Chiara Tersigni†, <sup>1</sup>Donatella Lucchetti, <sup>2</sup>Rita Franco, <sup>1</sup>Alessandro Sgambato, <sup>2</sup>Giovanni Scambia, <sup>1</sup>Nicoletta Di Simone. <sup>1</sup>Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; <sup>2</sup>Università Cattolica del Sacro Cuore, Rome, Italy.
- T-189 Cell-Free Membrane-Bound and Membrane-Unbound Mitochondrial DNA in Maternal Circulation in Preeclampsia.**  
Spencer C Cushen†, <sup>1</sup>Contessa A Ricci, <sup>1</sup>Danielle Reid, <sup>1</sup>Jessica L Bradshaw, <sup>1</sup>Talisa Silzer, <sup>1</sup>Blessing Alexandra, <sup>1</sup>Jie Sun, <sup>1</sup>Sabrina M Scroggins, <sup>2</sup>Mark K Santillan, <sup>2</sup>Donna A Santillan, <sup>2</sup>Nicole R Phillips, <sup>1</sup>Styliani Gouloupoulou\*. <sup>1</sup>University of North Texas Health Science Center, Fort Worth, TX, United States; <sup>2</sup>University of Iowa Health Care, Iowa City, IA, United States.
- T-190 Maternal Microchimerism in Umbilical Cord Blood in Preeclampsia Cases versus Controls.**  
Stephen A McCartney†, <sup>1</sup>Sami B Kanaan, <sup>2</sup>Angel Chae, <sup>1</sup>Hilary S Gammill, <sup>1</sup>J L Nelson, <sup>2</sup>Raj Shree\*. <sup>1</sup>University of Washington, Seattle, WA, United States; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, United States.
- T-191 The Risks Associated with Acute Organ Rejection Compared to Preeclampsia in Pregnancies after Liver Transplantation.**  
Ophelia Yin†, <sup>1</sup>Aneesh Kallapur†, <sup>1</sup>Lisa Coscia\*, <sup>2</sup>Serban Constantinescu\*, <sup>3</sup>Michael Moritz\*, <sup>4</sup>Yalda Afshar\*. <sup>1</sup>University of California, Los Angeles, Los Angeles, CA, United States; <sup>2</sup>Gift of Life Institute, Philadelphia, PA, United States; <sup>3</sup>Temple University, Philadelphia, PA, United States; <sup>4</sup>Lehigh Valley Health Network, Morsani College of Medicine, Allentown, PA, United States.
- T-192 Resolved Low Placentation and Risk of Hypertensive Disorder of Pregnancy.**  
Henri M Rosenberg†, Chelsea Debolt, Minhazur Sarker†, Geeta Rao, Jacqueline Roig, Angela Bianco\*. *Icahn School of Medicine Mount Sinai, New York, NY, United States.*
- T-193 Polymorphism of ESR1 Gene in Pregnants with Hypertension State.**  
Bakhodir Kurbanov. *Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan.*
- T-194 Hypertensive Disorders of Pregnancy Share Common cfDNA Methylation Profiles.**  
Jarmila A Zdanowicz†, Marialuigia Spinelli, Daniel Surbek, Martin Mueller\*. *University of Bern, Bern, Switzerland.*
- T-195 Factors Associated with Diagnosis of Gestational Hypertension or Preeclampsia in Women with Gestational Diabetes.**  
Vishmayaa Saravanan†, <sup>1</sup>Rachel Harrison, <sup>2</sup>Lauren Pavlik, <sup>1</sup>Meredith Cruz, <sup>1</sup>Anna Palatnik. <sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>2</sup>Advocate-Aurora Medical Group, Chicago, IL, United States.

## REPRODUCTIVE ENDOCRINOLOGY

- T-196 In Situ Mechanical Characterization Predicts the Developmental Potential of Oocytes and Embryos.**  
 Oren Wintner\*, <sup>1</sup>Naama Srebnik, <sup>1</sup>Dorit Kalo, <sup>2</sup>Zvi Roth, <sup>2</sup>Amnon Buxboim\*. <sup>1</sup>The Hebrew University of Jerusalem, Jerusalem, Israel; <sup>2</sup>The Hebrew University of Jerusalem, Rehovot, Israel.
- T-197 TOP5300, an Orally Active FSH Receptor Agonist, May Better Treat Specific Infertility Patient Populations.**  
Joie Z Guner†, Diana Monsivais, Fabio Stossi, Hannah Johnson, William E Gibbons, Martin M Matzuk, Stephen S Palmer\*. *Baylor College of Medicine, Houston, TX, United States.*
- T-198 Current Practices and Knowledge Surrounding Ovarian Stimulation in Transgender Men.**  
Samuel K Yost†, Emily K Kobernik, Molly B Moravek\*. *University of Michigan, Ann Arbor, MI, United States.*
- T-199 Is Conception with Assisted Reproductive Technology Associated with Increased Maternal Psychological Stress in Nulliparous Women?**  
Amir Lueth\*, Nathan Blue, Robert Silver. *University of Utah, Salt Lake City, UT, United States.*
- T-200 The Influence of Hashimoto Thyroiditis in the Metabolism of Follicle Microenvironment.**  
Diana C S Bastos†, <sup>1</sup>Maria Isabel Chiamolera\*, <sup>2</sup>Renata E. C. Silva†, <sup>2</sup>Maria C B Souza\*, <sup>3</sup>Roberto A Antunes\*, <sup>3</sup>Marcelo M Souza\*, <sup>3</sup>Ana C A Mancebo\*, <sup>3</sup>Patricia C F Arêas\*, <sup>3</sup>Fernando M Reis\*, <sup>4</sup>Flavia F Bloise\*, <sup>1</sup>Tania M Ortega-Carvalho\*. <sup>1</sup>Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; <sup>2</sup>Federal University of São Paulo, São Paulo, Brazil; <sup>3</sup>Fertipraxis Centro de Reprodução Humana, Rio de Janeiro, Brazil; <sup>4</sup>Federal University of Minas Gerais, Belo Horizonte, Brazil.
- T-201 Dose-Dependent Trend toward Increased Menstrual Cycle Length with Chronic Marijuana Use in Rhesus Macaques.**  
Kimberly Ryan†, <sup>1</sup>Shruthi Mahalingaiah, <sup>2</sup>Lily Campbell, <sup>3</sup>Jon Hennebold, <sup>4,1</sup>Jamie Lo\*. <sup>1,4</sup>Oregon Health & Science University, Portland, OR, United States; <sup>2</sup>Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>3</sup>Boston University, Boston, MA, United States; <sup>4</sup>Oregon National Primate Research Center, Beaverton, OR, United States.

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- T-202**      **Transfer Lift: Early Evaluation of the Optimal Day of Embryo Transfer by Causal Inference.**  
Yoav Kan Tor†, Zabari Nir†, Matan Gavish\*, Amnon Buxboim\*. *Hebrew University of Jerusalem, Jerusalem, Israel.*
- T-203**      **Novel Compound Heterozygous Variants in PATL2 Associate with Oocyte Meiotic Arrest.**  
Beatriz Rodríguez-Alonso†, Hakan Cakmak\*, Aleksandar Rajkovic\*. *UCSF, San Francisco, CA, United States.*
- T-204**      **Oh Boy! Exploring the Effect of Microfluidic Sperm Separation on Embryonic Sex.**  
Safina Usmani†, Caroline Peschansky†, Sarah Dynia†, Sonia Patel†, Jawaria Amir†, Royi Lynn†, Kayla Vitale†, Lauren Grimm†, Elizabeth Dulle†, Erica Louden\*, Roohi Jeelani\*, Angeline Beltsos\*. *Vios Fertility Institute, Chicago, IL, United States.*
- T-205**      **Identifying the Optimal, Multistep and Adaptive Embryo Transfer Strategy for Improving IVF Outcome.**  
Yoav Kan Tor†, Deborah Wolhandler†, Buxboim Amnon. *Hebrew University of Jerusalem, Jerusalem, Israel.*
- T-206**      **Does More Really Mean More? Comparing Pregnancy Rates and Number of Gestational Sacs Visible on Ultrasound Following Transfer of 1 vs. 2 PGT-Normal Embryos.**  
Anisa Hussain†, Abeer Sahlia\*, Lauren Grimm†, Jacqueline Sehring†, Jody Esguerra†, Angeline Beltsos\*, Roohi Jeelani\*. *Vios Fertility Institute, Chicago, IL, United States.*
- T-207**      **The Perfect Embryo: The Relationship between BMI and AMH and Resultant Embryo Quality in PCOS Patients.**  
Jawaria Amir\*,<sup>1</sup> Lauren Grimm†,<sup>2</sup> Caroline Peschansky†,<sup>2</sup> Sonai Patel†,<sup>2</sup> Sarah Dynia†,<sup>2</sup> Safina Usmani†,<sup>2</sup> Royi Lynn†,<sup>2</sup> Erica Louden\*,<sup>2</sup> Angeline Beltsos\*,<sup>2</sup> Roohi Jeelani\*.<sup>2</sup> *<sup>1</sup>Rush University, Chicago, IL, United States; <sup>2</sup>Vios Fertility Institute, Chicago, IL, United States.*
- T-208**      **Prevalence of Hirsutism and Polycystic Ovarian Syndrome (PCOS) in Latina/Latinx Females: Findings from the Environment, Leiomyomas, Latinas and Adiposity Study (ELLAS).**  
Amanda R Schwartz†,<sup>1</sup> Anne Waldo,<sup>1</sup> Amanda Manorot,<sup>1</sup> DeBlanc Jennie,<sup>1</sup> Maricella Castillo MacKenzie,<sup>1</sup> Samantha Schon,<sup>1</sup> Donna Baird,<sup>2</sup> Erica E Marsh\*.<sup>1</sup> *<sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States.*
- T-209**      **Towards the Molecular Understanding of PCOS Pathogenesis by RNA-seq Analysis of Multiple Tissues of Two Rat PCOS Models.**  
Qiong Lin, Joerg Mueller, Martin Fritsch, Ralf Lesche, Jorge Kageyama, Thomas M Zollner. *Pharma R&D, Bayer AG, Berlin, Germany.*
- T-210**      **Identification of Distinct Seminal Plasma Cytokine Profiles Associated with Male Age and Lifestyle Characteristics in Unexplained Recurrent Pregnancy Loss.**  
Nadia A. du Fossé†, Eileen E.L.O Lashley\*, Els van Beelen\*, Tess Meuleman\*, Saskia le Cessie\*, Jan M.M. van Lith\*, Michael Eikmans\*, Marie-Louise P van der Hoorn\*. *Leiden University Medical Center, Leiden, Netherlands.*
- T-211**      **Membrane Lipid Rich Freezing Medium Improves Pre-Pubertal Testicular Tissue Cryosurvival.**  
Guruprasad Kalthur\*, Reyon Dcunha, Ananda Hanumappa, Satish K Adiga. *Kasturba Medical College, Manipal, Manipal, India.*
- REPRODUCTIVE BIOLOGY**
- T-212**      **Elucidating the Role of SYCP2L in Oocyte Quality and Fecundity in Humans.**  
Caterina Clementi, Karen Hunter Cohn, Genevieve Galameau, Piraye Yurttas Beim\*. *Celmatix Inc., New York, NY, United States.*
- T-213**      **Does Culture Medium Used in IVF-Treatment Impact Post-Implantation Embryonic Growth and Developmental Trajectories with Sex-Specific Modification? The Rotterdam Periconception Cohort.**  
Linette van Duijn†, Régine PM Steegers-Theunissen\*, Esther B Baart\*, Sten P Willemsen\*, Joop SE Laven†, Melek Rousian\*. *Erasmus University Medical Centre, Rotterdam, Netherlands.*
- T-214**      **Using Serum Metabolomics to Identify Biomarkers of Viable Early, Intrauterine Pregnancy: An Untargeted <sup>1</sup>H NMR-Based Approach.**  
Christopher James Hill,<sup>1</sup> Marie Phelan,<sup>1</sup> Andrew Horne,<sup>2</sup> Kristina Gemzell-Danielsson,<sup>3</sup> Nicola Tempest,<sup>1</sup> Dharani Hapangama\*.<sup>1</sup> *<sup>1</sup>University of Liverpool, Liverpool, United Kingdom; <sup>2</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>3</sup>Karolinska Institutet, Stockholm, Sweden.*
- T-215**      **Endometrial Mitochondrial DNA Secreted in Extracellular Vesicles: A Novel Mechanism Modulating Maternal-Embryo Bioenergetics.**  
David Bolumar†,<sup>1</sup> Alicia Amadoz,<sup>1</sup> Inmaculada Moreno,<sup>1</sup> Carlos Marin,<sup>1</sup> Antonio Diez,<sup>1</sup> Jorge Jiménez-Almazán,<sup>1</sup> Carlos Simón,<sup>1,2</sup> Felipe Vilella.<sup>1</sup> *<sup>1</sup>Igenomix Foundation/INCLIVA, Paterna (Valencia), Spain; <sup>2</sup>POG Department, University of Valencia, Valencia, Spain.*
- T-216**      **High Cortisol Levels in Endometrium Impair Receptivity While Increased Estrone Levels Could Favor Pregnancy.**  
Almudena Devesa-Peiro†,<sup>1,2</sup> Diana Marti-García†,<sup>1</sup> Elena Labarta,<sup>3,1</sup> Marina Lopez-Nogueroles,<sup>4</sup> Patricia Sebastian-Leon,<sup>1</sup> Patricia Diaz-Gimeno\*.<sup>1</sup> *<sup>1</sup>IVI Foundation - Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain; <sup>2</sup>University of Valencia, Valencia, Spain; <sup>3</sup>IVI-RMA IVI Valencia, Valencia, Spain; <sup>4</sup>Analytical Unit Platform, Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain.*
- T-217**      **Mercury Disturb Reproductive Functions of Primary Endometrial Stromal Cells (ESC).**  
Roberto Gonzalez-Martin†,<sup>1</sup> Andrea Palomar†,<sup>2</sup> Silvia Pérez-Deben†,<sup>1</sup> Alicia Quiñero,<sup>1</sup> Francisco Domínguez\*.<sup>2</sup> *<sup>1</sup>IVI Foundation-RMA Global, Valencia, Spain; <sup>2</sup>IIS La Fe - IVI Foundation, Valencia, Spain.*
- T-218**      **Cabergoline Stimulates Human Endometrial Stromal Cell Decidualization and Reverses Inhibitory Effects of Interleukin-1 $\beta$  In Vitro.**  
Jie Yu,<sup>1</sup> Sarah L Berga,<sup>2</sup> Qingying Meng,<sup>3</sup> Mingjing Xia,<sup>4</sup> Trudy Kohout,<sup>3</sup> Marcel van Duin,<sup>3</sup> Robert N Taylor\*.<sup>2</sup> *<sup>1</sup>Wake Forest School of Medicine, Winston-Salem, NC, United States; <sup>2</sup>University at Buffalo, Buffalo, NY, United States; <sup>3</sup>Ferring Research Institute, San Diego, CA, United States; <sup>4</sup>Emory University School of Medicine, Atlanta, GA, United States.*
- T-219**      **Entosis Occurs in Human Embryo Implantation.**  
Andrea Palomar†,<sup>1</sup> Roberto Gonzalez-Martin†,<sup>2</sup> Stefania Salsano,<sup>2</sup> Silvia Pérez-Deben†,<sup>2</sup> Alicia Quiñero,<sup>2</sup> Francisco Domínguez\*.<sup>1</sup> *<sup>1</sup>IIS La Fe - IVI Foundation, Valencia, Spain; <sup>2</sup>IVI Foundation-RMA Global, Valencia, Spain.*
- T-220**      **In Vitro Decidualization and Molecular Characterization of Murine Endometrial Stromal Cells.**  
Jungwoo Kim,<sup>1</sup> Yoon Young Kim,<sup>1</sup> Yong Jin Kim,<sup>2</sup> Sung Woo Kim,<sup>1</sup> Hoon Kim,<sup>1</sup> Seung-Yup Ku.<sup>1</sup> *<sup>1</sup>Seoul National University Hospital, Seoul, Korea, Republic of; <sup>2</sup>Korea University Guro Hospital, Seoul, Korea, Republic of.*

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- T-221**      **Depletion of *Fkbp5* Gene in Mice Protects against Maternal Stress-Induced Age-Related Decline in Live Births.**  
Monica C Moore†, Xiofang Guo, Nihan Semerci, Asli Ozmen, Kellie Larson, Frederick Schatz, Umit Kayisli, Michael N. Teng, Charles Lockwood\*, Ozlem Guzeloglu-Kayisli\*. *USF Health Morsani College of Medicine, Tampa, FL, United States.*
- T-222**      **Single-Cell RNA Sequencing of Ovaries Reveals Transcriptional Networks Underlying Follicular Quiescence Regulated by Mullerian Inhibiting Substance.**  
Marie-Charlotte L Meinsohn†, Hatice Duygu Saatcioglu, Lihua Zhang, Maeva Chauvin, Nicholas Nagykeri, Esther Oliva, Patricia Donahoe, David Pépin. <sup>1</sup>*Massachusetts General Hospital - Harvard Medical School, Boston, MA, United States;* <sup>2</sup>*Massachusetts General Hospital, Boston, MA, United States.*
- T-223**      **Chronic Interferon Gamma Expression Drives Manifestation of Ovarian Dysfunction.**  
Enitome E Bafort†, Megan M Hess, Julio C Valencia, Loretta Smith, John Fenimore, Rebecca Erwin-Cohen, Michael Sanford, Bérénice A Benayoun\*, Howard A Young\*. <sup>1</sup>*National Cancer Institute, Frederick, MD, United States;* <sup>2</sup>*University of Southern California, Los Angeles, CA, United States.*
- T-224**      **Fetal Immune Development in Mice Is Promoted by Maternal Microchimeric Cells.**  
Christopher Urbschat†, Steven Schepanski, Maria E. Solano, Ina A. Stelzer, Nicole Fischer, Denise Ohnzeit, Victor Puelles, Kristin Thiele, Petra C. Arck\*. <sup>1</sup>*University Medical Center Hamburg-Eppendorf, Hamburg, Germany;* <sup>2</sup>*Stanford University School of Medicine, Stanford, CA, United States.*
- T-225**      **Uncomplicated Oocyte Donation Pregnancies Display Elevated CD163 Positive Type 2 Macrophage Load in the Decidua, Which Is Associated with Fetal-Maternal HLA Class II Mismatches.**  
Xuezi Tian†, Kaveri T.S. Aiyer†, Hanneke M. Kapsenberg\*, Dave L. Roelen\*, Marie-Louise van der Hoorn\*, Michael Eikmans\*. *Leiden University Medical Center, Leiden, Netherlands.*
- T-226**      **RNA Sequencing of the Fetal Inflammatory Response Syndrome Type I and Type II.**  
Robert Parat†, Roberto Romero\*, Derek Miller†, Jose Galaz†, Bogdan Done, Azam Peyvandipour, Meyer Gershater†, Li Tao†, Douglas Ruden\*, Jenna Isherwood, Roger Pique-Regi\*, Adi L Tarca\*, Nardhy Gomez-Lopez\*. <sup>1</sup>*Wayne State University SOM, Detroit, MI, United States;* <sup>2</sup>*Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, United States.*
- T-227**      **Recurrent Stillbirth Due to Chronic Histiocytic Intervillositis: Discovery of a Purified Maternal Alloantibody against Placental Protein.**  
Emily F Cornish†, Thomas McDonnell, David Williams\*. <sup>1</sup>*EGA Institute of Women's Health, University College London, London, United Kingdom;* <sup>2</sup>*Faculty of Engineering Science, University College London, London, United Kingdom.*
- T-228**      **Utilizing Primary HLA-G+ EVT and EVT-Like Cell Lines to Study Maternal Fetal Interactions.**  
 Sarika Kshirsagar, Tamara Hagen, Tamara Tilburgs\*. <sup>2,3</sup>*Harvard University, Cambridge, MA, United States;* <sup>2</sup>*Cincinnati Childrens Hospital, Cincinnati, OH, United States;* <sup>3</sup>*University of Cincinnati College of Medicine, Cincinnati, OH, United States.*
- T-229**      **Ozone Therapy and Pulsed Electro-Magnetic Field (PEMF) Could Improve Female Reproductive Potential.**  
Zaher Merhi\*, Subasinghe Ashini R Dias†, Daniella Emdin, Lisa Bosman, Andre Hugo Smith. <sup>3</sup>*SUNY Downstate Health Sciences University, Brooklyn, NY, United States;* <sup>2</sup>*Seton Hall University, South Orange, NJ, United States;* <sup>3</sup>*HOCATT, South Africa, South Africa.*
- T-230**      **Establishing Human Trophoblast Stem Cell Derived Organoids to Model Early Maternal-Fetal Interactions.**  
Jie Zhou, Yuchen Tian, Kylie J Dahlgren, Mark J Messler, Sehee Choi, Laura C Schulz, Toshihiko Ezashi, Bret D Ulery, R Michael Roberts, Danny J Schust. *University of Missouri, Columbia, MO, United States.*
- T-231**      **Extracellular Matrix Hydrogels from Decellularized Endometrium Promote Tissue Regeneration and Fertility Restoration in a Murine Model of Endometrial Damage.**  
Sara López-Martínez†, Adolfo Rodríguez-Eguren†, Lucía de Miguel-Gómez†, Amparo Faus, Emilio Francés-Herrero†, Antonio Pellicer\*, Hortensia Ferrero\*, Irene Cervelló\*. <sup>1</sup>*IVI Foundation - IIS La Fe, Valencia, Spain;* <sup>2</sup>*University of Valencia, Valencia, Spain;* <sup>3</sup>*IVIRMA, Roma, Italy.*
- T-232**      **Potential Molecules and Pathways Involved in Ovarian Rescue by Bone Marrow Derived Stem Cells in Human Ovarian Tissue.**  
Anna Buigues†, Maria Marchant†, Patricia Diaz-Gimeno, Jessica Martinez, Antonio Pellicer\*, Sonia Herraiz. <sup>1</sup>*IIS La Fe- IVI Foundation, Valencia, Spain;* <sup>2</sup>*University of Valencia - IVI Foundation, Valencia, Spain;* <sup>3</sup>*IVI-RMA Rome, Rome, Italy.*

### EPIDEMIOLOGY

- T-233**      **Stillbirth Rates Are Falling Faster Than Perceived.**  
Roshan Selvaratnam†, Daniel Rolnik\*, Mary-Ann Davey\*, Euan M Wallace\*. <sup>1,2</sup>*The Ritchie Centre, Monash University, Melbourne, Australia;* <sup>2</sup>*Safer Care Victoria, Melbourne, Australia.*
- T-234**      **Resilience, Coping Styles and Cognitive Appraisal Moderate Disaster Effects on Maternal Posttraumatic Stress: The Fort McMurray Wood Buffalo Wildfire Study.**  
Barbara Verstraeten†, Guillaume Elgbeili, Ashley Hyde†, Suzanne King\*, David Olson\*. <sup>1</sup>*University of Alberta, Edmonton, AB, Canada;* <sup>2</sup>*Douglas Mental Health University Institute, Montreal, QC, Canada;* <sup>3</sup>*McGill University, Montreal, QC, Canada.*
- T-235**      **Roles of Maternal and Fetal Vascular Pathology in a Case-Control Study of Autism.**  
Christine Chen, Jillamika Pongsachai, Jennifer S Feng†, Joan Krickellas, Sadia F Chowdhury†, Adwoa Nantwi†, Sylvia Dygulska, Hannah Bromberg, Ruchit Shah, Michael Joyce, Mehrin Jan, Serena Chen, Beata Dygulska, Carolyn Salafia\*. <sup>1,2,3</sup>*Placental Analytics, New Rochelle, NY, United States;* <sup>2</sup>*NYPBMH, Brooklyn, NY, United States;* <sup>3</sup>*CUNY Hunter College, New York, NY, United States;* <sup>4</sup>*NYU CGPH, New Rochelle, NY, United States;* <sup>5</sup>*Institute of Basic Research, Staten Island, NY, United States.*
- T-236**      **The Contribution of Social and Environmental Determinants of Health to Racial Differences in Preterm Birth Risk.**  
Julia J Brittain†, Shawn J Latendresse, Timothy P York\*, University of Richmond, Richmond, VA, United States; Baylor University, Waco, TX, United States; Virginia Commonwealth University, Richmond, VA, United States.

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### T-237 Exposure to Consumer Product Chemicals and Changes in Plasma Oxylipins in Pregnant Women.

Barrett M. Welch†, Alexander P Keil,<sup>2</sup> Paige A Bommarito,<sup>1</sup> Thomas J van t Erve,<sup>1</sup> Leesa J Deterding,<sup>1</sup> Jason G Williams,<sup>1</sup> David E Cantonwine,<sup>3</sup> Thomas F McElrath,<sup>3</sup> Kelly K Ferguson\*.<sup>1</sup> <sup>1</sup>NIH/NIEHS, Research Triangle Park, NC, United States; <sup>2</sup>UNC, Chapel Hill, NC, United States; <sup>3</sup>Harvard Medical School, Boston, MA, United States.

### T-238 Recent Trends in Gestational Diabetes Based on Maternal Age, Race/Ethnicity, and Pre-Pregnancy Weight Categories.

Darios Getahun,<sup>1</sup> Michael J Fassett,<sup>2</sup> Morgan R Peltier,<sup>3</sup> Chantal C Avila,<sup>4</sup> Xia Li,<sup>4</sup> Vicki Y Chiu,<sup>4</sup> David A Sacks.<sup>5</sup> <sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States; <sup>3</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>4</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States.

### T-239 Design and Methods of the Apple Women's Health Study: A Digital Longitudinal Cohort Study.

Shruthi Mahalingaiah,<sup>1</sup> Victoria Fruh,<sup>1</sup> Erika Rodriguez,<sup>1</sup> Sai Charan Konanki,<sup>1</sup> Jukka-Pekka Onnela,<sup>1</sup> Alexis de Figueiredo Veiga,<sup>1</sup> Anne Marie Z Jukic,<sup>2</sup> Kelly K Ferguson,<sup>2</sup> Donna D Baird,<sup>2</sup> Allen J Wilcox,<sup>2</sup> Curry L Christine,<sup>3</sup> Suharwardy Sanaa,<sup>3</sup> Fischer-Colbrie Tyler,<sup>3</sup> Agrawal Gracee,<sup>3</sup> Brent A Coull\*,<sup>1</sup> Russ B. Hauser\*,<sup>1</sup> Michelle A Williams\*.<sup>1</sup> <sup>1</sup>Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>2</sup>National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States; <sup>3</sup>Apple Inc., Cupertino, CA, United States.

### T-240 Does Fetal Placental Weight Ratio Impact Childhood Growth of Term Newborns in a Low-Risk Community Based Setting?

Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>5</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongsachai,<sup>1,2</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*.<sup>1,2,6</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New Rochelle, NY, United States; <sup>5</sup>Institute of Basic Research, Staten Island, NY, United States; <sup>6</sup>Institute for Basic Research, Staten Island, NY, United States.

### T-241 Early Childhood Growth in Autism Spectrum Disorders: A Case Control Study.

Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongsachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>5,1</sup> Michael Joyce,<sup>1</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*.<sup>1,2,5</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute for Basic Research, Staten Island, NY, United States.

## POPULATION HEALTH

### T-242 Periconceptional Maternal Obesity and Underweight Have a Negative Impact on Post-Implantation Embryonic Growth and Developmental Trajectories: The Rotterdam Periconception Cohort.

Linette van Duijn†, Melek Rousian\*, Joop SE Laven\*, Régine PM Steegers-Theunissen\*. *Erasmus University Medical Centre, Rotterdam, Netherlands.*

### T-243 Knowledge May Not Be the Barrier to Care for Pregnant Women with Opioid Use Disorder: The ACOG District II Education Bundle.

Neil Seligman,<sup>1</sup> Kathleen Dermady,<sup>2</sup> David Garry,<sup>3</sup> Leah Kaufman,<sup>2</sup> Darcy Dreyer,<sup>4</sup> Marilyn Kacica,<sup>5</sup> Cassie Leonard,<sup>6</sup> Kelly Gilchrist,<sup>7</sup> Christa Christakis.<sup>7</sup> <sup>1</sup>Univ. of Rochester, Rochester, NY, United States; <sup>2</sup>Upstate Medical Center, Syracuse, NY, United States; <sup>3</sup>Stony Brook Medicine, Stony Brook, NY, United States; <sup>4</sup>March of Dimes, Rochester, NY, United States; <sup>5</sup>NYS DOH, Albany, NY, United States; <sup>6</sup>Hudson Headwaters Health Network, Queensbury, NY, United States; <sup>7</sup>ACOG District II, Albany, NY, United States.

### T-244 Trends in Emergency Department Visits among Reproductive Age Women in the United States, 2006-2018.

Marissa S Weiss,<sup>1</sup> Li Jiang,<sup>1</sup> Courtney Townsel,<sup>1</sup> Martina T Caldwell,<sup>2</sup> Dee Fenner,<sup>1</sup> Erica E Marsh\*.<sup>1</sup> <sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>Henry Ford Health System, Detroit, MI, United States.

## WOMEN'S HEALTH DISPARITIES AND INEQUITIES

### T-245 Comparison of Ovarian Aging Markers Does Not Reveal Differences between Black and White Women.

Hannah Anvari†, Kara Goldman\*, Mary Ellen G. Pavone\*, Jian-Jun Wei\*, Melissa Simon\*, Francesca Duncan\*. *Northwestern University, Chicago, IL, United States.*

### T-246 Characterization of Human Uterine Leiomyoma-Derived Exosomes and Its Impact on Endometrium.

Antonia Navarro, Maria Victoria Bariani†, Hang-Soo Park†, Ayman Al-Hendy\*. *University of Chicago, Chicago, IL, United States.*

### T-247 Higher Prevalence of SGA in Highly Vulnerable Women the Mothers of Rotterdam Study.

Kajal SC Mohabier, Hanneke JP de Graaf, Eric AP Steegers, Loes CM Bertens. *Erasmus University Medical Center, Rotterdam, Netherlands.*

## COVID-19

### T-248 Association between SARS-CoV-2 Infection and Adverse Pre- and Postnatal Outcomes by Severity of Illness.

Michael J Fassett\*,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiachao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Darios Getahun.<sup>7</sup> <sup>1</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente West Los Angeles Medical Center; Keck School of Medicine, Los Angeles, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.

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- T-249 Association between SARS-CoV-2 Infection and Adverse Pre- and Postnatal Outcomes by the Trimester of Diagnosis.**  
Darios Getahun,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> Sacks A Sacks,<sup>4</sup> Alex Fong,<sup>5</sup> Neha Trivedi,<sup>6</sup> Jiaxiao Shi,<sup>7</sup> Vicki Y Chiu,<sup>7</sup> Morgan R Peltier,<sup>8</sup> Michael J Fassett.<sup>9</sup>  
<sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Irvine Medical Center, Irvine, CA, United States; <sup>6</sup>Kaiser Permanente San Diego Medical Center, San Diego, CA, United States; <sup>7</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>8</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>9</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.
- T-250 Increased Fetal Demise/Stillbirth within a USA COVID-19 Epicenter.**  
Ryan Zahn†,<sup>1</sup> Hadeer Eltahan†,<sup>1</sup> Debra Heller\*,<sup>2</sup> Nichole Cerone†,<sup>1</sup> Themba Nyirenda\*,<sup>1</sup> Judy Urgo\*,<sup>1</sup> Emre Kayaalp\*,<sup>1</sup> Manuel Alvarez\*,<sup>1</sup> Stacy Zamudio\*,<sup>1</sup> Abdulla Al-Khan\*.<sup>1</sup> <sup>1</sup>Hackensack University MC, Hackensack, NJ, United States; <sup>2</sup>Rutgers University, Newark, NJ, United States.
- T-251 SARS-CoV-2 Colonization of Maternal and Fetal Cells of the Human Placenta Promotes Alteration of Local Renin-Angiotensin System.**  
Sonam Verma†, Ebony B Carter, Indira U Mysorekar\*. Washington University SOM, St. Louis, MO, United States.
- T-252 COVID-19 in Pregnancy: Placental Histopathology Demonstrates Evidence of Acute Infection.**  
Courtney Olson-Chen\*, Ponnilla Marinescu†, Stefanie Hollenbach, Eva K Pressman, Philip Katzman. University of Rochester, Rochester, NY, United States.
- T-253 Characteristics, Risk Factors, and Outcomes for Pregnant and Postpartum Patients with COVID-19 Disease.**  
Felicia LeMoine†,<sup>1</sup> Kaitlyn Taylor†,<sup>1</sup> Elizabeth Sutton\*,<sup>2</sup>  
<sup>1</sup>LSUSOM OBGYN Residency, Baton Rouge, LA, United States; <sup>2</sup>Woman's Hospital, Baton Rouge, LA, United States.
- T-254 Placental Abnormalities in COVID-19. Maryland Study Group Report on COVID-19.**  
Liviu Cojocaru†,<sup>1</sup> Irina Burd\*,<sup>2</sup> Autusa Pahlavan†,<sup>1</sup> Ramya Reddy†,<sup>2</sup> Ozhan M Turan\*,<sup>1</sup> Katelyn Uribe†,<sup>2</sup> Meghna Ramaswamy†,<sup>1</sup> Sifa Turan\*.<sup>1</sup> <sup>1</sup>University of Maryland School of Medicine, Baltimore, MD, United States; <sup>2</sup>Johns Hopkins University, Baltimore, MD, United States.
- T-255 Single-Cell RNA Sequencing of SARS-CoV-2 Cell Entry Factors in the Preconceptional Human Endometrium.**  
Felipe Vilella\*,<sup>1</sup> Wanxin Wang,<sup>2</sup> Inmaculada Moreno,<sup>3</sup> Beatriz Roson,<sup>1</sup> Steve R Quake,<sup>4</sup> Carlos Simon.<sup>1</sup>  
<sup>1</sup>Igenomix Foundation INCLIVA, Paterna (Valencia), Spain; <sup>2</sup>Stanford University, Stanford, CA, United States; <sup>3</sup>Igenomix Foundation, Paterna (Valencia), Spain; <sup>4</sup>Stanford University, Stanford, CA, United States.
- T-256 Stress Decreases Host Viral Resistance and Increases Covid Susceptibility in Embryonic Stem Cells.**  
Daniel A Rappolee\*,<sup>1</sup> Mohammed Abdulhasan\*,<sup>1</sup> Ximena Ruden\*,<sup>1</sup> Benjamin Rappolee\*,<sup>2</sup> Sudipta Dutta\*,<sup>3</sup> Katherine Gurdziel†,<sup>1</sup> Douglas M Ruden\*,<sup>1</sup> Awoniyi O Awonuga\*,<sup>1</sup> Steven Korzeniewski\*,<sup>1</sup> Elizabeth E Puscheck\*.<sup>1</sup> <sup>1</sup>Wayne State University, Detroit, MI, United States; <sup>2</sup>Reproductive Stress 3M Inc, Grosse Pointe Farms, MI, United States; <sup>3</sup>Texas A&M University, Detroit, TX, United States.

# Abstracts

**Figures will be available only online**

*Underline represents presenting author; Asterisk represents senior author; Dagger represents an in-training author.*



O-001

**Simvastatin Inhibits Estrogen Signaling by Modulating Receptor Trafficking in Leiomyoma Cells.** *Sadia Afrin†, Malak El Sabeh†, Mostafa Borahay\*. Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Uterine fibroids or leiomyomas are the most common benign tumors in the female reproductive tract. Estrogen (E2), a steroid-derived hormone, and its receptors (ER), ER $\alpha$  and ER $\beta$ , are known to be key modulators in the development of leiomyomas by their upregulation of growth factors and activation of signaling pathways. Multiple in vitro and in vivo studies have shown the beneficial effect of simvastatin, a hypolipidemic drug, on uterine leiomyomas. Here, we hypothesized that simvastatin would modulate estrogen-dependent proliferation, downstream signaling, transcriptional activity as well as ER $\alpha$  trafficking. **Methods:** The primary and immortalized leiomyoma cells were treated with simvastatin (0.001, 0.01, 0.1, and 1  $\mu$ M) and E2 (10 nM), alone or in combination, for 48 h to examine cell viability by MTT assay, protein levels by western blotting, and cellular intensity by cytoimmunofluorescence. Estrogen response elements (ERE)-Luc activity was examined by luciferase assay. Palmitoylation and ubiquitination assay was performed by resin assisted capture and pull down assay. For the leiomyoma xenograft mouse model, one group was treated with the vehicle control (n = 10) and another group was treated with simvastatin (n = 10, 20  $\mu$ g/gm body weight) subcutaneously for 28 days, and ER expression was assessed using immunohistochemistry (IHC). Human leiomyoma tissue samples were obtained from a double-blind, phase II, randomized control trial (NCT03400826) with simvastatin (40 mg daily) or a placebo (starch 1500 encapsulated) for a total of 12 weeks. At the end of the treatment, the samples were collected after surgery and IHC staining was performed.

**Results:** Simvastatin treatment significantly decreased E2-induced cell proliferation and PCNA expression in leiomyoma cells. Simvastatin altered receptor trafficking by decreasing the localization of ER- $\alpha$  to the membrane and nucleus. The downstream targets of E2-ER signaling, ERK1/2 and AKT expression, and ERE-Luc activity were suppressed after simvastatin treatment. Notably, simvastatin inhibited ER- $\alpha$  palmitoylation, which links ER- $\alpha$  with the membrane and initiates ER signaling. Simvastatin increased ER- $\alpha$  degradation via ER- $\alpha$  ubiquitination. Finally, simvastatin treatment reduced ER- $\alpha$  expression in clinical fibroid tissue and xenograft animal model.

**Conclusion:** The results of experiments on leiomyoma cells, animal models, and clinical tissue validated the effectiveness of simvastatin to treat uterine leiomyoma growth. Palmitoylation and ubiquitination modulate ER- $\alpha$  stability and trafficking, and simvastatin's effect on ER- $\alpha$  through blocking palmitoylation and enhancing its ubiquitination, results in ER- $\alpha$  degradation, which highlights the potential of altering these post-translational modifications as a strategy for therapeutic applications. *Supported by NIH grant 1R01HD094380*

O-002

**Genetically-Induced Placental Endocrine Malfunction Alters the Maternal Liver and Whole Body Metabolic Function in Pregnancy.** *J Lopez-Tello†, E Salazar, T Napso, HEJ Yong, ER Christoforou, I Sandovici, M Constancia, Amanda N Sferruzzi-Perri\*. University of Cambridge, Cambridge, United Kingdom.*

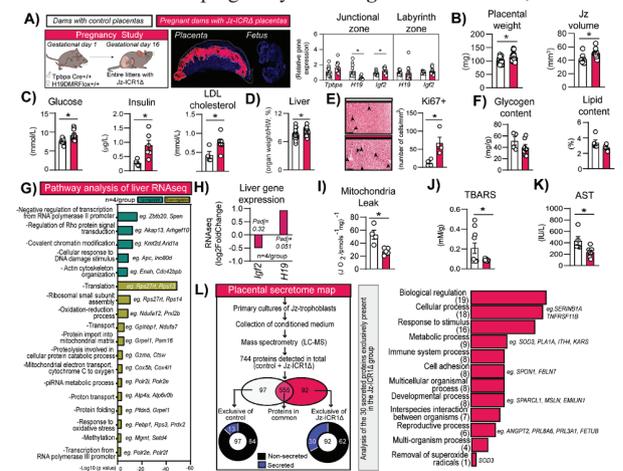
**Introduction:** The placenta is an active organ that secretes hormones into the maternal circulation. In turn, the maternal liver is involved in regulating whole body glucose-lipid metabolism. However, the impact of placental hormone output on maternal hepatic function during gestation has yet to be explored. Previous work has shown that the *Igf2-H19* locus is involved in controlling placental endocrine function in mice (Aykroyd et al., 2020). This study used manipulations of the *Igf2-H19* locus to induce placental endocrine malfunction and examine its consequences for hepatic metabolic function in pregnancy.

**Methods:** Mice were crossed to produce entire litters with reduced levels of the *H19* gene and activation of the normally silent maternal *Igf2* gene in the placental endocrine junctional zone (Jz-ICR1 $\Delta$ ; **A**). On day 16 of gestation, maternal blood was collected for metabolite analysis, maternal

liver for histology, biochemical, respirometry and RNAseq analyses, and placentas taken for qPCR, histology or for endocrine cell culture followed by LC-MS on the conditioned media.

**Results:** Jz-ICR1 $\Delta$  dams had litters with heavier placentas due to enlarged junctional zones (**B**). Additionally, Jz-ICR1 $\Delta$  dams had elevated circulating glucose, insulin and LDL-cholesterol levels (**C**) and heavier livers with increased hepatocyte proliferation (**D-E**). There was no change in hepatic glycogen or fat content in Jz-ICR1 $\Delta$  dams (**F**). However, genes involved in translation, mitochondrial homeostasis and response to oxidative stress, among others were affected in the maternal liver by Jz-ICR1 $\Delta$  (**G**). No differences were observed in the expression of *Igf2-H19* genes in the maternal liver (**H**). Mitochondrial proton leak and lipid peroxidation (TBARS) were reduced in the liver of Jz-ICR1 $\Delta$  dams (**I-J**). Circulating AST, which indicates liver damage, was lower in dams with Jz-ICR1 $\Delta$  (**K**). A total of 744 proteins were identified in the media from placental endocrine cell cultures, of which 92 were exclusively detected in the Jz-ICR1 $\Delta$  group with 30 previously identified to be secreted (**L**). Gene ontology showed that these secreted hormones from the Jz-ICR1 $\Delta$  group are involved in a variety of biological processes (**L**), including reproduction, metabolism and removal of superoxide radicals.

**Conclusion:** Altered production of placental hormones due to conditional *Igf2-H19* locus manipulation alters maternal hepatic and whole body metabolic function in pregnancy. **Funding:** Wellcome Trust, MRC.



**Figure 1.** (A) Experimental design. Ttpoa-Cre is active exclusively in the endocrine cells of the mouse placenta, and qPCR levels for *H19-Igf2* manipulation in separated placental zones. (B) The impact of Jz-ICR1 $\Delta$  on placental phenotype. (C) Circulating metabolites in maternal plasma. (D-F) Hepatic phenotype in response to Jz-ICR1 $\Delta$ . Glycogen content was determined by amyloglucosidase assay. Lipid content was done using the Folch assay. (G) RNA-seq of maternal liver in response to Jz-ICR1 $\Delta$  deletion. (H) Fold change levels of *Igf2* and *H19* genes obtained in liver RNAseq (m4group). (I) Mitochondrial respirometry (J) Levels of Thiobarbituric acid reactive substances (TBARS) in liver. (K) Circulating levels of Aspartate aminotransferase (AST) in maternal circulation. (L) Number of proteins detected by LC-MS in mouse Jz-ICR1 $\Delta$  cells (Venn diagram). Uniprot was used for biological processes. Uniprot analysis only evaluated the 30 Jz-ICR1 $\Delta$  proteins with established secreted capacity. Data analysed by t-test and shown as mean and standard error of the mean (\*p<0.05, q<0.05 RNAseq).

O-003

**Low-Dose of IL-2 Normalizes Hypertension and Mitochondrial Function in Response to Placental Ischemia.** *Evangelina Deer†, Lorena Amaral, Nathan Campbell, Sarah Fitzgerald, Owen Herroek, Tarek Ibrahim, Babbette LaMarca\*. University of Mississippi Medical Center, Jackson, MS, United States.*

**Introduction:** IL-2 is a cytokine released from CD4+T cells that regulates many inflammatory responses depending on its circulating concentration. IL-2 potentiates anti-inflammatory actions in order to quell a chronic inflammatory response. IL-2 is elevated in many chronic inflammatory conditions and has been shown to be increased during preeclampsia (PE), and in Reduced Uterine Perfusion Pressure (RUPP) rats, a rat model of PE. PE is characterized by new onset hypertension during pregnancy and increasing evidence indicates that proinflammatory cytokines interact with blood pressure regulatory systems to cause hypertension in various animal models of PE. Our most recent studies indicate inflammatory mediators cause mitochondrial (mt) dysfunction which contributes to hypertension in response to placental ischemia. The objective of the study was to determine the effects of Low Dose IL-2 on blood pressure and mt function in the RUPP rat model of PE.

**Methods:** We infused IL-2 (0.05 ng/ml) into RUPP rats on GD14 and examined hypertension and renal and placental and endothelial cell mt

function compared to control RUPP. On GD19, mean arterial pressure (MAP) was analyzed and fetal, and placental weights were measured and blood, placentas, and kidneys were collected for analysis of mitochondrial function.

**Results:** Mean arterial pressure was elevated  $122 \pm 5$  mmHg in RUPP rats ( $n=6$ ,  $p<0.05$ ) versus NP controls ( $102 \pm 3$  mmHg,  $n=5$ ), but was normalized with administration of RUPP + IL-2 ( $107 \pm 1$  mmHg,  $n=9$ ,  $p<0.05$ ) when compared to control RUPPs. Body, placental, and fetal weights were reduced in both RUPP and RUPP + IL-2 compared to NP controls. Placental mtROS, as measured by production of  $H_2O_2$ , was significantly elevated in RUPP rats ( $144.6 \pm 14.18$  % gated,  $n=5$ ,  $p<0.05$ ) compared to NP rats ( $100 \pm 12.34$  % gated,  $n=5$ ), but was reduced in RUPP + IL-2 rats ( $108.7 \pm 7.38$  % gated,  $n=9$ ,  $p<0.05$ ). Renal mtROS was elevated in RUPP rats ( $127.1 \pm 2.81$  % gated,  $n=5$ ,  $p<0.05$ ) compared to NP rats ( $100 \pm 5$  % gated,  $n=5$ ), but was improved with administration of RUPP + IL-2 ( $63.26 \pm 3.57$  % gated,  $n=9$ ,  $p<0.05$ ). In HUVECS, mtROS was significantly elevated in HUVECS treated with RUPP sera ( $6.38 \pm 1.81$  % gated,  $n=4$ ,  $p<0.05$ ) compared to NP control sera ( $1.86 \pm 0.6$  % gated,  $n=5$ ), but endothelial cell mtROS was attenuated in HUVECS treated with RUPP + IL-2 rat sera ( $2.69 \pm 0.53$  % gated,  $n=7$ ,  $p<0.05$ ).

**Conclusion:** These data indicate that Low Dose IL-2 normalized multi-organ mt function and hypertension in response to placental ischemia during pregnancy. Overall, our study indicates a role for the administration of IL-2 to improve mitochondrial dysfunction in the placenta and decrease blood pressure, which indicates its use as a potential therapeutic for PE.

#### O-004

**DHES0815A, a Novel Antibody-Drug Conjugate Targeting HER2/neu, Is Highly Active Against Uterine Serous Carcinomas *In Vitro* and *In Vivo*.** Joan Rose Tymon-Rosario<sup>†</sup>, Elena Bonazzoli, Bellone Stefania, Aranzazu Manzano, Silvia Pelligra, Adele Guglielmi, Barbara Gnutti, Burak Zeybek, Paola Manara, Luca Zammataro, Justin Harold, Dennis Mauricio, Natalia Buza, Pei Hui, Gary Altwerger, Gulden Menderes, Ratner Elena, Mitchell Clark, Vaagn Andikyan, Gloria Huang, Masoud Azodi, Peter E Schwartz, Alessandro D Santin\*. *Yale, New Haven, CT, United States.*

**Introduction:** Uterine serous carcinoma (USC) is an aggressive histologic variant of endometrial cancer which portends a poor prognosis. DHES0815A is a novel antibody-drug-conjugate (ADC) which binds specifically to HER2 overexpressing tumors at a distinct epitope from that bound by trastuzumab and pertuzumab after which it delivers the toxic payload, PBD-MA, a DNA mono-alkylating agent. The objective of this study was to evaluate the preclinical activity of DHES0815A against primary USC cell lines and xenografts.

**Methods:** Twelve primary USC cell lines were assessed by immunohistochemistry (IHC) for HER2 protein expression and for *C-erbB2* gene amplification using fluorescent in situ hybridization (FISH) analysis. Cell viability and bystander killing in USC cell lines after exposure to DHES0815A, the non-targeted ADC, and the unconjugated antibody (i.e. MHES0488A) were evaluated using flow cytometry-based assays. *In vivo* activity of DHES0815A was tested against HER2/neu overexpressing USC xenografts.

**Results:** High HER2/neu protein expression was seen in 25% (3/12) of the primary USC cell lines. USC cell lines overexpressing HER2/neu were significantly more sensitive to DHES0815A when compared to the non-targeted control ADC ( $p<0.001$ ). DHES0815A did not induce significant bystander killing of HER2/neu negative tumors when admixed with HER2/neu positive tumors. DHES0815A caused growth-inhibition and increased survival in USC HER2/neu overexpressing xenografts when compared to controls ( $p<0.01$ ).

**Conclusion:** DHES0815A is both highly selective and toxic to USC tumors overexpressing HER2/neu both *in vitro* and *in vivo*. HER2-directed ADCs, alone or in combination with other HER2/neu targeted agents may represent a novel treatment option for patients with tumors harboring HER2/neu overexpression refractory to trastuzumab and traditional chemotherapy.

#### O-005

**Molecular Mechanisms Underlying Selective Sorting of miRNAs into Small Extracellular Vesicles in Placental Cells in Gestational Diabetes Mellitus (GDM).** Soumyalekshmi Nair<sup>†</sup>,<sup>1</sup> Andrew Lai,<sup>1</sup> Nanthini Jayabalan,<sup>1</sup> Dominic Guanzon,<sup>1</sup> Katherin Scholz-Romero,<sup>1</sup> David McIntyre,<sup>2</sup> Martha Lappas,<sup>3,4</sup> Carlos Salomon.<sup>1,5</sup> <sup>1</sup>University of Queensland Centre for Clinical Research, Brisbane, Australia; <sup>2</sup>University of Queensland, Mater Health, South Brisbane, Australia; <sup>3</sup>University of Melbourne, Melbourne, Australia; <sup>4</sup>Mercy Hospital for Women, Victoria, Australia; <sup>5</sup>University of Concepcion, Concepción, Chile.

**Introduction:** Gestational Diabetes Mellitus (GDM) is the glucose intolerance that develops during pregnancy, and has short and long-term effects on maternal and fetal health. Extracellular vesicles (EVs)-associated miRNAs are key regulators of gene expression in target cells. The aim of the present study is to identify the mechanism by which miRNAs are selectively packaged into EVs from placental cells, in normal pregnancy and GDM.

**Methods:** Small EVs like exosomes were isolated from the cell-conditioned media of Primary Human Trophoblast (PHT) cultures from normal glucose tolerant (NGT), and GDM patients. The miRNA and protein profile in PHT cells and EVs were analysed using next generation sequencing and high throughput mass spectrometry, respectively. The over-represented sequence motifs in the miRNAs were identified using the Motif Discovery on Short Sequence (MDS2) tool. To identify proteins associated with EVs miRNA sorting, trophoblast cellular extracts were incubated with biotin beads coated with candidate miRNAs, and pulled-down proteins were analysed by liquid chromatography mass spectrometry.

**Results:** We identified a specific set of proteins and miRNAs that were differentially expressed in PHT cells in GDM compared to NGT. In EVs, miR-10a-5p was upregulated whereas miR-574-5p and miR-181-5p were downregulated in GDM compared to NGT ( $p$  value  $\leq 0.05$ ). A specific set of miRNAs were highly enriched in EVs compared to their cells of origin in NGT and GDM. Among these, 29 miRNAs were unique to NGT; 17 miRNAs were unique to GDM; and 26 miRNAs were upregulated in EVs in both NGT and GDM. We identified unique miRNA motifs in these three groups based on their coverage and  $p$  value. Candidate miRNAs were chosen for each group (miR-150-5p and miR-1246 for NGT; miR-1285-5p for GDM; and miR-486-5p for shared), based on their abundance and enrichment in EVs. Finally, we identified a repertoire of unique proteins that were interacting with miRNAs within each group. Based on the peptide count and coverage, the top highly represented proteins were Nucleolin, Neuroblast differentiation-associated protein, Heterogeneous nuclear ribonucleoprotein, and RNA helicases.

**Conclusion:** These findings provide insights into the differential mechanisms through which miRNAs are specifically sorted into EVs in normal pregnancy and GDM

#### O-006

**Associations of Dietary Glycemic Index and Load during Pregnancy with Blood Pressure, Placental Hemodynamic Parameters and the Risk of Gestational Hypertensive Disorders.** Clarissa J. Wiertsema<sup>†</sup>, Rama J. Wahab, Annemarie G.M.G.J. Mulders, Romy Gaillard. *Erasmus MC, Rotterdam, Netherlands.*

**Introduction:** In non-pregnant populations, adherence to diets with a low-glycemic index and low-glycemic load seems to lower blood pressure. We hypothesized that maternal adherence to a lower dietary glycemic index and load during pregnancy improves gestational hemodynamic adaptations and reduces the risk of gestational hypertensive disorders. We examined the associations of dietary glycemic index and load with maternal blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders.

**Methods:** In a population-based cohort among 3378 pregnant Dutch women, dietary glycemic index and load were assessed from food frequency questionnaires at median 13.4 (95% range 9.9-22.9) weeks gestation. Blood pressure was measured in early, mid and late pregnancy.

Placental hemodynamic parameters were measured in mid and late pregnancy by ultrasound. Data on gestational hypertensive disorders was acquired from medical records.

**Results:** Mean dietary glycemic index (SD) was 58 (3) and mean dietary glycemic load (SD) was 155 (47). Dietary glycemic index was not associated with blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders. Higher dietary glycemic load SDS was associated with a higher diastolic blood pressure in early-pregnancy, remaining after adjustment for socio-demographic and lifestyle factors ((0.98 (95% CI 0.35-1.61) mmHg per SDS increase in glycemic load). No other associations of glycemic load with blood pressure or placental hemodynamic parameters and the risk of gestational hypertensive disorders were present.

**Conclusion:** Within this low-risk pregnant population, we did not find consistent associations of dietary glycemic index and load with blood pressure, placental hemodynamic parameters and the risk of gestational hypertensive disorders. Further studies need to assess whether the effects on gestational hemodynamic adaptations are more pronounced among high-risk women with an impaired glucose metabolism.

### O-007

**Metabolomic Signatures of Low and High Adiposity Neonates Differ Based on Maternal BMI.** Begum Aydogan Mathyk†,<sup>1</sup> Brian Piccolo,<sup>2</sup> Kartik Shankar,<sup>3</sup> Perrie O'Tierney-Ginn\*,<sup>4</sup> <sup>1</sup>Brandon Regional Hospital, Brandon, FL, United States; <sup>2</sup>USDA-ARS Arkansas Children's Nutrition Center, Little Rock, AR, United States; <sup>3</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>4</sup>Tufts Medical Center, Boston, MA, United States.

**Introduction:** Maternal obesity (body mass index, BMI>30kg/m<sup>2</sup>) is associated with high infant adiposity, cord blood (CB) insulin levels, and a pro-inflammatory phenotype at birth, all of which contribute to risk of future cardiometabolic disease in the offspring. Variation in neonatal adiposity within maternal BMI groups is underappreciated, and it remains unclear whether the metabolic impairments at birth are an outcome of maternal obesity or excess fetal fat accrual. Using untargeted metabolomics on CB samples stratified by maternal BMI and neonatal adiposity, we examined the hypothesis that CB metabolites associated with fetal fat accrual differ between offspring of normal weight and obese women.

**Methods:** Umbilical venous blood was collected at the time of scheduled cesarean delivery from 50 normal weight (LE; pre-gravid BMI=22.3±1.7 kg/m<sup>2</sup>), and 50 obese (OB; BMI=34.5±3.0 kg/m<sup>2</sup>) women. Neonatal adiposity was estimated from flank skinfold thickness. The first (low adiposity, LA) and third (high adiposity, HA) tertiles of neonatal %body fat (BF) were used to create four groups: LE women with low (LELA, 8.5±1.5 %BF) and high (LEHA, 15.5±1.8 %BF) adiposity neonates; OB women with low (OBLA, 8.6±2.0 %BF) and high (OBHA, 16.1±1.2 %BF) adiposity neonates. CB metabolites were measured via GC/MS, group differences were analyzed by Univariate analysis, PERMANOVA and Enrichment Analysis.

**Results:** OBLA metabolomic profile was different from other groups (OBLA vs LELA, *FDR*=0.027 and OBLA vs OBHA, *FDR*=0.027 via pairwise PERMANOVA). Lauric acid (C12:0) was 82-118% higher in OBLA vs all other groups (*Adj p* <0.0001 via Kruskal-Wallis). Several other fatty acids were higher in OBLA vs OBHA group (stearic acid, linoleic acid, azelaic acid, *FDR*=0.03; palmitic acid, arachidic acid, *FDR*=0.04). 'β-Oxidation of Very Long Chain Fatty Acids', 'Mitochondrial β-Oxidation of Medium-Chain Saturated Fatty Acids' and 'Fatty Acid Biosynthesis' were the top three enriched pathways between OBHA and OBLA groups (*all FDR*<0.01). 'Valine, Leucine and Isoleucine Degradation' (branched-chain amino acids) was the top enriched pathway between LEHA and LELA groups (*FDR*=0.01).

**Conclusion:** CB metabolites associated with neonatal adiposity differed between offspring of normal weight and obese women. Several fatty acids were higher in LA neonates born to OB women. Notably, lauric acid, a medium chain FA, which has been associated with improved insulin sensitivity. Differences in such metabolically active lipids at birth may

have long-term consequences for offspring metabolism. We speculate that variations in placental lipid metabolism and delivery to fetus may mediate these differences in cord FA.

### O-008

**Adult Offspring of Obese Mice Have Reduced Resistance Artery Vasodilator Responses.** Ramón A Lorca\*, Julie A Houck, Jerad H Dumolt, Owen R Vaughan, Kelsey Barner, Theresa L Powell, Thomas Jansson, Lorna G Moore, Colleen G Julian. *University of Colorado Anschutz Medical Campus, Aurora, CO, United States.*

**Introduction:** Maternal obesity increases the risk of cardiovascular and metabolic disease in the offspring both during childhood and adult life. As compared to controls with normal body mass index, pregnant women and mice that are obese have lower circulating levels of adiponectin (ADN), an adipokine involved in regulating energy metabolism, vascular function and placental function. We hypothesized that offspring of obese mice have impaired resistance artery function, which is prevented by restoration of normal circulating ADN levels in obese dams during late pregnancy.

**Methods:** Adult female mice were fed with either control (CON) or obesogenic (OB) diet until the OB group gained 25% of their initial body weight and mated with CON males. A mini-osmotic pump was subcutaneously implanted in all pregnant dams at late pregnancy (embryonic day 14.5). CON dams received a continuous infusion of phosphate saline buffer (PBS) whereas OB females received either PBS or ADN (0.62 μg g<sup>-1</sup> day<sup>-1</sup>). Dams were maintained on their respective diets during pregnancy and throughout lactation. After weaning, offspring were fed CON diet. Between seven and nine months of age, adult offspring's resistance (mesenteric) and pulmonary arteries were dissected and mounted in a wire myograph. Contractile and dilator responses to phenylephrine or endothelin-1 and acetylcholine (ACh), bradykinin (BK) or an AMPK activator (A769662), respectively, were determined in the offspring of CON-PBS (n = 7), OB-PBS (n = 9) and OB-ADN (n = 5) dams. Concentration-response curves were compared by two-way ANOVA.

**Results:** Mesenteric (MsA) and pulmonary artery (PA) vasoconstrictor responses to phenylephrine and endothelin-1 were similar among all groups. Likewise, MsA vasodilator responses to BK and A769662 were comparable among groups but offspring from OB-PBS and OB-ADN dams had 35% and 25% less ACh-evoked vasodilation (*p*<0.05 and *p*=0.07), respectively, compared to MsA from CON-PBS animals. PA vasodilator responses to ACh were not affected by any treatment. BK and A769662 did not evoke vasodilation in PA from any group.

**Conclusion:** In mice, maternal obesity has a long-lasting impact on the cholinergic response of resistance vessels of the offspring. However, restoration of maternal ADN during pregnancy did not restore cholinergic responses in these vessels, suggesting they are independent of reduced ADN levels in pregnancy.

### O-009

**The Impact of Pre-Pregnancy Maternal Lipid Metabolism on Neonatal Adiposity.** Raziq Rojas-Rodriguez†, Patrick M Catalano\*. *Tufts Medical Center, Boston, MA, United States.*

**Introduction:** Changes in maternal glucose metabolism related to neonatal adiposity have been well-characterized. However, there are less data regarding the changes in maternal lipid metabolism and neonatal fat mass (FM). Since pregravid maternal BMI has as strong association with neonatal adiposity independent of gestational weight gain (GWG), it is necessary to examine maternal lipid metabolism longitudinally, beginning prior to conception. **Aim:** To investigate the longitudinal changes in maternal lipid metabolism in low (LA) and high (HA) adiposity women and their relationship with neonatal adiposity.

**Methods:** Thirteen women were evaluated pregravid (P), 12-14 (E), and 34-36 weeks (L) gestation. Body composition was estimated utilizing hydrodensitometry. There were 6 LA and 7 HA subjects, based on P body fat percent (BF%). Basal endogenous glucose production and glycerol turnover (GLYTO) were measured for 2hr using <sup>2</sup>H<sub>2</sub>-glucose and <sup>3</sup>H<sub>2</sub>-glycerol. Following the basal measures, insulin sensitivity (IS) and GLYTO were measured using the hyperinsulinemic-euglycemic clamp

with continued infusion of glucose and glycerol stable isotopes. Lipid oxidation (FATOX) was measured with indirect calorimetry. Neonatal body composition was performed using total body electrical conductivity.

**Results:** At P, the HA group had higher weight (kg) ( $p \leq 0.01$ ), fat free mass (FFM) (kg) ( $p \leq 0.05$ ), FM (kg) ( $p \leq 0.001$ ), and BF% ( $p \leq 0.01$ ), compared to LA cohort. Both cohorts had similar GWG and accrual of FFM and FM during pregnancy. IS (umol/kgFFM/min)/(pmol/L insulin) decreased from P to L in both cohorts ( $p \leq 0.0001$ ), with higher reductions in HA subjects ( $p \leq 0.01$ ). GLYTO (mmol/hr) during the basal period and with insulin infusion (104 pmol/m<sup>2</sup>/min) increased with advancing gestation ( $p \leq 0.05$ ,  $p \leq 0.01$ , respectively), indicating increased basal lipolysis and increased lipid insulin resistance with no significant differences between groups. There was a longitudinal increase of clamp FATOX in both groups ( $p \leq 0.01$ ). Neonates of HA mothers had higher FM ( $p \leq 0.01$ ) and BF% ( $p \leq 0.01$ ) compared to those from LA mothers. P FM was positively associated with L GLYTO at basal ( $r=0.67$ ,  $p \leq 0.01$ ) and insulin clamp ( $r=0.70$ ,  $p \leq 0.01$ ) conditions. There was a positive relationship between P and L GLYTO during both basal and insulin clamp ( $r=0.78$ ,  $p \leq 0.01$ ;  $r=0.82$ ,  $p \leq 0.01$ , respectively). L basal and clamp GLYTO were positively related to neonatal FM ( $r=0.65$ ,  $p \leq 0.01$ ;  $r=0.67$ ,  $p \leq 0.01$ , respectively). The % suppression of GLYTO at L was inversely associated with neonatal FM ( $r=-0.65$ ,  $p \leq 0.05$ ).

**Conclusion:** P maternal adiposity and GLYTO are significantly related to changes in lipid metabolism in L gestation. Maternal L basal and clamp GLYTO is significantly related to neonatal FM and accounts for 45% variance in neonatal adiposity at birth. Future studies should consider pregravid lifestyle interventions to improve maternal metabolic state and prevent transgenerational obesity in offspring.

#### O-010

##### Highly Atherogenic Lipid Particles Are Associated with Preeclampsia (Pre-E) in Obese Women with Unexplained Infertility Who Conceived during Ovarian Stimulation with Intrauterine Insemination (OS-IUI).

Robert A. Wild,<sup>1</sup> Rodney K Edwards,<sup>1</sup> David S Wrenn,<sup>2</sup> Yan D Zhao,<sup>1</sup> Karl R Hansen.<sup>1</sup> <sup>1</sup>University of Oklahoma HSC, Oklahoma City, OK, United States; <sup>2</sup>Quest Diagnostics, Secaucus, NJ, United States.

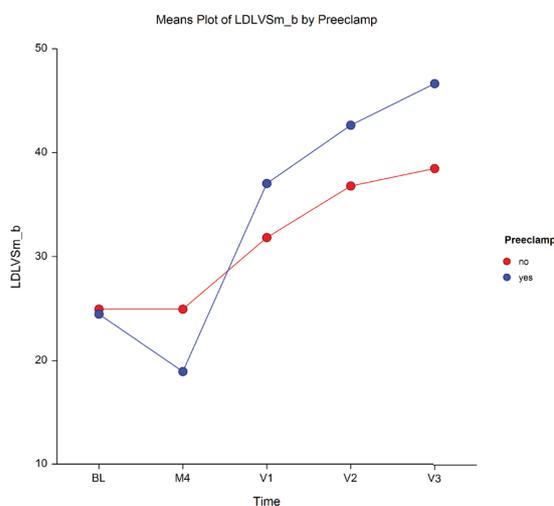
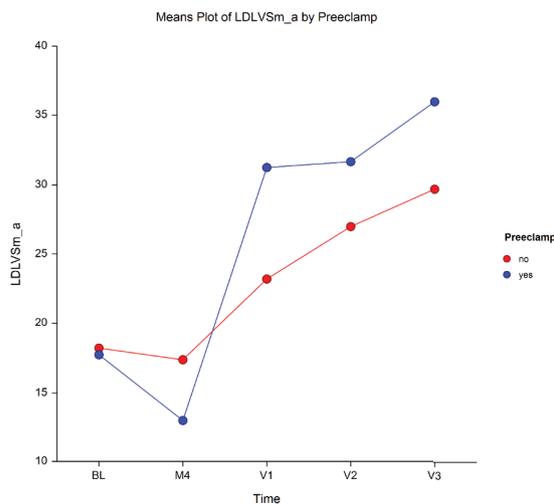
**Introduction:** Obesity is epidemic. Women with infertility are often obese. The FIT-PLEASE Reproductive Medicine Network RCT assessed whether pre-pregnancy diet plus exercise [orlistat + diet + exercise (D+E) vs. exercise alone (E)] before OS-IUI can improve live birth rates in obese women with unexplained infertility. Our **OBJECTIVE** was to determine if there is a relationship between highly atherogenic very small ldl lipid particle concentrations and Pre-E in this cohort

**Methods:** Frozen samples at each visit were blindly analyzed by ion mobility (Quest Diagnostics) at baseline (BL), after D+E or E (M4), and at 16, 24, and 36 weeks gestation. Mean values across the visits were analyzed using linear mixed models with repeated measures and 2 factor anova with repeated measures.

**Results:** Pre-E developed in 11 of 76 (14.5%) patients who had a live birth. Triglycerides (TGs) and Chol/HDL ratios (not shown) were higher across the visits for those who developed Pre-E ( $p < 0.001$ ). Levels of highly atherogenic very small LDL cholesterol particles (a, b, c) were higher during but not before pregnancy in those who developed Pre-E ( $p < 0.05$ ) with or without gestational diabetes.

**Conclusion:** Triglycerides are known to be elevated in preeclampsia; our data are consistent. This was found pre-pregnancy and throughout pregnancy in this cohort. Unlike the other lipids, highly atherogenic very small LDL cholesterol particles were elevated during, but not pre-pregnancy. Highly atherogenic dyslipidemia is a risk factor for short-(complications during) and long-term (cardiovascular complications later) maternal morbidity. The role of these very small LDL cholesterol particles in the pathogenesis of Pre-E (an acute vascular disease) deserves further investigation.

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#### O-011

##### Frequency and Correlates of Severe Chronic Hypertension (CHTN) 5 - 7 Years after Pregnancy Complicated by Mild CHTN. Ayamo Gina Oben†, Jeff Szychowski, Rachel Sinkey\*, Peter Ketch†, Cooper Elkins†, William Andrews\*, Alan Tita\*. University of Alabama at Birmingham, Birmingham, AL, United States.

**Introduction:** Mild CHTN affects 5-10% of reproductive age women, yet little data exist regarding the risks of progression from mild to severe CHTN or other cardiovascular diseases (CVDs). Our objective was to investigate the incidence of a composite of severe CHTN or other CVDs within 5 - 7 years after an index pregnancy complicated by mild CHTN.

**Methods:** This is a retrospective cohort of women with mild CHTN during an index pregnancy from 2012 - 2014. Women were included if they received prenatal care at our center, had mild CHTN during pregnancy (defined by ACOG as elevated blood pressure (BP) prior to pregnancy or newly diagnosed in pregnancy < 20 weeks gestation (GA) with systolic BP (SBP) 140-159 mmHg and/or diastolic BP (DBP) 90 - 109 mmHg). Women with Type 1 diabetes, SLE, cardiomyopathy, proteinuria (p/c ratio > 0.3 or 24hr > 300mg), severe CHTN, and creatinine levels > 1.1 mg/dL < 23 weeks GA were excluded. The primary outcome was a composite of severe CHTN (BP ≥ 160/110) more than 12 weeks after the index delivery or other CVDs (death, stroke, heart failure or cardiomyopathy, heart attack or angina, thromboembolism, renal failure (creatinine > 1.1 mg/dL) unrelated to preeclampsia in a subsequent pregnancy. We estimated the

cumulative incidence and 95%CI over the entire period from 2012-2019. We also examined differences in patient characteristics between those who progressed and those who do not. Other secondary CV outcomes (Table) were also collected.

**Results:** A total of 459 women with mild hypertension in the index pregnancy met inclusion criteria. Of these, 160 (35%) women experienced the primary outcome composite of severe CHTN within 5 - 7 years of the index pregnancy unrelated to a diagnosis of preeclampsia in a subsequent pregnancy. Race/ethnicity and smoking status differed between women with mild CHTN who progressed to severe CHTN versus those who did not, but notably, obesity was similar. The primary component of the composite outcome was severe HTN in 96.3% (Table). Average time for progression from mild to severe CHTN was 2.4 ± 1.9 years after delivery (median [Q1-Q3] = 2.0 [0.7-3.6] years). There were 5 maternal deaths (3.1%). Secondary CV outcomes of interest were rare.

**Conclusion:** In this cohort, one in three women with mild CHTN in an index pregnancy had progression to severe CHTN 5 - 7 years later. Because CHTN is a major risk factor for heart disease (a leading cause of death among women in the US), opportunities to mitigate this disease are urgently needed.

**Table: Outcomes 5-7 Years After Pregnancy Complicated by Mild Chronic Hypertension**

	N	Rate and 95% CI
<b>Primary composite outcome (includes 1 or more of the 7 items below)</b>	160	34.9% (30.5%-39.2%)
Severe CHTN by BP criteria	154	33.6% (29.2%-37.9%)
Any stroke	1	0.2% (0.01%-1.2%)
VTE (PE or DVT)	3	0.7% (0.1%-1.9%)
Heart Failure	2	0.4% (0.05%-1.6%)
Maternal death	5	1.1% (0.4%-2.5%)
Renal failure (serum creatinine >1.1)	1	0.2% (0.01%-1.2%)
<b>Secondary outcomes</b>		
Arrhythmia	1	0.2% (0.01%-1.2%)
Atrial fibrillation or flutter	1	0.2% (0.01%-1.2%)
Cardiovascular disease	2	0.4% (0.05%-1.6%)
Peripheral vascular disease	0	-
Carotid stenosis	1	0.2% (0.01%-1.2%)
Other	0	-

**O-012**

**A Prognostic Model for Early Risk Stratification of Spontaneous Preterm Birth.** Anadejda Landman<sup>†</sup>,<sup>1</sup> Marjon de Boer\*,<sup>1</sup> Marije Lamain-de Ruyter\*,<sup>2</sup> Martijn Heymans\*,<sup>1</sup> Arie Franx\*,<sup>3</sup> Martijn Oudijk\*,<sup>4</sup> Mireille Bekker\*,<sup>2</sup> Wendy Koster\*,<sup>3</sup> <sup>1</sup>Amsterdam UMC - VUmc, Amsterdam, Netherlands; <sup>2</sup>University Medical Center Utrecht, Wilhelmina Children's Hospital, Utrecht, Netherlands; <sup>3</sup>Erasmus MC, Rotterdam, Netherlands; <sup>4</sup>Amsterdam UMC - AMC, Amsterdam, Netherlands.

**Introduction:** To develop a prognostic model for spontaneous preterm birth (SPTB) based on maternal characteristics, obstetric history and placental biomarkers.

**Methods:** In this Dutch prospective multicenter cohort (RESPECT, ZonMw 50-50200-98-060) we recruited women <14 weeks of gestation at their initial prenatal visit. Maternal characteristics and obstetric history were collected from medical records and questionnaires. Pregnancy-associated Plasma Protein-A (PAPP-A) and Placental Growth Factor (PIGF) were collected between 9-14 weeks of gestation. The primary outcome was SPTB <37 weeks of gestation. Missing data were handled using multiple imputation and Rubin's Rules were used for pooled analyses. We used logistic regression with backward elimination to identify the most discriminative predictors. Internal validation was performed by bootstrapping. For clinical use, risk categories were calculated based on predicted probabilities: very low (<1%), low (1-5%), moderate (5-10%), and high risk (≥10%). We also evaluated the performance of the model for nulliparous women and multiparous women with and without a previous preterm birth.

**Results:** A total of 3,695 women with singleton pregnancies were included for analysis of which 1,642 (44.4%) were nulliparous. SPTB occurred in

128 (3.5%) pregnancies. The most discriminative predictors for SPTB were nulliparity (OR 3.68, 95% CI 2.33-5.84) and history of preterm birth <37 weeks (OR 6.90, 95% CI 3.62-13.16). Other predictors in the model were maternal age, smoking, vaginal blood loss in the first trimester and PAPP-A. Model performance was fair (AUC 0.687) and a calibration plot showed that the model was well calibrated. The classification in risk categories and performance measures of are shown in **Table 1**.

**Conclusion:** This prognostic model including maternal characteristics, obstetric history and the biomarker PAPP-A has a fair discriminative ability to predict the occurrence of SPTB. Perhaps this model could select women who may or may not benefit from follow-up management strategies such as cervical length screening.

Risk category	Predicted probability	Women per risk category	Women with SPTB <37 weeks	Women without SPTB <37 weeks	Sens	Spec	PPV	NPV	LR+	LR-
<b>a. TOTAL COHORT (N= 3,695)</b>										
Very low risk	<1%	317 (8.6%)	2 (0.6%)	315 (99.4%)	98.4%	8.8%	3.7%	99.4%	1.080	0.177
Low risk	1-5%	2578 (69.8%)	66 (2.6%)	2512 (97.4%)	46.9%	79.3%	7.5%	97.7%	2.3%	0.670
Moderate risk	5-10%	670 (18.1%)	45 (6.7%)	625 (93.3%)	11.7%	96.8%	11.5%	96.8%	3.635	0.912
High risk	≥10%	130 (3.5%)	15 (11.5%)	115 (88.5%)	-	-	-	-	-	-
<b>b. NULLIPAROUS WOMEN (N=1,642)</b>										
Very low risk	<1%	2 (0.1%)	0	2 (100%)	100%	0.13%	4.9%	100%	1.001	0
Low risk	1-5%	1025 (62.4%)	40 (3.9%)	985 (96.1%)	50.6%	63.2%	6.7%	96.1%	1.377	0.781
Moderate risk	5-10%	554 (33.7%)	35 (6.3%)	519 (93.7%)	43.2%	66.8%	6.3%	95.8%	1.300	0.851
High risk	≥10%	61 (3.7%)	6 (9.8%)	55 (90.2%)	-	-	-	-	-	-
<b>c. MULTIPAROUS WOMEN WITHOUT PREVIOUS PRETERM BIRTH (N=1,867)</b>										
Very low risk	<1%	315 (16.9%)	2 (0.6%)	313 (99.4%)	92.9%	17.0%	1.7%	99.4%	1.119	0.420
Low risk	1-5%	1546 (82.8%)	25 (1.6%)	1521 (98.4%)	3.6%	99.7%	16.7%	98.5%	13.13	0.967
Moderate risk	5-10%	6 (0.3%)	1 (16.7%)	5 (83.3%)	-	-	-	-	-	-
High risk	≥10%	0	0	0	-	-	-	-	-	-
<b>d. MULTIPAROUS WOMEN WITH PREVIOUS PRETERM BIRTH (N=186)</b>										
Very low risk	<1%	0	0	0	-	-	-	-	-	-
Low risk	1-5%	7 (3.8%)	1 (14.3%)	6 (85.7%)	94.7%	3.6%	10.1%	85.7%	0.983	1.465
Moderate risk	5-10%	110 (59.1%)	9 (8.2%)	101 (91.8%)	47.4%	64.1%	13.0%	91.5%	1.318	0.821
High risk	≥10%	69 (37.1%)	9 (13.0%)	60 (87.0%)	-	-	-	-	-	-

**Table 1** Performance measures for spontaneous preterm birth

**O-013**

**Loss of MIG-6 Results in Endometrial Progesterone Resistance and Endometriosis-Related Infertility through ERBB2 Overexpression.** Tae Hoon Kim,<sup>1</sup> Jung-Yoon Yoo,<sup>2</sup> Jung-Ho Shin,<sup>3</sup> Ryan Marquardt,<sup>1</sup> Ulrich Müller,<sup>4</sup> Asgerally Fazleabas,<sup>1</sup> Steven Young,<sup>5</sup> Bruce Lessey,<sup>6</sup> Ho-Geun Yoon,<sup>2</sup> Jae-Wook Jeong\*,<sup>1</sup> <sup>1</sup>Michigan State University, Grand Rapids, MI, United States; <sup>2</sup>Yonsei University College of Medicine, Seoul, Korea, Republic of; <sup>3</sup>Guro Hospital, Korea University Medical Center, Seoul, Korea, Republic of; <sup>4</sup>Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>5</sup>University of North Carolina, Chapel Hill, NC, United States; <sup>6</sup>Wake Forest Health, Winston-Salem, NC, United States.

**Introduction:** Fertility problems are highly associated with endometriosis. Although the exact etiology of endometriosis-related infertility is unknown, endometrial progesterone resistance has recently been suggested as a crucial element in the development of endometrial diseases. MIG-6 acts as a key P4 signaling mediator in the endometrium of the human and mouse. Uterine-specific Mig-6 knock-out mice (*Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>*) are infertile due to non-receptive endometrium. We hypothesized that MIG-6 loss causes endometriosis-related infertility due to endometrial progesterone resistance.

**Methods:** We examined MIG-6 levels in eutopic endometrium from infertile women with endometriosis. We utilized a non-human primate endometriosis model to track MIG-6 expression in eutopic endometrium through disease progression. To determine the effect of MIG-6 loss on endometriosis development, we surgically induced endometriosis with endometrium from control and *Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>* mice. To investigate the effect of ERBB2 targeting on progesterone resistance and infertility, we introduced *ErbB2* ablation in the *Mig-6* knockout mice (*Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>ErbB2<sup>fl/fl</sup>*).

**Results:** The expression of MIG-6 mRNA and proteins were significantly reduced in eutopic endometrium of early secretory phase infertile women with endometriosis. Induction of endometriosis in a non-human primate model of endometriosis progressively reduced the expression of MIG-6. Endometrial tissue from *Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>* mice formed a significantly

increased number of endometriotic lesions in the mouse compared to the control. ERBB2 overexpression was identified in non-receptive endometrium from *Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>* mice. *Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup> Erbb2<sup>fl/fl</sup>* double mutant mice reversed the endometrial progesterone resistance as well as all phenotypes including infertility and increased endometriosis development seen in *Pgr<sup>cre/+</sup>Mig-6<sup>fl/fl</sup>* mice. Remarkably, the transcriptomic analysis showed that altered genes in *Mig-6<sup>fl/fl</sup>* mice reverted to their normal expression levels in *Mig-6<sup>fl/fl</sup>Erbb2<sup>fl/fl</sup>* mice.

**Conclusion:** Together, our results demonstrate that MIG-6 loss causes progesterone resistance through ERBB2 overexpression in non-receptive endometrium of endometriosis-related infertility. Our results suggest that ERBB2 is a potential target for progesterone resistance and endometriosis-related infertility.

#### O-014

**The Role of ARID1a in Mediating Endometriosis Disease Progression.** Valerie Flores†, Tran Dang, Hugh S Taylor,\* Gloria Huang. *Yale School of Medicine, New Haven, CT, United States.*

**Introduction:** Endometriosis is a chronic gynecologic disease affecting 10% of reproductive-aged women. While medical therapy and surgery are the cornerstones of treatment, each has associated failure rates. We have shown that non-responders (NRs) to progestins have decreased progesterone receptor (PR) expression; surprisingly estrogen receptors (ERs) alpha and beta were often decreased as well. The decreases in ERs and PR levels in endometriosis may be analogous to receptor negative breast cancer, suggesting other non-hormonal mechanisms are involved in disease progression. As several cancer-associated mutations, including ARID1a, are seen in endometriosis, we aimed to determine if the tumor suppressor gene ARID1a was differentially expressed, and contributing to treatment-resistant disease in our previously described endometriosis subjects.

**Methods:** Endometriotic lesions were obtained from 43 subjects in this retrospective cohort study. Matched eutopic endometrium was obtained from 10 subjects. IHC was performed using a rabbit polyclonal IgG for detection of ARID1a, and the Histo-score used to quantify ARID1a expression—total and glandular. PR, ER alpha and ER beta expression were also assessed by IHC. Two blinded investigators independently scored IHC data. Data regarding hormonal therapy use and response to progestins were determined from the medical record. Mann-Whitney U and Spearman's rank correlation used for statistical analysis.

**Results:** ARID1a levels were significantly lower in NRs (n=29) compared to responders (n=14) to progestin-based therapy (H score 15 vs 48; p=0.0003). When comparing only glandular H scores in responders vs NRs, there were again lower levels of ARID1a in NRs compared to responders (H score 90 vs 175; p=0.027). There was no correlation between ARID1a levels in matched ectopic and eutopic specimens. ARID1a levels did not correlate with PR or ERs, but 9 NRs had low levels of PR, ERs and ARID1a.

**Conclusion:** We found lower levels of ARID1a in endometriosis subjects who were NRs to progestins. In addition to its function as a tumor suppressor, ARID1a is also necessary for decidualization and embryo implantation in the endometrium. In a conditional knock-out model, mice with uterine loss of ARID1a lacked decidualization, indicating loss of key features of progestin response and correlating with our findings of low ARID1a and progesterone resistance. Our prior findings of loss of PR and ERs in NRs to progestin-based therapy suggest that treatment-resistant endometriosis may have diminished requirement for estrogen for proliferation. In addition, the decrease in ARID1a in NRs show that endometriosis pathogenesis extends beyond progesterone-resistance alone and may involve altered expression of tumor suppressors. Instead, this work suggests several aberrant pathways are involved, highlighting the complexity and variability of this disease and the need for novel non-hormonal therapies for effective treatment.

#### O-015

**Endometriosis Is a Possible Risk Factor for Premature Cardiovascular Disease.** Jessica N Blom†, Maria P Velez\*,<sup>1,2</sup> Chad McClintock†, Jessica Pudwell\*, Susan Brogly\*,<sup>3,2</sup> Olga Bougie\*.<sup>1</sup> *Kingston Health Sciences, Dept OBGYN, Queen's University, Kingston, ON, Canada;* <sup>2</sup>*Kingston Health Sciences, ICES Queen's, Kingston, ON, Canada;* <sup>3</sup>*Kingston Health Sciences, Dept Surgery, Queen's University, Kingston, ON, Canada.*

**Introduction:** Although cardiovascular disease (CVD) remains the leading cause of premature death in women worldwide, screening, diagnosis and treatment strategies were developed based on the male CVD experience. Female specific risk factors for CVD must be identified to improve patient outcomes. Endometriosis affects up to 10% of the female population, and may increase one's risk for CVD through chronic inflammation and early menopause. The OBJECTIVE of this study was to determine the association between a diagnosis of endometriosis and subsequent risk of CVD.

**Methods:** We conducted a population-based cohort study using administrative health data from ICES in Ontario. The incidence of CVD and cardiovascular health outcomes was compared between women with endometriosis (exposed) and 2 age-matched women without endometriosis (unexposed) from 1993-2015 who were 18-50 years old upon enrollment. Women were considered to have endometriosis if they had a confirmed surgical diagnosis at any time (ICD9-617, ICD10-N80), or if they had ≥ 2 codes indicating a medical diagnosis (OHIP dx617). Exclusion criteria included CVD parameters at baseline. The primary outcome was composite hospitalizations due to CVD events. Secondary outcomes included composite CVD events of interest. Outcomes were based on previously validated composites. Cox-proportional hazards models were used to estimate hazard ratios (HR) by endometriosis status while adjusting for sociodemographic and prior health factors.

**Results:** A total of 500,559 patients were enrolled in the study (166,853 exposed and 333,706 unexposed). Average age of enrolment was 36.4 years for both groups. Women with endometriosis had a higher incidence of hospitalization for CVD (197 cases / 100,000 person-years) as compared to unexposed (164 cases / 100,000 person-years) and a significantly reduced time to event (HR 1.16; 95% CI 1.11-1.20; p<0.001). Similarly, the incidence of secondary CVD events was higher in the exposed group (292 cases / 100,000 person-years) as compared to unexposed (224 cases / 100,000 person-years) and the time to event was reduced (HR 1.25; 95% CI 1.21-1.29; p<0.001).

**Conclusion:** This is the first population-based study to examine the association between endometriosis and CVD. Our results indicate that endometriosis may be a risk factor for the development of premature CVD. Further studies will need to be conducted to elucidate the potential mechanism, including the mediating role of surgical menopause.

#### O-016

**Randomized, Placebo-Controlled Trial of Botulinum Toxin for Endometriosis-Associated Chronic Pelvic Pain: A Longitudinal Assessment.** Pamela Stratton\*,<sup>1</sup> Hannah K Tandon†,<sup>2,1</sup> Vy Phan†,<sup>1</sup> Jacqueline V Aredo†,<sup>1,3</sup> Ninet Sinaii,<sup>1</sup> Jay P Shah,<sup>1</sup> Barbara I Karp\*.<sup>1</sup> *<sup>1</sup>NIH, Bethesda, MD, United States;* *<sup>2</sup>University of Nebraska Medical Center, Omaha, NE, United States;* *<sup>3</sup>Stanford University, Stanford, CA, United States.*

**Introduction:** Women with endometriosis-associated chronic pelvic pain (endo-CPP) often have persistent pain despite optimal surgical and hormonal management of lesions. Pelvic floor muscle spasm may contribute to pain persistence. Here, we evaluated the longitudinal efficacy of botulinum toxin (BTX) in relieving endo-CPP.

**Methods:** Women (18-50yrs) with endo-CPP (surgically diagnosed, optimized hormonal treatment) in a randomized, double-masked, placebo-controlled trial received 100U onabotulinumtoxinA or placebo into pelvic floor muscles with spasm (NCT01553201). An optional open BTX (2<sup>nd</sup>) injection was offered any time 1-12 months after masked injection at subject's request. Baseline, 1-month, and up to 1-year evaluation included pelvic exam for pelvic floor muscle spasm, pain rating, medication tracking, and Oswestry Disability Index. Adverse effects were recorded.

Subjects rated response to injection as percent (%) improvement and benefit duration. T-tests, non-parametric tests, Fisher's exact test, logistic regression, and survival analysis compared data.

**Results:** Of 29 randomized women, all identified pelvic floor spasm as a major focus of endo-CPP. No subject dropped out before 1-year follow-up. At 1 month after masked injection, 11/15 (73%) who received BTX and 4/14 (29%) placebo reported benefit ( $p=.027$ ). BTX reported a greater % improvement ( $p=.034$ ) and longer-lasting benefit compared to placebo ( $p=.023$ ). The number of pelvic floor muscles in spasm decreased in BTX only ( $p=.019$ ). Adjusting for treatment and benefit, higher baseline disability predicted request for 2<sup>nd</sup> injection at 1-month ( $p=.022$ ). Controlling for treatment, those without benefit more often requested open BTX at 1-month ( $p=.032$ ). Prior to 2<sup>nd</sup> injection, placebo group had more days with no benefit than BTX ( $p=.02$ ). Women with focal pelvic pain at baseline exam had longer lasting benefit compared to those with diffuse ( $p=.02$ ), especially in BTX group ( $p=.004$ ). In all subjects, benefit was briefer with 1<sup>st</sup> (masked) than 2<sup>nd</sup> (open) injection ( $p=.02$ ), particularly in placebo ( $p=.002$ ). Over time, those receiving BTX used less pain medication than placebo ( $p=.037$ ). VAS did not reflect benefit seen due to wide pain rating variability at baseline and after injection in both groups. Adverse events were mild and not serious, with no difference in incidence between groups ( $p=.11$ ).

**Conclusion:** We demonstrate pelvic floor muscle spasm as part of endo-CPP and relief of pain/spasm from BTX compared to placebo that persisted over time. BTX was well tolerated and not associated with more/worse adverse events than placebo. Those with focal pelvic pain were more likely to have longer benefit.

#### O-017

##### **Bleeding Patterns in Women with Endometriosis-Associated Pain Treated with Relugolix Combination Therapy: SPIRIT Program.**

Andrea S Lukes,<sup>1</sup> Sawсан As-Sanie,<sup>2</sup> Christian M Becker,<sup>3</sup> Mauricio S Abrao,<sup>4</sup> Claudia Mehedintu,<sup>5</sup> Galyna Reznichenko,<sup>6</sup> Linda Giudice,<sup>7</sup> Furong Wang,<sup>8</sup> Viatcheslav G Rakov,<sup>9</sup> Qurratul A Warsi.<sup>8</sup> <sup>1</sup>Carolina Woman's Wellness Center, Durham, NC, United States; <sup>2</sup>University of Michigan, Ann Arbor, MI, United States; <sup>3</sup>Nuffield Department of Women's and Reproductive Health, Oxford, United Kingdom; <sup>4</sup>Sao Paulo University, Sao Paulo, Brazil; <sup>5</sup>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania; <sup>6</sup>Clinical Maternity Hospital # 4, Zaporizhzhya, Ukraine; <sup>7</sup>University of California, San Francisco, CA, United States; <sup>8</sup>Myovant Sciences, Inc., Brisbane, CA, United States; <sup>9</sup>Myovant Sciences GmbH, Basel, Switzerland.

**Introduction:** Relugolix combination therapy (Rel-CT: relugolix 40 mg, estradiol 1 mg, norethindrone acetate 0.5 mg) significantly improved endometriosis-associated pain vs placebo in the pivotal SPIRIT 1 and 2 studies. Clinical effect was maintained over 52 weeks (wks) in the long-term extension (LTE) study. Rel-CT has also been shown to inhibit ovulation and maintain estradiol concentrations as in the early follicular phase of the menstrual cycle. Here, we describe bleeding patterns in women with endometriosis treated up to 52 wks with Rel-CT.

**Methods:** SPIRIT 1 and 2 were Phase 3, randomized, double-blind, 24-wk, placebo-controlled studies of Rel-CT, delayed Rel-CT (relugolix 40 mg monotherapy then Rel-CT, both for 12 wks), or placebo in premenopausal women (age 18-50 years) with surgically diagnosed endometriosis, moderate-to-severe dysmenorrhea and non-menstrual pelvic pain. Women completing SPIRIT 1 or 2 were eligible to enroll in the open-label LTE of once-daily Rel-CT. Bleeding patterns (no bleeding, spotting, light, moderate, heavy, extremely heavy) were based on women's daily eDiary records. Amenorrhea was defined as lack of bleeding for at least 56 consecutive days after starting treatment. Women who received Rel-CT in the pivotal studies and continued in the LTE were the focus of this analysis.

**Results:** Of 1261 randomized women, 1044 completed the pivotal studies; 802 enrolled in the LTE. Baseline demographics/characteristics were balanced across treatment groups. Increasing proportions of women treated with Rel-CT experienced amenorrhea at Wk 24 and 52: 67.9% (95% CI: 62.0, 73.3) and 76.6% (70.7, 81.9), respectively. The average number of bleeding days per cycle (standard deviation) decreased from 5.8 (2.4) at baseline to 1.7 (3.6) and 1.4 (3.6) at Wk 24 and 52; days with

heavy/extremely heavy bleeding reduced from 1.9 at baseline to 0.1 at Wk 24 and 52. Similar changes were observed in women initially receiving placebo after transitioning to Rel-CT at Wk 24.

**Conclusion:** Most women with endometriosis-associated pain treated with Rel-CT over 52 wks achieved amenorrhea. There was a reduction of bleeding days per cycle, including days with heavy/extremely heavy bleeding. Findings are consistent with the mechanism of action of Rel-CT and may support patient counseling.

#### O-018

##### **Single-Cell Sequencing Reveals Novel Cell Type and Heterogeneous Non-Monoclonal Cell Populations in Human Leiomyomas.** Jyoti Goad†,<sup>1</sup> Joshua Rudolph,<sup>1</sup> Jian-Jun Wei,<sup>2</sup> Serdar E Bulun,<sup>2</sup> Debabrata Chakravarti,<sup>2</sup> Aleksandar Rajkovic\*.<sup>1</sup> <sup>1</sup>University of California, San Francisco, CA, United States; <sup>2</sup>Northwestern University, Chicago, IL, United States.

**Introduction:** Uterine leiomyomas or fibroids are considered monoclonal. However, recent histology and flow cytometry experiments indicate presence of cellular heterogeneity in leiomyomas. Leiomyomas associate with somatic genetic variants and chromosomal abnormalities. Primarily somatic variants in *MED12* are present in 70% of leiomyomas. Previous studies have indicated that leiomyomas carrying *MED12* variant have increased accumulation of extracellular matrix compared to *MED12* variant negative leiomyomas. We hypothesized that underlying leiomyoma genotype, affects cellular composition and gene expression.

**Methods:** We collected uterine leiomyomas (n=8) and matched normal myometrium (n=5) from eight patients undergoing hysterectomy. The tissue collected was dissociated to prepare a single-cell suspension. Single cells were processed through 10X Chromium system (10X Genomics). Part of tissue collected was used to perform genotyping and histology. Data analysis was performed using the Seurat pipeline. Data were validated using immunohistochemistry or in-situ hybridization.

**Results:** We analyzed a total of 34,435 high-quality cells (11,235 cells from myometrium, 15,417 from *MED12* positive, and 7,783 cells from *MED12* negative leiomyoma). Our analysis revealed presence of 18 different clusters across lineages in myometrium and leiomyomas. These cell clusters were highly reproducible, as nearly all clusters were represented in all patient samples. We identified heterogeneity in smooth muscle (5 distinct populations) and fibroblasts populations (2 distinct populations) in myometrium. We identified transcriptional changes in smooth muscle and fibroblast cell clusters of both *MED12* positive and *MED12* negative leiomyomas compared to myometrium. Furthermore, we discovered novel lymphatic endothelial cell populations in both *MED12* positive and *MED12* negative leiomyomas. Our data shows that immune cell population infiltration in leiomyomas is genotype-dependent. We found increased infiltration of T-cells and NK cells in *MED12* positive leiomyomas while B-cells and macrophages infiltrate *MED12* negative leiomyomas. Moreover, our analysis revealed that *MED12* positive leiomyomas are composed of cells carrying the *MED12* mutant variant as well as cells carrying wild type *MED12* allele, indicating that leiomyoma cellular moiety is not monoclonal.

**Conclusion:** We generated a single cell atlas for myometrium and leiomyomas. Our data reveals previously unknown cellular heterogeneity in myometrium and leiomyomas. We discovered novel lymphatic endothelial cell populations in leiomyomas and found that immune cell infiltration in leiomyomas is genotype dependent. Furthermore, our data indicates that *MED12* positive leiomyomas are not monoclonal.

#### O-019

##### **Long Noncoding RNA MIAT Modulates the Extracellular Matrix Deposition in Leiomyomas via Sponging MiR-29 Family.** Tsai-Der Chuang, Derek Quintanilla, Drake Boos, Omid Khorram\*. LA Biomed at UCLA Medical Center, Torrance, CA, United States.

**Introduction:** To determine the expression and functional roles of a long noncoding RNA (lncRNA) MIAT (myocardial infarction associated transcript) in leiomyoma pathogenesis.

**Methods:** Paired myometrium and leiomyoma tissue samples (N=67) from patients without any treatments for at least 3 months prior to surgery

were collected for isolation of leiomyoma smooth muscle cells (LSMC) and gene analysis. The collected tissue specimens included Caucasians (N=12), African Americans (N=25), Hispanics (N=23) and Asians (N=7). The menstrual cycle phase was determined by histologic analysis with 32 specimens being identified as in the proliferative phase and 17 specimens in the secretory phase. The MED12 (Mediator of RNA polymerase II transcription, subunit 12 homolog) mutation status was determined by PCR amplification and Sanger sequencing. Of the specimens sequenced, 43 fibroids had the MED12 mutations (64.2%). MIAT and microRNA 29 family (miR-29a, -b and -c) were assessed by qRT-PCR. The direct interaction of MIAT and miR-29 family was determined by the luciferase activity assay and RNA immunoprecipitation (RIP). Using LSMC three-dimensional spheroid culture system knockdown of MIAT was done by siRNA transfection and protein abundance of collagen type I (COL1A1) and collagen type III (COL3A1) was determined by western blot analysis. Results were analyzed by Student's t-tests and one-way ANOVA with Tukey's HSD for post hoc analysis.

**Results:** Leiomyoma expressed significantly higher levels of MIAT which was independent of race/ethnicity and menstrual cycle phase. However, MIAT was more abundant, while miR-29 family was expressed significantly less in leiomyomas bearing the MED12 mutation as compared with wild type leiomyomas. Using luciferase reporter activity and RNA immunoprecipitation analysis we confirmed MIAT has sponge activity over miR-29 family. Transient transfection of MIAT siRNA in LSMC spheroids resulted in up-regulation of miR-29 family with an inverse relationship with expression of their targets COL1A1 and COL3A1. Treatment of LSMC spheroids with TGF- $\beta$ 3 (Transforming Growth Factor  $\beta$ -3) through TGF- $\beta$  receptor signaling induced expression of COL1A1 and COL3A1, and MIAT levels, while repressed miR-29 family expression. Overall, knockdown of MIAT in LSMC spheroids partially blocked the effects of TGF- $\beta$ 3 on the induction of COL1A1 and COL3A1 expression.

**Conclusion:** MIAT expression is induced in leiomyoma in a positive correlation with MED12 mutation status, resulting in a decrease in miR-29 family levels via sponging mechanism and accumulation of their targets COL1A1 and COL3A1. Collectively, these results indicate a significant role of MIAT in leiomyoma pathogenesis.

## O-020

**Reduction in Menstrual Blood Loss in Patients Treated with Relugolix Combination Therapy: LIBERTY Long-Term Extension Study.** [Ayman Al-Hendy](#),<sup>1</sup> [Andrea S Lukes](#),<sup>2</sup> [Alfred Poindexter III](#),<sup>3</sup> [Roberta Venturella](#),<sup>4</sup> [Claudio Villarreal](#),<sup>5</sup> [Rachel B Wagman](#),<sup>6</sup> [Yulan Li](#),<sup>6</sup> [Laura McKain](#),<sup>6</sup> [Elizabeth A Stewart](#).<sup>7</sup> <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>Carolina Woman's Wellness Center, Durham, NC, United States; <sup>3</sup>Baylor College of Medicine and St. Luke's Episcopal Hospital, Houston, TX, United States; <sup>4</sup>University Magna Graecia, Catanzaro, Italy; <sup>5</sup>University of Chile, Santiago, Chile; <sup>6</sup>Myovant Sciences, Inc., Brisbane, CA, United States; <sup>7</sup>Mayo Clinic, Rochester, MN, United States.

**Introduction:** Once-daily relugolix combination therapy (Rel-CT) reduced menstrual blood loss (MBL) in women with uterine fibroids (UF) and was well tolerated, through 24 weeks (wks) in LIBERTY 1 and 2. We report treatment effect on bleeding patterns in patients (pts) treated with Rel-CT for up to 52 wks or transitioning from placebo (PBO) to Rel-CT after 24 wks.

**Methods:** Women with UF-associated heavy menstrual bleeding (HMB) who completed the 24-wk double-blind, PBO-controlled LIBERTY 1 and 2 trials were eligible to enroll in a 28-wk long-term extension (LTE) study. All received once-daily Rel-CT (40 mg relugolix, estradiol 1 mg, norethindrone acetate 0.5 mg). Primary efficacy endpoint: proportion of pts who achieved or maintained an MBL volume <80 mL and a  $\geq$ 50% reduction from pivotal study baseline to the last 35 days of treatment. Secondary endpoints: mean % MBL reduction and amenorrhea rate. Outcomes were analyzed by baseline LIBERTY treatment assignment (Rel-CT and PBO) using descriptive statistics without statistical testing for treatment comparison. In LIBERTY, study drug was initiated within the first 7 days of menses onset. At time of rollover into the LTE, women in

the PBO group started treatment later in the menstrual cycle because they were required to complete the Week (Wk)24 feminine product collection before transitioning to Rel-CT.

**Results:** With Rel-CT (N=163), a rapid reduction in MBL was observed at Wk4 (least square (LS) mean % change from baseline: -52.8%), reaching -81.2% at Wk8, which was maintained through Wk52 (-89.9%). With PBO (N=164), there was no meaningful change in MBL during the first 24 wks (-17.6%) nor decrease in MBL after the first 4 wks of the LTE, because Rel-CT was initiated later in the menstrual cycle. At Wk32, a rapid reduction in MBL (LS mean % change: -79.6%) was observed, progressing to -91.9% at Wk52, confirming the effect of Rel-CT on MBL shown in the pivotal studies. At Wk52, MBL <80 mL was observed in 87.1% and 75.6% pts in the Rel-CT and PBO→Rel-CT groups and, in 89.0% and 81.1% of pts, a  $\geq$ 50% reduction of MBL was reported in the Rel-CT and PBO→Rel-CT groups, respectively. The proportion of pts achieving amenorrhea over the last 35 days of treatment was 70.6% (Rel-CT group) and 57.9% (PBO→Rel-CT group).

**Conclusion:** Rel-CT had a rapid and clinically meaningful impact on MBL reduction in women with UF, which was maintained through Wk52. The onset of treatment effect was faster when Rel-CT was initiated at the start of menses.

## O-021

**Upregulation of miR-877-3p and Downregulation of miR-186-3p in the Placentas of Patients with Idiopathic Preterm Birth.** [Duygu Mutluay](#), [Xiaofang Guo](#),<sup>†</sup> [Ozlem Guzeloglu Kayisli](#), [Frederick Schatz](#), [Umit Kayisli](#), [Charles Lockwood](#). *University of South Florida, Morsani College of Medicine, Tampa, FL, United States.*

**Introduction:** MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by degrading target mRNAs and/or by suppressing translation. We previously showed increased FKBP51 and decreased progesterone receptor (PR) levels in decidual cells at the maternal-fetal interface of laboring vs. non-labor specimens. Several miRNAs are known to be involved in the pathogenesis of preterm birth (PTB). We hypothesize that idiopathic PTB is associated with dysregulation of specific miRNAs that target *FKBP5* and *PR* transcripts. **Methods:** Global RNA sequencing (RNA-Seq) was used to detect differentially expressed miRNA profiles in placental specimens obtained from term labor (n=7) or idiopathic PTB (n=5) or gestational age (GA)-matched controls obtained from pre-eclamptic patients (n=5) with indicated PTBs. Differentially expressed miRNAs with  $\geq$  2-fold changes among the groups were analyzed by TargetScan software to identify relevant miRNAs targeting *FKBP5* or *PR* transcripts. Expression levels of these newly identified miRNAs were then assessed by miRNA-specific reverse transcription, followed by qPCR analysis and normalization to U6 snoRNA levels. Data were compared using One-Way ANOVA,  $P < 0.05$  was considered statistically significant.

**Results:** RNA-Seq analysis revealed that idiopathic PTB placentas exhibited 144 and 161 upregulated miRNAs and 342 and 299 downregulated miRNAs, compared to term or GA-matched control specimens, respectively. Among these differentially expressed miRNAs, TargetScan software identified 21 downregulated miRNAs targeting *FKBP5* and 14 upregulated miRNAs targeting *PR*. Moreover, highest fold changes in miRNA-877 upregulation and miRNA-186 downregulation were found in idiopathic PTB vs. term or GA-matched controls. Thus, further analysis by qPCR confirmed that: 1) miRNA-877-3p was significantly upregulated in idiopathic PTB vs. term or GA-matched controls (Mean $\pm$  SEM; 2.31 $\pm$  0.3 vs. 1.29 $\pm$  0.4 or 0.72 $\pm$  0.1, respectively;  $P < 0.05$ ); and 2) miRNA-186-3p was significantly downregulated in idiopathic PTB vs. term or GA-matched controls (0.41 $\pm$  0.04 vs. 1.28 $\pm$  0.3 or 1.12 $\pm$  0.2, respectively;  $P < 0.05$ ).

**Conclusion:** These results identified several novel miRNAs expressed in the placenta linked to idiopathic PTB. Increased miRNA-877-3p and decreased miRNA-186-3p levels likely contribute to the induction of idiopathic PTB by down-regulating *PR* and up-regulating *FKBP5* transcripts, respectively. Moreover, these miRNAs can be used as potential

biomarkers to predict idiopathic PTB and/or as therapeutic targets. Future functional studies are required to determine the role(s) of these miRNAs in the pathogenesis of PTB.

## O-022

**Iron-Dependent Apoptosis Causes Embryotoxicity in Inflamed and Obese Pregnancy.** Allison L Fisher<sup>†</sup>,<sup>1</sup> Veena Sangkhae,<sup>2</sup> Tomas Ganz,<sup>2</sup> Elizabeth Nemeth\*.<sup>2</sup> <sup>1</sup>Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States; <sup>2</sup>Center for Iron Disorders, UCLA, Los Angeles, CA, United States.

**Introduction:** Iron is essential during pregnancy. Iron supplements are commonly recommended without screening for iron disorders, yet most women in developed countries have adequate iron stores. Maternal iron marker ferritin is linked to pregnancy complications, which signals the potential harm of supplementation in iron-replete women. As ferritin is also induced by inflammation, it is unclear if iron, inflammation, or their interaction cause injury. We addressed this question using mouse models.

**Methods:** Two models of maternal iron excess were used and compared to dams with normal iron status. Dietary iron supplementation was modeled by feeding C57BL/6 mice high iron diet (2,500-5,000ppm iron) for 1 week prior to and in pregnancy. C57BL/6 mice deficient in the iron-regulatory hormone hepcidin modeled genetic iron loading and were fed normal chow (185ppm). Models of maternal systemic inflammation included acute inflammation induced by a subcutaneous injection of LPS on E8.5 or 15.5, and chronic inflammation caused by diet-induced obesity. We evaluated the dam, placenta, and embryo response to inflammation at various time points.

**Results:** In the LPS model, we saw potentiation of adverse embryo outcomes when dams were both iron-loaded and LPS-injected compared to either condition alone. In iron-loaded dams, E8.5 LPS injection caused embryo loss and malformations, and E15.5 LPS injection caused embryo resorption. Western blot and immunostaining showed increased apoptotic marker cleaved caspase 3 ( $P<0.001$ ) in placenta and embryo endothelia, only in the iron-loaded and LPS-injected groups. In human endothelial cells, cytokine screen identified TNF $\alpha$  as the signal that potentiated apoptosis in iron-laden cells ( $P<0.001$ ). Treatment of iron-loaded dams with TNF $\alpha$  neutralizing antibody prior to LPS protected embryos from death ( $P=0.009$ ). RNA Seq of mouse placental endothelial cells showed enrichment of oxidative stress pathways with iron loading, which preceded apoptosis. Treatment of iron-loaded dams with antioxidant vitamin E prevented LPS-induced embryo death and endothelial apoptosis. In the obesity model, iron excess worsened embryopathy, causing subcutaneous hemorrhaging and malformations, and potentiated placental cleaved caspase 3 expression ( $P<0.05$ ). Iron-loaded obese dams had higher serum TNF $\alpha$  levels ( $P=0.015$ ), and neutralizing TNF $\alpha$  prevented embryopathy.

**Conclusion:** In summary, maternal iron excess greatly worsened LPS- and obesity-induced embryo injury. Iron excess caused oxidative stress, which sensitized the fetal endothelia to lethal apoptotic damage by maternal TNF $\alpha$ . Iron-dependent embryo injury was prevented by maternal anti-TNF $\alpha$  or antioxidant therapy. Our models suggest that women could be at risk for adverse pregnancy outcomes when exposed to both excess iron and inflammation.

## O-023

**A Broad Spectrum Chemokine Inhibitor Prevents Infection-Induced Myometrial Inflammation and Activation.** Adam Boros-Rausch<sup>†</sup>,<sup>1,2</sup> Tsung-Yen Wu,<sup>3</sup> Kristina Adams Waldorf,<sup>3</sup> Oksana Shynlova\*,<sup>1,2,4</sup> Stephen Lye\*,<sup>1,2,4</sup> <sup>1</sup>The Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; <sup>2</sup>Department of Physiology, The University of Toronto, Toronto, ON, Canada; <sup>3</sup>The University of Washington, United States, Washington, WA, Canada; <sup>4</sup>Department of Obstetrics & Gynecology, The University of Toronto, Toronto, ON, Canada.

**Introduction:** We have reported earlier that prophylactic administration of a broad-spectrum chemokine inhibitor (BSCI, FX125L) blocks infection (Group B Streptococcus)-induced preterm labor (PTL) in a nonhuman primate (NHP, *Macaca nemestrina*) model. We hypothesized that a BSCI

prevents contraction of uterine smooth muscle (myometrium) by inhibiting the expression of pro-inflammatory cytokines and contraction-associated proteins (CAPs).

**Methods:** Human myometrial biopsies (N=4) were collected from women at term (not in labor), and primary myocytes were isolated by enzymatic digestion. Myometrial cells were treated with LPS (bacterial lipopolysaccharide, 100ng/mL), FSL-1 (bacterial lipopeptide, 100ng/mL), or vehicle for 8 or 24 hours to mimic the infectious process w/w BSCI (250nM). Conditioned media was collected and secreted cytokines IL6, CCL2, and CXCL8 were analyzed by ELISA. For in vitro contraction assays, myometrial cells were embedded in collagen gels ( $1.5 \times 10^5$  per well in a 6-well plate) for 48 hours and stimulated with inflammatory mimics (LPS, FSL-1, or vehicle) w/w BSCI (100nM) for 96 hours. Chronically catheterized NHP at 117-125 days gestation received choriodecidual inoculations of either (1) saline (N=7), (2) GBS ( $5 \times 10^8$  colony forming units (CFU)/mL; N=5), and (3) GBS with BSCI (10 mg/kg intravenous and intra-amniotic; N=4). Cesarean sections were performed at PTL or 4 days after inoculation, myometrial biopsies were collected, and total RNA was extracted for RT-qPCR.

**Results:** 1) BSCI significantly decreased CCL2 and CXCL8 secretion ( $P<0.05$ ) by LPS-induced primary human myocytes in vitro. 2) LPS-induced myocytes embedded in 3D collagen gel caused a significant ( $P<0.05$ ) decrease of gel area (70%), while FSL-1 administration caused a significant (53%) decrease of gel area, as compared to vehicle-treated control. Pre-treatment of myocytes with BSCI significantly prevented collagen gel contraction induced by bacterial mimics. 3) Compared with saline controls, GBS induced PTL in 4 animals, but in only 1 of 4 receiving BSCI. *CCL2*, *CXCL8*, *IL1B*, *IL1RN*, *IL10*, and *GJAI* transcript levels were significantly elevated in NHP myometrium during PTL. BSCI significantly ( $P<0.05$ ) inhibited GBS-induced expression of cytokines and CAP gene *GJAI*.

**Conclusion:** BSCI can inhibit uterine smooth muscle cell contraction and cytokine expression in vivo and in vitro. BSCI could represent a novel therapeutic for reducing dysregulated myometrial inflammation and potentially suppressing preterm labour in women.

## O-024

**Exposure to Experimental Fetal Inflammatory Response Syndrome Results in IL-6 Dependent Upregulation of Interferon Lambda Expression and Subsequent Loss of Neonatal Paneth Cells.** Sarah Nichole Watson<sup>†</sup>, Huiyu Gong, Brian Juber<sup>†</sup>, Steven Mcelroy\*. *University of Iowa, Iowa City, IA, United States.*

**Introduction:** Fetal exposure to chorioamnionitis-driven fetal inflammatory response syndrome (FIRS) is a known risk factor for subsequent development of necrotizing enterocolitis (NEC). NEC remains a leading cause of mortality and morbidity in premature infants, however, mechanisms remain elusive. Our previous work has shown that murine fetal exposure to maternal inflammation (FEMI) decreases neonatal Paneth cell function and density. Paneth cells are key to intestinal innate immunity and disruption of their biology is associated with NEC. Thus, understanding how FEMI disrupts Paneth cell biology may have important implications in understanding chorioamnionitis-associated NEC. Interferon lambda (IFN- $\lambda$ ) has recently been found to induce Paneth cell necroptosis through STAT 1 signaling. As IL-6 can upregulate STAT-1, we hypothesized that fetal exposure to prenatal maternal inflammation decreases Paneth cell function through IL-6 dependent upregulation of IFN- $\lambda$  expression.

**Methods:** IL6<sup>-/-</sup> mice on a C57BL/6J background were given a single intraperitoneal injection of 100 ug/kg of Escherichia coli O55:B5-derived LPS on day 15.5 of gestation and compared to wild type and sham controls. Neonatal small intestine was harvested on day P7 or P14 following spontaneous delivery. Tissue expression of the Paneth cell-specific antimicrobial peptide  $\alpha$ -defensin-1 was quantified by real time rtPCR. IL-6 receptor (IL-6R) and IFN- $\lambda$  expression was determined using in situ hybridization. Placental samples were harvested on day 19.5 for IL-6R evaluation. Statistical significance was determined using ANOVA.

**Results:** FEMI significantly decreased  $\alpha$ -defensin-1 expression compared to sham controls ( $0.6692 \pm 0.042$  vs  $1.03 \pm 0.06$ ,  $n \geq 5$  per group,  $p < 0.0001$ ).

However, this reduction was IL-6-dependent as IL6<sup>-/-</sup> mice had elevated levels compared to shams ( $1.55 \pm 0.08$  n $\geq 5$  per group,  $p < 0.0001$ ). IL-6R was visualized diffusely through the placenta and at the base of intestinal crypts where Paneth cells reside (N=3). Neonates exposed to FEMI had increased IFN- $\lambda$  RNA staining at the base of intestinal crypts compared to controls at P7 and P14 (N=3).

**Conclusion:** FEMI decreases neonatal Paneth cell function in an IL-6-dependent manner, likely due to robust expression of IL-6-R in placental tissue and at the base of intestinal crypts. Further, FEMI upregulates IFN- $\lambda$  expression within intestinal crypts that persists for several weeks of life after birth. Given our laboratory's prior findings that FEMI induces IL-6 dependent intestinal injury, our novel findings that FEMI increases IFN- $\lambda$  expression in intestinal crypts may help explain chorioamnionitis-associated development of NEC in preterm infants.

## O-025

**Establishment of a Mouse Uterine Explant Model to Study Inflammation in Pregnancy and Parturition.** Madeline Snedden,<sup>1</sup> Chandrashekar Kyathanahalli,<sup>1,2</sup> Emmet Hirsch\*,<sup>1,2</sup> <sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States.

**Introduction:** Inflammation has been implicated in the mechanisms of spontaneous labor at term and preterm. Most existing *in vitro* inflammation models use single cell types that fail to replicate the complex microenvironment and crosstalk at play within tissues *in vivo*. Here we developed a pregnant uterine explant model to study inflammatory processes in labor. We also examined the effects of 17  $\beta$ -estradiol (E2) and low-dose bacterial lipopolysaccharide (LPS) on the production of cytokines and contraction-associated proteins (CAPs).

**Methods:** Virgin female CD1 mice (8-16 weeks old, n=3) were paired with adult males. Uterine horns were removed from pregnant mice at gestation day (GD) 14.5 and 18.5, incised longitudinally, cleared of all fetal material, and divided into segments, each containing an intact decidual cap. These were halved, diced, and transferred into DMEM complete medium with or without E2 (10-100 pM) for 2h followed by stimulation with LPS (10 ng/ml) or control for 6h. Fresh-frozen tissues from the same animals (t=0) were also stored. Lactate dehydrogenase (LDH) release into culture media was measured as a marker of cellular viability. RNA and protein were extracted after 8-24h in culture. Quantitative PCR was performed for interleukin (*Il-1 $\beta$* ) and tumor necrosis factor (*Tnfa*). Western blotting was performed for CAPs: oxytocin receptor (OXTR), connexin 43 (Cx43), and pERK1/2. Statistical differences were assessed by Kruskal-Wallis and Dunn's post hoc test.

**Results:** Cellular viability was similar between treatment and control. Expression of ubiquitin C and myosin light chain 20 (used as endogenous, stably expressed controls for qPCR and western blot, respectively), was similar across all treatment groups. Compared to t=0, time spent in culture with or without E2 did not alter baseline inflammatory cytokine mRNA or CAPs. LPS significantly increased the expression of *Il-1 $\beta$*  and *Tnfa* mRNA (GD14.5 = E18.5) by about 15-fold compared to corresponding controls, but did not modulate OXTR, Cx43, or pERK1/2 (protein) levels. E2 priming did not further modulate the LPS-induced changes in inflammatory cytokine mRNAs and CAP proteins.

**Conclusion:** With optimized conditions for maintaining tissues *ex vivo*, the pregnant mouse uterine explant system may fill the gap between cell monolayer culture and *in vivo* conditions. The explants can be set up within 1h of euthanasia and are easily maintained in standard tissue culture medium without altering baseline expression of inflammatory cytokines or CAPs. These tissues retain their viability for at least 24h. Using LPS, we demonstrate here that the tissue explants can be used to study inflammation-associated changes pertinent to labor.

## O-026

**Inflammatory-Related Mir-612 Is Increased in Circulating Exosome-Like Vesicles from Women Undergoing Preterm Birth.** Bruna Ribeiro de Andrade Ramos\*,<sup>1</sup> Júlia Abbade Tronco†,<sup>1</sup> Márcio de Carvalho\*,<sup>1</sup> Patrícia Pintor dos Reis\*,<sup>1</sup> Juliano Coelho da Silveira\*,<sup>2</sup> Márcia Guimarães da Silva\*,<sup>1</sup> <sup>1</sup>Sao Paulo State University, Botucatu, Brazil; <sup>2</sup>Sao Paulo University, Pirassununga, Brazil.

**Introduction:** Preterm Labor (PTL) and Preterm Premature Rupture of Membranes (PPROM) lead to severe perinatal morbidity/mortality worldwide. Exosomes act in cell communication and contain microRNAs (miRNAs) that are potential biomarkers for these complications. We aimed to compare the expression, in exosome-like vesicles (sEV) from peripheral blood, of miRNAs between term and preterm pregnancies.

**Methods:** Case-control study with women with PTL, PPROM, term pregnancy with and without labor (TL and T) seen at the Botucatu Medical School Hospital, SP, Brazil. sEV were isolated from plasma using Total Exosome Isolation reagent (Invitrogen) and visualized by transmission electron microscopy (TEM). Western blot (WB) for detection of exosomal protein CD9 and Nanoparticle Tracking Analysis (NTA) were performed. Total RNA was extracted using DNA/RNA/Protein Purification kit (Norgen), quantified, then purified/concentrated using Amicon columns. The expression of over 800 miRNAs was performed by nCounter Human V3 miRNA Assay (NanoString). Clinical and sociodemographic data were compared by t-test and X<sup>2</sup>. miRNA counts were normalized by background threshold using miR-6721 as the endogenous control. A comparison of miRNAs expression and relative risk was performed by generalized linear model (Poisson or negative binomial distribution) and adjusted by Wald's correction.

**Results:** Samples from 31 pregnant women - 15 preterm (8 PTL and 7 PPROM) and 16 term (9 T and 8 TL) were included in this study. Mean gestational age was 34w5d  $\pm$  2w3d and 39w2d  $\pm$  1w for preterm and term groups, respectively. TEM, WB, and NTA confirmed sEV isolation, the median size of the particles was 115.5  $\pm$  4.5 nm. Expression of miR-612 was increased in preterm groups compared to term groups [PTL: 187.7  $\pm$  27.4 vs. TL: 155.7  $\pm$  29.9,  $p = 0.003$  RR=1.20 (1.06-1.36) and PPROM: 168.7  $\pm$  22.7 vs. T: 135.3  $\pm$  20.5,  $p < 0.001$  RR=1.25 (1.09-1.42)]. miR-612 has been shown to increase apoptosis in tumor cells and to regulate NF-KB inflammatory pathway, processes involved in PTL/PPROM pathogenesis. miR-1253, miR-1283, miR378e and miR-579-3p were down-regulated in PPROM compared to term (T) pregnancies. miR-1283 is involved in trophoblast proliferation while there is limited data on miR-1253, miR-378e and miR-579-3p.

**Conclusion:** miRNAs from circulating exosome-like vesicles are differently expressed between term and preterm pregnancies and have the potential to be used as non-invasive biomarkers for pregnancy complications and therapeutic purposes. Future *in vitro* studies will allow elucidating the exact role played by inflammatory-related and other microRNAs in the pathophysiology of PTL and PPROM.

## O-027

**Dose-Response Profile of a Novel Anti-Interleukin-1 Therapeutic, Rytvela, for Prevention of Preterm Birth.** Tiffany Habelrih†,<sup>1,2</sup> Sarah-Eve Loiselle†,<sup>1,2</sup> France Côté†,<sup>1,2</sup> Xin Hou\*,<sup>2</sup> Christiane Quiniou\*,<sup>2</sup> Sylvain Chemtob\*,<sup>1,2</sup> <sup>1</sup>Université de Montréal, Montreal, QC, Canada; <sup>2</sup>CHU Sainte-Justine, Montreal, QC, Canada.

**Introduction:** Preterm birth (PTB) is the leading cause of neonatal morbidity and mortality. Studies have shown that interleukin-1 (IL-1) plays an important role in the pathophysiology of PTB as it participates in inducing the production of pro-inflammatory mediators and uterine activating proteins (UAPs [FP, CX43, COX2, OTR]) leading to labour. More importantly, uteroplacental inflammation, associated with PTB's parturition pathways, is detrimental to the fragile fetus and can lead to long term sequelae. Our group has developed an allosteric antagonist of the IL-1 receptor, namely Rytvela, found to be potent and safe in preventing PTB by inhibiting the MAP-Kinase pathway while preserving the NF-kB pathway (important in innate immunovigilance), as it suppresses inflammation. Rytvela has been shown to inhibit inflammatory upregulation and uterine

activation. This study aims to further pre-clinical development of Rytvela by evaluating the optimal dose to inhibit the inflammatory cascade, prolong gestation and protect the fetus.

**Methods:** Pregnant CD-1 mice were injected with LPS (10 ug intra-peritoneal) or IL-1b (1 ug/kg intra-uterine) on gestational day (G) 16.5 to induce preterm labour. Rytvela was injected at different doses (0.1, 0.5, 1, 2, 4 mg/kg/day s.c.) from G16 to G19. Rate of prematurity (<G18.5), neonate survival, and newborn weight were evaluated. Gestational tissues (placenta, maternal plasma, fetal membrane, uterus, amniotic fluid) were collected on G17.5 to quantify cytokines, pro-inflammatory mediators, and UAPs by RT-qPCR and ELISA.

**Results:** Rytvela exhibited a dose-response profile and achieved Emax at a dose of 2 mg/kg/day by inhibiting 70% of LPS-induced PTB and 60% of IL-1b-induced PTB ( $p < 0.05$ ). Rytvela also attained Emax at a dose of 2 mg/kg/day for increasing neonatal survival by up to 65% in both *in vivo* models of PTB ( $p < 0.01$ ). Neonate weight and inhibition of pro-inflammatory mediators (up to 500-fold decrease) and UAPs in all gestational tissues achieved Emax at a dose of 1 mg/kg/day ( $p < 0.05$ ).

**Conclusion:** Emax of Rytvela at improving birth outcome and preventing inflammatory upregulation was achieved at 2 mg/kg/day. Rytvela exhibits desirable properties for the safe prevention and treatment of PTB. This information is critical as we proceed in the pre-clinical (and ultimately clinical) development of Rytvela.

### O-028

**Placenta Serum Amyloid A-2 (SAA-2) Plays an Important Role in Maternal Inflammation-Induced Adverse Fetal Outcomes.** Yang Liu†, Jin Liu†, Anguo Liu†, Irina Burd\*, Lei Jun\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** The intrauterine inflammation (IUI) and associated inflammatory cytokines during pregnancy are thought to be a major cause of abortion and preterm birth (PTB). Serum amyloid A (SAA) is one of the notable markers of inflammation. However, the potential role of SAA in maternal inflammation is not fully understood. In this study, we hypothesized that increased SAA induced by IUI is involved in the mechanisms of abortion and PTB, inhibition of SAA alleviates adverse fetal outcomes via regulating inflammatory cytokines.

**Methods:** CD-1 dams ( $n=36$ ) were sacrificed at embryonic (E) days 10-18 for evaluation of SAA expression. In another E17 group, using an established mouse model of IUI (LPS, 25mg/dam), dams ( $n=32$ ) were randomly allocated to four groups: PBS+PBS, LPS+PBS, LPS+SAA and PBS+SAA. SAA (5 mg/kg) or PBS was intraperitoneally infused one hour post injection (hpi) of LPS. Furthermore, 41 dams into four groups (PBS+PBS, LPS+PBS, LPS+ siRNA (SAA) and PBS+ siRNA (SAA)) received intravenous injection of either siRNA-SAA (1.5 mg/kg) or vehicle 1hpi following IUI. The PTB and viability were observed. Placentas were harvested daily from E10-18 or 24hpi after the surgery. Western blot and qRT-PCR were performed to characterize the levels of *Saa* and inflammatory cytokines. Standard statistical analyses were employed.

**Results:** There was no detectable expression noted of SAA2 in placenta from E10-18 while SAA1, 3 and 4 were consistently expressed. At 24 hpi, LPS+ PBS significantly decreased the fetal viability compared to PBS+PBS ( $p < 0.05$ ); LPS+SAA showed an even lower fetal viability compared to LPS+PBS ( $p < 0.05$ ). SAA2 was significantly increased in LPS+PBS ( $p < 0.01$ ) and LPS+SAA ( $p < 0.05$ ). There were no significant changes for SAA1, 3 and 4 between groups. Validation on the effect of siRNA of SAA2 demonstrated that SAA2 expression was inhibited while SAA1, 3 and 4 were not. When treated with siRNA of SAA2, the viability was markedly increased in LPS+ siRNA compared to LPS+PBS ( $p < 0.01$ ). Furthermore, there was a significant difference in preterm birth between LPS+PBS and LPS+ siRNA (40% vs 0%). The levels of *Il6* ( $p < 0.0001$ ), *Tnf- $\alpha$*  ( $p < 0.0001$ ) mRNA were increased in LPS+ PBS compared to PBS+PBS, while siRNA significantly decreased *Il6* ( $p < 0.001$ ) and *Tnf- $\alpha$*  ( $p < 0.001$ ) expressions.

**Conclusion:** Our study demonstrated that increased SAA2 in the placenta may be associated with increased PTB and abortion during

IUI. Administration of siRNA alleviated PTB and abortion following IUI. These changes may occur through the regulation of inflammatory immune responses.

### O-029

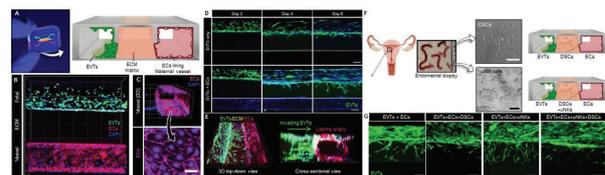
**A Microphysiological Model of Human Trophoblast Invasion during Early Embryo Implantation.** Sneha Mani,<sup>1</sup> Ju Young Park,<sup>1</sup> Jeremy Clair,<sup>2</sup> Cassidy Blundell,<sup>1</sup> Rachel Young,<sup>1</sup> Jessica Kanter,<sup>1</sup> Scott Gordon,<sup>3</sup> Yoon-Suk Yi,<sup>1</sup> Dan Dongeun Huh\*,<sup>1</sup> Monica Mainigi\*.<sup>1</sup> <sup>1</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>2</sup>Pacific Northwest National Laboratory, Richland, WA, United States; <sup>3</sup>The Children's Hospital of Philadelphia, Philadelphia, PA, United States.

**Introduction:** During embryo implantation, extravillous trophoblasts (EVTs) invade into the uterus and remodel maternal spiral arteries (SA). Abnormal invasion underlies many adverse pregnancy outcomes, but studying this process is challenging. We present a novel device, 'Implantation-on-a-chip' (IOC), which incorporates human cells and models EVT invasion. Using the IOC, we examine fetal-maternal cellular interactions and reveal previously unknown effects of pre-implantation maternal immune cells on early pregnancy.

**Methods:** The IOC device contains three lanes defined by two microfabricated rails (1A). The center lane containing extracellular matrix (ECM) hydrogel separates two channels seeded with EVT, isolated from first trimester placental tissue, and maternal endothelial cells (ECs) (1B), that form vessel-like structures (1C). To examine the influence of individual maternal cell types on EVT invasion, we added decidualized stromal cells (DSCs) and uterine NK (uNK) cells obtained from non-pregnant endometrial biopsies to the ECM hydrogel (1F). Proteomic analysis on effluent from the IOC identified factors that influence invasion.

**Results:** In 6 days, EVTs sprouted into the ECM scaffold and reached the vascular compartment. Adding ECs to the device markedly promoted EVT movement (1D). Invading EVTs infiltrated the vascular compartment (1E), resulting in endothelial disruption and apoptosis. Adding DSCs to the hydrogel led to inhibition of EVT movement, while incorporation of pre-implantation uNK cells promoted invasion (1F&G). Adding DSCs and uNK cells led to inhibition of EVT invasion suggesting that DSC contribution was dominant. Proteomics analysis identified ECs as a predominant source of soluble factors that influence EVT invasion.

**Conclusion:** The IOC device successfully mimics directional EVT invasion and SA remodeling. ECs play an active role in EVT migration, possibly through diffusion-mediated soluble factors. DSC addition inhibited EVTs, supporting a 'gatekeeper' role. Pre-implantation uNK cells, representing the bulk of the native immune environment encountered by the implanting embryo, promoted invasion. The IOC device provides a novel research platform, and could fulfill the urgent need for predictive preclinical models of human pregnancy.



### O-030

**The Utero-Placental Vascular Skeleton: A New Image Processing Technique to Estimate Utero-Placental Vascular Morphologic Development and the Association with Maternal Hemodynamic Adaptation to Pregnancy.** Eline S de Vos†, Anton HJ Koning\*, Régine PM Steegers-Theunissen\*, Sten P Willemsen\*, Bas B van Rijn\*, Eric AP Steegers\*, Annemarie GMGJ Mulders\*. *Erasmus Medical Centre, Rotterdam, Netherlands.*

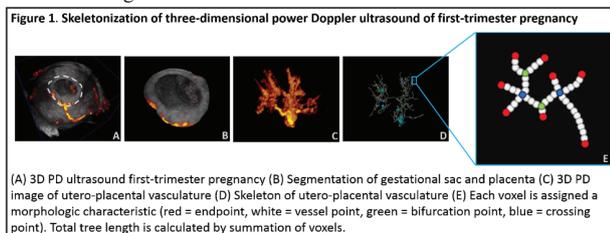
**Introduction:** Inadequate maternal hemodynamic adaptation to pregnancy, involving impaired systemic and local vascular remodeling, is associated with reduced first-trimester placental development and placenta-related complications. Using a new image processing technique,

we aimed to estimate utero-placental vascular morphologic development and investigated the association with maternal hemodynamic adaptation in the first trimester.

**Methods:** In 214 ongoing pregnancies, maternal hemodynamic adaptation was assessed by mean arterial blood pressure (MAP) and uterine artery (UtA) blood flow pulsatility (PI) and resistance indices (RI) at 7-9-11 weeks of gestation. At each visit, three-dimensional (3D) power Doppler (PD) volumes of the gestational sac and placenta were obtained. Using virtual reality segmentation and skeletonization, we developed a new image processing technique for the construction of the utero-placental vascular tree (Figure 1). Quantification of utero-placental vascular morphology was performed by assigning a morphologic characteristic to each voxel in the skeleton (i.e. end-, vessel-, bifurcation- or crossing point) and calculating total tree length. Linear regression analysis with adjustments for confounders was used to evaluate associations between MAP, UtA PI and RI, and utero-placental vascular morphology.

**Results:** In the total cohort, reduced first-trimester hemodynamic adaptation, estimated by high random intercepts of the UtA PI and RI, was negatively associated with utero-placental vascular morphologic development: UtA PI: vessel point:  $\beta = -17.93$ , 95%CI -31.24;-4.62,  $p = 0.009$ ; bifurcation point:  $\beta = -7.57$ , 95%CI -13.36;-1.78,  $p = 0.011$ ; crossing point:  $\beta = -5.49$ , 95%CI -9.67;-1.32,  $p = 0.010$ ; tree length:  $\beta = -16.19$ , 95%CI -27.77;-4.62,  $p = 0.006$ ; Similar associations were found between UtA RI and the skeleton parameters. Associations persisted after exclusion of pregnancies with placenta-related complications.

**Conclusion:** Virtual reality segmentation and skeletonization are used as new processing techniques and confirm associations between inadequate maternal hemodynamic adaptation to pregnancy and reduced first-trimester utero-placental vascular morphologic development. The predictive value of the utero-placental vascular skeleton markers needs further investigation.



## O-031

**Placental Cell-Specific Extracellular Vesicle Profiles from Syncytiotrophoblasts and Extravillous Trophoblasts in the First Trimester.** Terry Morgan, Mayu Morita, Leslie Myatt. *Oregon HS University, Portland, OR, United States.*

**Introduction:** Placental extracellular vesicles (EVs) are the subject of intense interest because they reflect relative placental health and provide insights into placental:maternal signaling. To date, most studies have focused on placental alkaline phosphatase (PLAP) positive EVs either by direct imaging or inference derived from size fractionation. We hypothesized that multiplexing multiple antibody markers on each EV would increase PLAP profile specificity and provide insights into relative contributions from syncytiotrophoblasts and extravillous trophoblasts (EVTs) into the maternal circulation during the first trimester.

**Methods:** Retrospective analysis of banked platelet poor plasma uniformly collected, processed, and frozen from 20 Caucasian women collected at 6, 8, and 10 weeks' gestation. Antibodies were validated using histologic sections to characterize syncytiotrophoblasts (PLAP/CD66f/CD63), EVT (PLAP/HLA-C/E-cadherin/CD63), and spiral artery "plug" cells (PLAP/CD56/E-cadherin/CD63). Cell- and size-specific EVs were imaged and quantitated per ul of plasma using nanoscale high resolution flow cytometry on a FacsAria Fusion (BD Biosciences) validated by our group. All samples were run in duplicate in a single batch. Plasma samples were simultaneously stained for CD41/CD61/CD9 platelet EV and CD31+/CD41- endothelial EV internal controls present in all plasma samples in the detectable range. Male plasma and stained 0.1  $\mu$ m PBS and

EV depleted plasma served as negative controls. EV size was estimated relative to Megamix polystyrene beads (100nm-900nm in size). Known quantities of flow sorted 200nm beads were used as sample dilution buffer to serve as concentration controls to normalize test volumes.

**Results:** Similar to others, we observed the greatest number of PLAP+ EVs/maternal plasma volume at 6 weeks gestation. Syncytiotrophoblast counts trended downward from 6-10 weeks ( $p < 0.05$ ) as did CD56+ plug cell EVs. In contrast, HLA-C+ EVT EVs represented the majority of PLAP+ events and counts remained constant from 6-10 weeks. Platelet and endothelial EVs were also constant.

**Conclusion:** Multiplex nanoscale flow cytometry provides a method to examine placental cell-specific EV profiles in maternal plasma, which we suspect may vary relative to uteroplacental blood flow and pregnancy outcome.

## O-032

**Impaired Cell Polarity and Bi-Directional Endothelial Cell-Matrix Interactions Mediate Disrupted Fetoplacental Angiogenesis.** Shuhan Ji\*, Diane Gumina†, Emily J Su\*. *University of Colorado School of Medicine, Aurora, CO, United States.*

**Introduction:** Placentas of pregnancies complicated by severe, early-onset fetal growth restriction with abnormal umbilical artery Doppler velocimetry (FGRadv) have sparse villous vascularity secondary to impaired angiogenesis. We have previously shown that FGRadv placental endothelial cells (ECs) exhibit deficient migration in the absence of substrate. However, this model did not account for potential effects of the placental microenvironment. To address this, we developed a novel model of placental fibroblast (FB) cell-derived matrices (CDM), allowing us to interrogate how intrinsic EC impairment and matrix differences influence EC function. We hypothesize that deficiencies in FGRadv ECs and matrix individually and synergistically contribute to impaired fetoplacental angiogenesis.

**Methods:** Human fetoplacental ECs and villous stromal FBs were isolated from placentas of FGRadv or term, uncomplicated pregnancies. Intrinsic EC function was further evaluated by assessing cell polarity through wound scratch assays followed by immunofluorescence for actin. To generate CDM, control or FGRadv FBs were seeded to confluence, subjected to ascorbic acid, and then decellularized. Subsequently, control or FGRadv ECs were plated on control or FGRadv CDM, with comparison of four groups: [1] Control ECs on control CDM, [2] Control ECs on FGRadv CDM, [3] FGRadv ECs on control CDM, and [4] FGRadv ECs on FGRadv CDM. EC polarity and migration were assessed via confocal or serial live-cell imaging, respectively. One-way ANOVA with Tukey post hoc testing was utilized for statistical analyses.

**Results:** In the absence of substrate, FGRadv ECs failed to establish appropriate polarity in response to a wound scratch, with actin fibers oriented in parallel to the wound, whereas actin was arranged perpendicularly in control ECs. To delineate the role of CDM, FGRadv ECs grown on FGRadv CDM demonstrated strikingly impaired polarity, similar to FGRadv ECs plated without substrate, when compared to the other three experimental EC-CDM groups. Intriguingly, control ECs exhibited a similar disruption of cell polarity when grown on FGRadv CDM, while control CDM partially rescued FGRadv EC polarity. Live cell migration tracking also showed significant impairments in EC velocity ( $p < 0.001$ ), accumulated distance ( $p < 0.001$ ), and Euclidean distance ( $p < 0.0001$ ) for both control and FGRadv ECs plated on FGRadv CDM, with control CDM rescuing FGRadv EC migratory properties.

**Conclusion:** FGRadv ECs exhibit inherent defects in polarity and migration, which support our previous findings. These intrinsic EC impairments persist when subjected to FGRadv CDM. In contrast, FGRadv CDM impairs control EC migratory properties, while control CDM rescues FGRadv EC angiogenesis. These data highlight the importance of bi-directional EC-matrix signaling on fetoplacental angiogenesis.

## O-033

**Activation of Amnion Signaling during Intrauterine Inflammation Is TNF-Dependent.** Pietro Presicce†, M Cappelletti†, M Morselli†, P Senthamaraikannan†, L Miller\*, M Pellegrini\*, A Jobe\*, C Choungnet\*, S Kallapur\*. <sup>1</sup>UCLA David Geffen School of Medicine, Los Angeles, CA, United States; <sup>2</sup>Inst for Quantitative and Computational Biosciences UCLA, Los Angeles, CA, United States; <sup>3</sup>Cincinnati Children's Hospital, Cincinnati, OH, United States; <sup>4</sup>UCD, Davis, CA, United States.

**Introduction:** Intrauterine infection/inflammation (IUI) is characterized histologically by neutrophilic infiltration of the placenta and fetal membranes and is frequently associated with preterm labor. As the amnion is in contact with the amniotic fluid (AF), it is strategically located to transduce inflammatory signals to mount the immune response. However, the role of amnion in the pathophysiology of IUI is poorly understood. We hypothesize that during IUI amnion expresses neutrophil chemoattractants and inflammatory mediators in a TNF-dependent manner.

**Methods:** We modeled IUI in Rhesus macaque (*Macaca mulatta*) at ~80% of gestation: 1. intra-amniotic (IA) saline (ctrl, n=17); 2. IA *E. coli* lipopolysaccharide (LPS) (O55:B5, 1mg) (LPS, n=16); and 3. Adalimumab maternal subcutaneous (40mg 3h prior to IA LPS) + IA (40mg 1h prior to IA LPS) (Adal+LPS, n=11). Adalimumab is a recombinant IgG1 anti-human TNF monoclonal Ab that reduces TNF levels in AF. Fetuses were delivered surgically 16h later. Amnion was peeled away from the underlying chorion and decidua in the extraplacental fetal membranes, and RNAseq analysis was performed.

**Results:** RNAseq analysis of amnion (ctrl n=2; LPS n=4, Adal+LPS n=3) showed that principal component analysis (PCA) of global gene-expression profiles segregated according to prenatal exposures (Fig 1A, DEGs, adjusted p value < 0.05). LPS exposure induced 247 genes (>2 fold change vs. ctrl). Of those genes, 31 were downregulated by Adalimumab (>2 fold change vs. LPS) and 12 are upregulated by Adalimumab (>2 fold change vs. LPS). 36 genes were downregulated by LPS (>2 fold change vs. ctrl). Specific genes involved in neutrophil activation/migration and regulation of inflammatory cytokines were induced by LPS and downregulated by TNF-blockade (Fig 1B). We validated RNAseq data by qPCR (ctrl n=5-17; LPS n=5-16; Adal+LPS n=5-11). TNF-blockade decreased the LPS-induced IL-1 $\beta$ , IL-6, IL-8/CXCL8, TNF $\alpha$ , CCL2/MCP-1 and CSF3 mRNAs (Fig 1C).

**Conclusion:** Amnion expression of neutrophil chemokines and inflammatory mediators during LPS induced IUI is TNF-dependent. TNF-signaling in the amnion may be an important component in the initiation of IUI. These results shed light on the pathophysiology of IUI.

## O-034

**Role of GATA2 and GATA3 in Syncytiotrophoblast Development during Mammalian Placentation.** Ananya Ghosh†. University of Kansas Medical Center, Kansas City, KS, United States.

**Introduction:** The placenta establishes a maternal-fetal exchange interface essential for nutrient and gas exchange between the mother and the developing fetus. The establishment of this exchange interface is essential for the successful progression of pregnancy and mammalian reproduction. Trophoblast cells of the placenta establish a vascular connection between the mother and the fetus and express hormones essential for the successful progression of pregnancy. A critically conserved step in the establishment of the maternal-fetal exchange surface in mammals is the fusion of trophoblast cells into a multinucleated syncytiotrophoblast layer (SynT). We discovered that GATA transcription factors, GATA2 and GATA3, are essential for trophoblast development and differentiation. In the early stages of mammalian development, GATA factors are selectively expressed in the trophoblast cells, and simultaneous global deletion of *Gata2* and *Gata3* genes in mice (**GATA-DKO**) abrogates placentation leading to embryonic death ~ embryonic day 8.0 (**E8.0**), a stage similar to first-trimester human pregnancy. The abnormal phenotype was accompanied by severe blood loss in the GATA-DKO placenta and a reduction in the labyrinth layer composed mainly of two layers of syncytiotrophoblast cells.

**Methods:** We, therefore, hypothesize that **GATA factors are essential to establish developmental stage-specific conserved transcriptional**

**programs in trophoblast cells and are essential for vascular remodeling at the maternal-fetal interface.** To address this, we have used trophoblast subtype-specific Cre-mouse models that would specifically ablate *Gata2* and *Gata3* genes in these trophoblast subpopulations and then study its effect on overall placental and embryonic development. We used SynT progenitor-specific marker *Gcm1* as the promoter driving the Cre recombinase, **Gcm1-Cre mice**, and crossed it with mice having *LoxP* sites flanking specific exons in *Gata2* allele and *Gata3* allele, **GATA-Floxed mice, to generate a conditional GATA-knockout mouse model.** The overall changes as a consequence of the knockout on the placental development were analyzed using single-cell RNA-Seq and confirmed via immunohistochemistry and in-situ hybridization.

**Results:** Our study shows that in *Gcm1<sup>Cre</sup>* mediated dual knockout of *Gata2* and *Gata3* arrest the growth of the embryos at E9.5 due to developmental abnormalities observed in the placenta particularly in the SynT layers. This was further confirmed by immunostaining for the specific SynT markers. Using single-cell RNA-Seq analyses, we also show that the loss of GATA factors in the SynT progenitors result in the loss of differentiated trophoblast populations, immune cells, and membrane transporters.

**Conclusion:** Based on the observations we infer that GATA2 and GATA3 plays an important role in the labyrinth progenitor cells and are essential for proper placentation and embryonic survival post-implantation.

## O-035

**Females Are Not Just 'Protected' Males: Sex-Specific Vulnerabilities in Placenta and Brain after Prenatal Immune Disruption.** Amy E Braun†, Pamela A Carpentier†, Brooke A Babineau†, Aditi R Narayan†, Michelle L Kielhold†, Hyang Mi Moon†, Jennifer Su†, Vidya Saravanapandian†, Theo D Palmer\*. <sup>1</sup>Stanford, Stanford, CA, United States; <sup>2</sup>Northwestern, Chicago, IL, United States; <sup>3</sup>Trinity Biosciences, Brisbane, CA, United States; <sup>4</sup>Oregon Health & Science University, Portland, OR, United States; <sup>5</sup>University of California San Diego, La Jolla, CA, United States.

**Introduction:** Current perceptions of genetic and environmental vulnerabilities in the developing fetus are biased towards poor male outcomes. An argument is made that males are more vulnerable to gestational complications and neurodevelopmental disorders, the implication being that an understanding of disrupted development in males is sufficient to understand causal mechanisms that are assumed to be similar but attenuated in females. Here we examine this assumption in the context of a maternal immune disruption of fetal development and its postnatal outcomes in female and male mice.

**Methods:** Pregnant C57Bl/6 mice were treated with low dose (60 $\mu$ g/kg) lipopolysaccharide (LPS) at embryonic day 12.5. Acute changes in placenta and fetal brain were measured, along with long-term changes in adult offspring cortex cytoarchitecture, densities and ratio of excitatory (Satb2+) to inhibitory (Parvalbumin+) neuronal subtypes, postnatal growth and behavior outcomes were compared between male and female offspring.

**Results:** Males show a significant reduction in proliferation in the ventricular zone of the developing cortex at 2 hours after LPS (24.84 phH3+ cells/ $\mu$ m<sup>2</sup>  $\pm$  5.21 vs. 19.38  $\pm$  4.83 phH3 cells/ $\mu$ m<sup>2</sup>; p=0.0414; n  $\geq$  13), and reduced placental weight at 24 hours following LPS (103 $\mu$ g  $\pm$  15 vs. 96 $\mu$ g  $\pm$  15; p = 0.0182; n  $\geq$  44) while females were not significantly affected by LPS. In adult cortex, males exposed to prenatal LPS show markedly depleted Parvalbumin (PV+) and Satb2+ cell densities (p < 0.0001 all regions; n=6), alongside social and learning-related behavioral abnormalities. In contrast, females exhibit unique acute inflammatory signaling in fetal brain (730.11 pg/ $\mu$ g  $\pm$  67.25 vs. 937.61 pg/ $\mu$ g  $\pm$  139.92; p=0.0207; n=6), delayed postnatal body growth (p < 0.0001; n  $\geq$  13), and opposite changes in PV+ density (p = 0.0126; posterior; n  $\geq$  5), alongside changes in juvenile behavior in response to LPS (84.85 initiations/trial  $\pm$  34.69 vs. 104.9  $\pm$  16.34 initiations/trial; p = 0.0425; n  $\geq$  6), as well as elevated anxiety-related behavior as adults.

**Conclusion:** While males are more severely impacted by prenatal immune disruption in certain measures, females exposed to the same insult exhibit a unique set of vulnerabilities and developmental consequences absent

in males. Our results reveal disparate sex-specific features of prenatal vulnerability to maternal inflammation and warn against the casual extrapolation of male disease mechanisms to females.

### O-036

**Derivation of Human Trophoblast Stem Cells from Pluripotent Stem Cells to Model Early Placental Development.** Francesca Soncin, Mariko Horii, Rob Morey†, Tony Buy, Daniela Requena, Virginia Chu Cheung†, Omar A Farah, Don Pizzo, Mana M Parast. *University of California San Diego, La Jolla, CA, United States.*

**Introduction:** The placenta is a fetal-derived organ required for proper embryo development. The investigation of early placental development in human is associated with restricting technical and ethical issues. Recently, human trophoblast stem cells (hTSC) were derived from first trimester placental tissues (Okae et al. 2018); however, aside from ethical challenges, the unknown disease potential of these cells raises questions about their scientific utility. Therefore, in this study, we applied culture conditions for derivation of hTSCs, to pluripotent stem cell (hPSC)-derived trophoblast for the establishment of hPSC-derived TSC.

**Methods:** Two embryonic stem cell lines (hESC), WA09/H9 (female) and WA01/H1 (male), and one induced pluripotent stem cell line (iPSC), derived from human dermal fibroblasts, were differentiated into cytotrophoblast (CTB)-like cells by treatment with BMP4 and IWP2 for 4 days. Cells were then re-plated on collagen IV-coated plates in hTSC media. Cells were maintained at sub-confluency and passaged as necessary up to 10 passages. Cells were tested for surface EGFR expression by flow cytometry and for multiple trophoblast markers by qPCR.

**Results:** All cell lines tested were maintained in a proliferative state under hTSC culture conditions for over 10 passages. Over this period, cells went through an adaptation period and gradually changed morphology from cuboidal, CTB-like cells, to small round cells forming tight colonies, similar to placenta-derived hTSC. Expression of surface EGFR was over 90% at time of re-plating in hTSC media. Its expression oscillated during the adaptation period with levels as low as 30-50% before increasing and stabilizing around 80-90%. hPSC-derived TSC showed expression of TSC markers such as TP63, VGLL1, ELF5 and GATA3 with low or no expression of syncytiotrophoblast (STB) and extravillous trophoblasts (EVT) markers.

**Conclusion:** We were able to establish TSC from both hESC and iPSC after initial differentiation into CTB-like cells. We are currently assessing the differentiation potential of the hPSC-derived TSC and comparing both gene expression and epigenetic profiles of these cells to placenta-derived hTSCs. Since iPSCs can be derived by reprogramming placental cells collected at delivery, when the pregnancy outcome is known, this model would allow for modeling both normal and abnormal trophoblast differentiation in the context of any placental pathology.

### O-037

**Percutaneous Electrical Stimulation of Skeletal Muscle Attenuates Insulin Resistance in Pregnant Rats with Diet-Induced Obesity.** David Coggin-Carr†,1,2 Keara McElroy-Yaggy\*,3 Paul D Taylor\*,4 Anna L David\*,2 Elisabet Stener-Victorin\*,5 Tom Jetton\*.3 <sup>1</sup>University of Vermont, Burlington, VT, United States; <sup>2</sup>University College London, London, United Kingdom; <sup>3</sup>University of Vermont, Colchester, VT, United States; <sup>4</sup>Kings College London, London, United Kingdom; <sup>5</sup>Karolinska Institutet, Stockholm, Sweden.

**Introduction:** Maternal obesity is common worldwide, conferring elevated risks of maternal/fetal complications and adult-onset disease in offspring. Pregnancy/obesity are states of increased insulin resistance (IR). Percutaneous electrical stimulation (ES) of skeletal muscle mitigates IR in polycystic ovary syndrome and diabetes; its effect during pregnancy is unknown. We hypothesized that ES in pregnant rats with diet-induced obesity (DIO) would attenuate increased IR, quantified *in vivo* by (gold standard) euglycemic hyperinsulinemic clamps.

**Methods:** We established DIO in female Wistar rats (weight 230±3g, age 76±1d) with a high fat (45%) high sucrose (20%) diet (TestDiet 58V8) given for 44d [IQR 27-47] before timed mating. Obese pregnant dams (n=38) were randomized to receive 12-14 ES exposures under 1-2%

isoflurane anesthesia (DIO+ES group, n=19) or no ES but equivalent anesthesia/handling (DIO group, n=19). ES was administered daily from embryonic day (E)1-4 using filiform needles inserted into rectus abdominis, tibialis anterior and triceps surae, stimulated at 3/15Hz frequency (10mA intensity) for 30 min/d. On E18±1d, after an 18h fast, clamps were performed by investigators blind to treatment group after cannulating the left carotid artery for sampling and right external jugular vein for insulin/glucose infusion. Clamps were also performed (during estrus) in a contemporaneous non-pregnant control group of 10 non-obese same-age female Wistars fed standard chow. Serum corticosterone was measured by ELISA as a marker of stress. Groups were compared by one-way ANOVA and post-hoc LSD tests.

**Results:** By mating, obesogenic diet-exposed rats weighed 19% more than control-fed rats (300±3 vs 277±6g, p<0.001; >2SD above mean). Pregnancy + obesity resulted in marked IR, reflected by a lower steady state glucose infusion rate (GIR) during the clamp in the DIO group vs non-pregnant non-obese controls (13.3±1.33 vs 18.2±1.37mg/kg/min, p=0.014). GIR was restored in DIO+ES vs (untreated) DIO groups (16.8±1.01 vs 13.3±1.33mg/kg/min, p=0.029), reflecting enhanced whole-body insulin sensitivity, and did not differ from non-pregnant non-obese controls (p>0.05). Corticosterone was increased in DIO+ES/DIO groups vs non-pregnant non-obese controls (122±10.5/132±11.8 vs 74±11.9ng/ml, p=0.011/p<0.001) but not significantly impacted by ES.

**Conclusion:** ES attenuated IR induced by pregnancy/obesity in rats without evoking a measurable stress response. If this can be replicated clinically, ES could have a role in the management of maternal obesity. Mechanisms of action and safety require further study.

### O-038

**Seasonality and the Immune System during Pregnancy: Impact of Seasonal Affective Disorder.** Cindy Xin Wen Zhang†, Robert Levitan, Stephen Matthews. *University of Toronto, Toronto, ON, Canada.*

**Introduction:** The immunology of pregnancy is complex, requiring a balance between heightened and suppressed maternal immune responses throughout gestation. Aside from internal cues provided by the fetus and placenta, the immune system also responds to environmental cues, including the change in seasons. One extreme manifestation of seasonality in humans is Seasonal Affective Disorder (SAD), a form of depression characterized by overeating, hypersomnia, and weight gain in the fall-winter period. There is evidence for altered immune function in SAD patients which has implications for understanding seasonal influences on psychopathology risk in children. We hypothesized that high maternal seasonality, as in SAD, is associated with a unique pattern of immune activity during pregnancy, and that season of sample collection moderates this effect.

**Methods:** All participants were enrolled in the Ontario Birth Study (OBS), the sample included 100 highly seasonal mothers with a lifetime history of SAD and 100 age- and BMI-matched control mothers. Maternal plasma c-reactive protein (CRP) was measured in the 1<sup>st</sup> and 3<sup>rd</sup> trimester using ELISAs. In a subsample (n=67), gene expression of pro- (*IL1β*, *IL6*, *TNFα*) and anti-inflammatory (*IL4*, *IL10*) cytokines were measured in peripheral leukocytes collected at the same times as plasma. Multiple regressions were conducted to predict CRP using the variables maternal seasonality group, gene expression of each cytokine, seasonality group by cytokine interaction, and BMI as a covariate.

**Results:** In the 1<sup>st</sup> trimester, there was a significant effect of maternal seasonality (p=0.044) but no effect of season (p=0.71), and no interaction effect (p=0.99). Highly seasonal women had significantly lower plasma CRP than controls at this time (p=0.041). There was no effect of maternal seasonality or season in the 3<sup>rd</sup> trimester. There was no change in plasma CRP across pregnancy (p=0.56). There was a significant increase in anti-inflammatory cytokine *IL10* expression from trimester 1 to trimester 3 in all subjects (p=0.002). Upon further investigation, this difference was only present in highly seasonal subjects (p=0.001). In the 1<sup>st</sup> trimester, multiple regression analyses revealed a significant interaction effect of *IL1β* gene expression and maternal seasonality group (p=0.028), and a main effect of *IL10* gene expression (p=0.047).

**Conclusion:** This is the first study to investigate effects of season and maternal SAD on immune function during pregnancy. The finding that highly seasonal women had lower levels of plasma CRP in trimester 1 has implications for understanding fetal neurodevelopment. Primary neurulation and neural proliferation occur during the first trimester and are susceptible to inflammatory insults that may lead to the aberrant cortical development associated with psychiatric disorders. Future studies will assess whether maternal prenatal data relate to offspring developmental outcomes.

### O-039

**Estradiol Stimulates Pregnancy-Dependent H<sub>2</sub>S Biosynthesis in Human Uterine Artery Endothelial Cells via ER $\alpha$ /ER $\beta$ -Mediated Upregulation of CBS Transcription.** Jin Bai†, Thomas J Lechuga†, Qian-rong Qi†, Yi-hua Yang†, Quan-wei Zhang†, Yan Li†, Dong-bao Chen\*. *University of California, Irvine, CA, United States.*

**Introduction:** Normal pregnancy is featured by dramatic rises in uterine blood flow, providing the bidirectional maternal-fetal exchanges of nutrients and respiratory gases obligated for *in utero* fetal growth and survival. Pregnancy-associated uterine vasodilation is accompanied with dramatically elevated estrogens and augmented production of a newly recognized uterine artery (UA) vasodilator hydrogen sulfide (H<sub>2</sub>S) in UA endothelial cells (UAECs) and smooth muscle cells by selectively upregulating the expression of its producing enzyme cystathionine  $\beta$ -synthase (CBS) but not cystathionine  $\gamma$ -lyase (CSE) *in vivo*. However, whether estrogens stimulate pregnancy-dependent UA H<sub>2</sub>S biosynthesis is unknown. This study using fully validated primary human UAECs isolated from both non-pregnant (NP) and pregnant (P) women was to determine if estrogens stimulate pregnancy-dependent UAEC H<sub>2</sub>S biosynthesis via specific estrogen receptor, ER $\alpha$  and/or ER $\beta$ , mediated CBS transcription.

**Methods:** Real-time PCR, Western blot and methylene blue assay were used to measure CBS/CSE mRNA expression, protein expression and H<sub>2</sub>S production in hUAECs, respectively. Putative estrogen-responsive elements (EREs) were identified by LASAGNA motif search tool. Luciferase reporter gene expression studies using human CBS promoter and its 5'-deletion constructs were used to locate  $\alpha$ / $\beta$ EREs in CBS proximal promoter and further verified by ChIP-qPCR using specific ER $\alpha$  and ER $\beta$  antibodies.

**Results:** Baseline H<sub>2</sub>S production and CBS mRNA/protein were higher ( $p < 0.05$ ) in P- vs. NP- hUAEC culture *in vitro*; all were stimulated by treatment with estradiol-17 $\beta$  (E<sub>2</sub> $\beta$ , 10 nM, 24-48 h) by 2-3 fold ( $p < 0.01$ ) in P- vs. NP- hUAECs and blocked by 1  $\mu$ M ER antagonist ICI 182,780. Human proximal CBS promoter contains 2  $\alpha$ EREs and 4  $\beta$ EREs, preferentially binding ER $\alpha$  and ER $\beta$ , respectively. Luciferase studies located ER $\alpha$  and ER $\beta$  interactions with specific  $\alpha$ EREs and  $\beta$ EREs that mediate E<sub>2</sub> $\beta$ -stimulated pregnancy-dependent *trans*-activation of CBS promoter; these results were further verified by ChIP-qPCR and sequencing analyses. In P-hUAECs, ER $\alpha$  or ER $\beta$  agonist alone *trans*-activated CBS promoter and stimulated mRNA/protein expressions to levels similar to that of E<sub>2</sub> $\beta$ , while ER $\alpha$  or ER $\beta$  antagonist alone abrogated E<sub>2</sub> $\beta$ -stimulated responses. E<sub>2</sub> $\beta$  did not stimulate either human CSE promoter *trans*-activation or mRNA/protein expressions in NP- and P- hUAECs. E<sub>2</sub> $\beta$  also stimulated ER-dependent CBS but not CSE mRNA/protein expressions in UA *ex vivo* explant ring cultures.

**Conclusion:** Estrogens stimulate pregnancy-dependent hUAEC H<sub>2</sub>S biosynthesis specifically by upregulating ER $\alpha$  and ER $\beta$  mediated CBS transcription (Supported by NIH RO1 HL70562 and R21 HD097498).

### O-040

**Placental MiRNAs Predicted to Target Insulin Signaling Pathways Are Associated with Maternal Insulin Resistance in Late Pregnancy.** Fernanda L Alvarado Flores†, William Beyer, Tomoko Kaneko-Tarui, Tianjiao Chu, Yoel Sadovsky, Patrick Catalano, Perrie O'Tierney-Ginn\*. <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, United States.

**Introduction:** Development of insulin resistance (IR) in late pregnancy is important for fetal growth. This increase in IR is associated with impairments in post-receptor insulin signaling (IS) in skeletal muscle -

specifically, a decrease in IRS1 expression and an excess of its inhibitory molecule p85 $\alpha$ . The primary initiator of these changes is unknown. We hypothesized that placental miRNAs are associated with maternal IR in late pregnancy, based on their predicted targets within the IS pathway.

**Methods:** 132 healthy women (70 lean (BMI<25); 62 obese (BMI>30)), were recruited at scheduled term C-section. TargetScan v.7 was used to predict 4 miRNAs targeting IRS1 and p85 $\alpha$ . Expression of miR126-3p, miR128-3p, miR424-5p and miR144-3p was measured in placental tissue using qPCR. Maternal HOMA-IR was calculated as a measure of IR. Data were log normalized; group differences were assessed by t-test. Multi-linear regression models were generated to adjust for relevant clinical variables.  $p < 0.05$  was considered significant. MiRNet 2.0 tool was used for functional interpretation and network analysis by using hypergeometric test.

**Results:** HOMA-IR was 62% higher in obese women at delivery ( $p < 0.001$ ). Placental expression of all selected miRNAs was higher in women with obesity ( $p < 0.001$ ). Placental miRNAs 126-3p, 128-3p and 424-5p were positively correlated with maternal HOMA-IR, conditional on fetal sex, gestational age and 4 other factors. After adjusting for pre-pregnancy BMI, none remained significant (Table). Network enrichment analysis for the 4 miRNAs found the IS pathway highly significant (adj. P-value= 0.004). Functional analysis verified that IRS1 is targeted by miR144-3p and miR126-3p, and p85 $\alpha$  is targeted by miR128-3p, miR424-5p, and miR126-3p.

**Conclusion:** Placental expression of miRNAs targeting the IS pathway was higher in women with obesity than those without. Three of these miRNA correlated with maternal IR at time of delivery, but not independently of pre-gravid BMI. These findings suggest that maternal adiposity may be an epigenetic modifier in any potential effect of these placental miRNA on maternal IR.

Table

miRNA	Obese vs Lean	Correlation HOMA-IR	Model 1			Model 2			Model 3		
			% change	All (r)	$\beta$	SE	P-value	$\beta$	SE	P-value	$\beta$
miR-126-3p	45*	0.32*	0.36	0.10	0.0004	0.37	0.10	0.001	0.16	0.12	0.18
miR-128-3p	22*	0.19*	0.34	0.14	0.02	0.33	0.15	0.03	0.16	0.14	0.27
miR-424-5p	32*	0.22*	0.18	0.11	0.04	0.16	0.09	0.09	0.04	0.09	0.61
miR-144-3p	45*	0.09	0.06	0.06	0.32	0.08	0.07	0.24	0.00	0.06	0.98

Model 1: adj. for fetal sex and GA

Model 2: adj. with model 1 + ethnicity, maternal age, smoking status, and parity

Model 3: adj. with model 2 + maternal pre-pregnancy BMI

\*P-value<0.05

### O-041

**Clot Formation Potential in the Late Pregnant and Postpartum Woman.** Elizabeth Barker†, Ira Bernstein, Thomas Orfeo, Kelley McLean, Maria Cristina Bravo. *Larner College of Medicine, University of Vermont, Burlington, VT, United States.*

**Introduction:** Microvesicles (MV) are phospholipid bilayer enclosed structures that bud off from various cell types, retaining the membrane composition (lipid and protein) of their originating cell. Endothelial cells, platelets, leukocytes, erythrocytes, and placental trophoblasts have all been identified as sources of plasma MVs. Some MVs have been shown to express tissue factor, and thus can potentially initiate the process of thrombin generation and fibrin clot formation. Here we report on the potential contributions of MVs to clot formation potential in healthy women during the peripartum period.

**Methods:** Healthy women (N=20) were prospectively enrolled in a longitudinal study at an academic hospital in a protocol approved by the Institutional Review Board. Two blood collections were obtained: Visit 1 (V1) was in the late third trimester, and Visit 2 (V2) was 18-48 hours postpartum (10 cesarean deliveries and 10 vaginal deliveries). Blood was collected into citrate and platelet poor plasma was prepared and frozen (PPP). The Thrombodynamics T2F analyzer was used to measure plasma clot formation from two locations within the measurement cuvette: 1) at the site from exogenous immobilized tissue factor (TF) source (Clot<sub>TF</sub>), and 2) at sites distant from the immobilized TF source (Clot<sub>sp</sub>). PPP was thawed in the presence of corn trypsin inhibitor to prevent contact pathway activation and either 1) immediately recalcified and assayed, or 2) centrifuged at 16,000x g for 45 minutes at 15°C to sediment potential

MVs, the supernate was collected (PPP<sub>desMV</sub>) and subsequently recalcified and assayed. Clot formation parameters describing onset time and rate were extracted, including: rate of Clot<sub>TF</sub> growth ( $V_{TF}$ ); onset time of Clot<sub>Sp</sub> ( $T_{Sp}$ ), and the rate of Clot<sub>Sp</sub> growth ( $V_{Sp}$ ). Comparisons were made across Visits using a paired Student's t-test. Data are presented as mean  $\pm$  SD or Pearson correlation values.

**Results:** Clot<sub>Sp</sub> parameters were more robust ( $p < 0.001$ ) at V2 compared to V1 in PPP as characterized by onset time ( $T_{Sp}$ , 14.6 $\pm$ 4.3 vs. 22.1 $\pm$ 6.5 min) and rate of formation ( $V_{Sp}$ , 4.9 $\pm$ 2.7 vs. 2.2 $\pm$ 1.1 %/min). Route of delivery did not appear to affect ClotSp formation. No Clot<sub>Sp</sub> was observed in any PPP<sub>desMV</sub> preparations. The  $V_{TF}$  in PPP was significantly ( $p < 0.005$ ) greater at V2 compared to V1, 59.1 $\pm$ 7.9 vs. 51.5 $\pm$ 2.5  $\mu$ m/min.  $V_{TF}$  was significantly greater in PPP compared to PPP<sub>desMV</sub> at both visits (35.5 $\pm$ 1.9  $\mu$ m/min at V1, 38.1 $\pm$ 3.2  $\mu$ m/min at V2). In PPP preparations, the  $V_{TF}$  correlated with  $V_{Sp}$  across all visits ( $r=0.77$ ).

**Conclusion:** Compared to late pregnancy, the immediate postpartum period demonstrates an increase in endogenous clot potential that derives from sedimentable material. This material also potentiates faster clot formation from immobilized TF. Together these findings may implicate procoagulant MVs as contributors to the increased prothrombotic risk postpartum.

#### O-042

**Identification and Isolation of Rare Microchimeric Cells in Maternal and Cord Blood.** Whitney Harrington\*,<sup>1</sup> Yonghou Jiang,<sup>2</sup> Whitney Harrington\*,<sup>2</sup> John Houck,<sup>2</sup> Marc Carlson,<sup>2</sup> Stephen McCartney†,<sup>1</sup> Kelsey Olerich†,<sup>1</sup> Sami B Kanaan,<sup>3</sup> J Lee Nelson,<sup>3,1</sup> Raj Shree.<sup>1</sup> <sup>1</sup>University of Washington, Seattle, WA, United States; <sup>2</sup>Seattle Children's Research Institute, Seattle, WA, United States; <sup>3</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

**Introduction:** During pregnancy cells are exchanged between the mother and fetus and may persist for many years in the recipient, a phenomenon known as microchimerism. In the mother, fetal Mc (FMc) can be detected many years after delivery and may contribute to future health and disease, including reproductive fitness. In the offspring, maternal Mc (MMc) can be detected in both immune and non-immune cells in peripheral blood and distributed throughout the body, however little is known about the functional and immunologic consequence of this maternal graft. The rare frequency of these microchimeric cells presents a challenge to their identification and isolation for down-stream studies.

**Methods:** We have developed an approach to identify rare microchimeric cells using antibodies specific to non-shared HLA Class-I antigens as part of a broad flow cytometry immunophenotyping panel. In validation studies utilizing a dual antibody labeling strategy in which both donor and recipient cells may be uniquely labeled, we are able to reliably detect donor cells as low as 0.01%. By comparison, single labeling donor cells allows for enrichment of rare cells but requires further confirmation of cell origin. Sorting rare donor cells for downstream single cell RNA sequencing (scRNAseq) allows confirmation of cell origin using divergent single nucleotide polymorphisms and recovery of microchimeric T cell receptor (TCR) sequences.

**Results:** We have applied this technique to identify both FMc and MMc in a cohort of US maternal-fetal pairs. In an example maternal-fetal pair utilizing the dual antibody strategy, we identified FMc in maternal peripheral blood mononuclear cells collected prior to delivery at a level of 0.034%. Fetal origin cells were comprised of 77% T cells, the majority of which were naive, 14% monocytes, 11% dendritic cells, and rare B-cells and NK cells. In two cord bloods utilizing single antibody labeling of maternal cells, we were able to identify presumptive MMc at levels of 0.014% and 0.020%, including 39% and 22% T cells respectively. Confirmation of cell origin and distribution along with microchimeric TCR repertoire and predicted antigen specificity from our scRNAseq data is ongoing.

**Conclusion:** We have developed a method to robustly phenotype and isolate rare microchimeric cells for downstream analysis and functional studies. We are now applying this technique to isolate rare microchimeric

fetal and maternal cells in the setting of both normal and abnormal pregnancy to better understand the immunologic consequence of these grafts.

#### O-043

**Women with Adverse Pregnancy Outcomes Have Higher Risk of Midlife Stroke: The PATH Study.** Eliza C Miller, Natalie A. Bello, Rindcy Davis, Alexander M Friedman, Mitchell S.V. Elkind, Ronald Wapner, Sarah E. Tom\*. *Columbia University, New York, NY, United States.*

**Introduction:** A history of adverse pregnancy outcomes (APO) has been associated with an increased risk of future cardiovascular disease, including stroke. Few large US population-based surveys included data on APO.

**Methods:** The Population Assessment of Tobacco and Health (PATH) study is a nationally representative survey of 45,971 US respondents. Female respondents  $\geq 50$  years old who reported pregnancy history at the 2013-2014 baseline interview were included in this cross-sectional analysis ( $n = 3303$ ; weighted  $n = 37,312,466$ ). The primary exposure was self-reported history of  $\geq 1$  APO, including preterm delivery, low birth weight, preeclampsia, placenta previa, placental abruption, and stillbirth. The primary outcomes were 1) self-reported stroke before age 60 and 2) any self-reported stroke. We used weighted logistic regression models to estimate odds ratios (OR) and 95% confidence intervals (95%CI) for the association between APO and stroke, adjusting for age, race/ethnicity, socioeconomic status, parity, and self-reported vascular risk factors.

**Results:** Among stroke-free respondents, 16 % reported  $\geq 1$  APO. Among women who reported a stroke before age 60, 40% reported  $\geq 1$  APO ( $p < 0.001$  compared to respondents who did not experience stroke  $< 60$  years); among women reporting stroke at any age, 26 % reported  $\geq 1$  APO ( $p = 0.01$  compared to stroke-free respondents). Controlling for covariates, women with APOs had increased odds of stroke before age 60 (adjusted OR 2.47, 95% CI 1.35, 4.51). Addition of hypertension to the model did not significantly change the effect size (adjusted OR 2.43, 95%CI 1.34, 4.40). The association of APOs with stroke at any age was not significant after controlling for covariates (adjusted OR 1.55, 95%CI 0.92, 2.62), with a similar relationship when including hypertension in the model.

Nested models: Odds ratios (95% confidence intervals)					
	Model 1 (un-adjusted)	Model 2 (+ age, race/ethnicity)	Model 3 (+ education, income)	Model 4 (+diabetes, cholesterol, parity, smoking)	Model 5 (+hypertension)
<b>Outcome 1: Stroke &lt;60 years</b>					
Any APO	3.56 (1.87, 6.77)	3.36 (1.75, 6.46)	3.18 (1.69, 5.97)	2.47 (1.35, 4.51)	<b>2.43 (1.34, 4.40)</b>
Preterm delivery	3.37 (1.43, 7.92)	3.30 (1.41, 7.73)	3.59 (1.54, 8.36)	2.54 (1.15, 5.56)	2.39 (1.12, 5.08)
Low birth weight	2.50 (1.06, 5.89)	2.35 (1.00, 5.54)	2.25 (1.00, 5.05)	1.91 (0.83, 4.40)	1.87 (0.83, 4.20)
Other APO	3.33 (1.66, 6.68)	3.32 (1.65, 6.66)	2.72 (1.30, 5.71)	2.24 (0.97, 5.15)	2.31 (1.00, 5.34)
<b>Outcome 2: Stroke at any age</b>					
Any APO	1.90 (1.12, 3.22)	1.94 (1.13, 3.30)	1.88 (1.13, 3.14)	1.55 (0.92, 2.62)	1.52 (0.89, 2.58)
Preterm delivery	2.48 (1.17, 5.24)	2.53 (1.18, 5.40)	2.63 (1.26, 5.49)	2.05 (0.99, 4.26)	1.98 (0.97, 4.01)
Low birth weight	1.20 (0.55, 2.63)	1.14 (0.51, 2.54)	1.10 (0.51, 2.36)	0.98 (0.44, 2.17)	0.92 (0.41, 2.03)
Other APO	1.61 (0.85, 3.08)	1.69 (0.88, 3.24)	1.52 (0.80, 2.89)	1.32 (0.68, 2.54)	1.39 (0.73, 2.68)

**Conclusion:** In this analysis of self-reported, US nationally representative survey data, APOs were independently associated with midlife stroke. Women with APOs are at higher risk for midlife stroke and should have targeted risk reduction interventions.

**O-044**

**Isolating Mechanisms on Future Cardio-Renal Risk in Mothers with Complicated Pregnancy.** Kimberley J. Botting, Wen Tong†, Youguo Niu†, Tessa A Garrud†, Lin Zhang†, Sage G Ford†, Qiang Lyu†, Olga V Patey†, Dino A Giussani\*. *University of Cambridge, Cambridge, United Kingdom.*

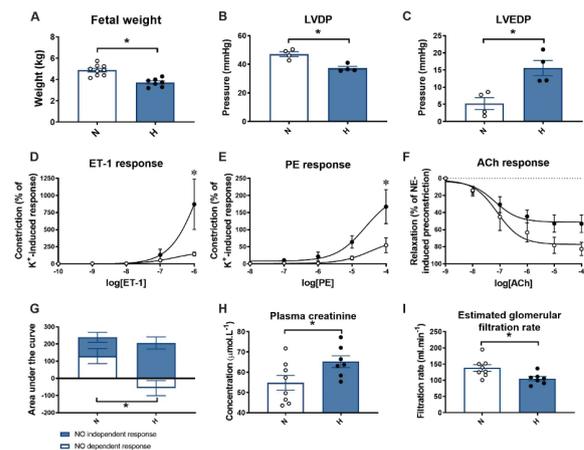
**Introduction:** Women who develop complicated pregnancy, such as preeclampsia and fetal growth restriction (FGR), are at greater risk of cardiovascular disease (Ray et al. *Lancet* 366:1797,2005). However, it is unclear if this is due to a shared cause, such as genetic predisposition, or because of damage during the adverse pregnancy. Healthy ewes develop utero-placental pathology similar to gestational hypertension and FGR, during hypoxic pregnancy (Tong et al. *Reprod Sci* 26(1):T-196, 2019). However, whether these ewes develop cardiovascular complications remains unknown. We have isolated the effects of hypoxic pregnancy in sheep on maternal cardiovascular function in late gestation.

**Methods:** Pregnant ewes carrying a singleton were exposed to normoxia (N) or hypoxia (H: 10% O<sub>2</sub> in maternal air) from 0.7 of gestation in isobaric chambers (Brain et al. *PLoS Biol* 17(1):e2006552, 2019). At 138 days gestational age (dGA; term at 145 dGA), maternal blood samples were taken for plasma creatinine and calculation of estimated glomerular filtration rate (eGFR). Following euthanasia, the maternal heart was isolated and left ventricle (LV) function investigated (Langendorff preparation). Third-order maternal femoral arteries were isolated and vascular function established via wire myography. Statistical significance ( $p < 0.05$ ) was determined with the Student's *t* test and two-way ANOVA + Sidak *post-hoc* test.

**Results:** Hypoxic pregnancy resulted in fetal growth restriction (A), and reduced LV developed pressure (LVDP; B) but enhanced LV end-diastolic pressure (LVEDP; C) in the maternal heart. Maternal femoral arteries from hypoxic pregnancy showed enhanced constriction in response to endothelin (ET-1; D) and phenylephrine (PE; E), but impaired endothelial-dependent dilatation to acetylcholine (ACh; F). This endothelial dysfunction was due to the absence of NO-dependent relaxation, as inhibition with the NO synthase blocker L-NAME reversed the ACh response to constriction (G). These vasoactive changes were associated with increased maternal plasma levels of creatinine, and decreased estimated GFR (H and I), in keeping with systemic endothelial dysfunction and impaired glomerular ultrafiltration.

**Conclusion:** The data support that mothers from complicated pregnancy are at greater risk of cardio-renal dysfunction because of lasting adverse effects originating during the sub-optimal pregnancy rather than a genetic predisposition.

*Support: British Heart Foundation*



**Figure. Hypoxic pregnancy impairs maternal cardio-renal function.** Values are mean ± S.E.M. for maternal heart weight relative to body weight (A), LVDP (B), LVEDP (C), the dose-dependent femoral artery vasoconstriction in response to ET-1 (D) and PE (E), the dose-dependent femoral artery vasodilatation in response to ACh (F) and the NO-dependent and NO-independent component of the femoral artery vasodilatation to ACh in the presence and absence of L-NAME, respectively (H), the maternal concentration of creatinine in plasma (H) and the maternal eGFR (I) at 138 dGA. Groups are N (○) and H (●). Significant differences ( $p < 0.05$ ) are \*N vs. H; Student's *t*-test for unpaired data or two-way RM ANOVA, where appropriate.

**O-045**

**Small NonCoding RNA Biotypes in the Human Preimplantation Embryo.** Sophie Petropoulos\*,<sup>1,2,3</sup> Stewart J Russell†,<sup>4</sup> Cheng Zhao†,<sup>2</sup> Karen Menezes†,<sup>4</sup> Clifford L Librach\*.<sup>4,5,6</sup> *1University of Montreal, Montreal, QC, Canada; 2Karolinska Institutet, Stockholm, Sweden; 3Centre de recherche du CHUM, Montreal, QC, Canada; 4CreATE Fertility Centre, Toronto, ON, Canada; 5University of Toronto, Toronto, ON, Canada; 6Women's College Hospital, Toronto, ON, Canada.*

**Introduction:** Human embryonic development during the first week is a critical window of development. During this time, the first lineages are established: 1) trophoblast (TE; prospective placenta), 2) primitive endoderm (PE; prospective yolk sac), and 3) pluripotent epiblast cells (EPI; prospective embryo proper). We have previously determined that lineage specification in human embryos occurs during late embryonic day (E) 5. Mechanism(s) underlying lineage segregation in the human remain unknown. To date, a comprehensive profile of all small ncRNAs during preimplantation development is lacking. With recent advancements in singlecell genomics, we can now measure the sncRNA content in an individual cell. We now hypothesize that 1) sncRNA biotypes are detected throughout development and increase with time. 2) sncRNAs, particularly the miRNA biotype, restrict expression of totipotency genes during late E5, ultimately causing a cascade of differentiation required for lineage specification.

**Methods:** Human embryos (E3E8, N=36 embryo, 601 cells) were dissociated into singlecells and libraries were prepared using smallseq. Data was processed using an in house pipeline that is an improvement on the established pipeline (<https://github.com/eyay/smallseq>).

**Results:** SncRNA biotypes are detected throughout preimplantation in human embryo (E3-E7). Expression of miRNAs, tRNAs and snRNAs increase with development. In contrast, the number of snoRNA molecules present decrease with developmental time. tRNA represents the most abundant biotype (15-20% of all biotypes). Further, 244 significantly differentially expressed miRNAs are detected between the TE and ICM lineage. These lineage specific signatures emerge at E5 and become progressively more pronounced with time. Candidate miRNAs (including *miR-200c-3p* and *miR-24-3p*) potentially responsible for driving lineage segregation have been identified. These miRNAs target pluripotency and known lineage genes, *SOX2* and *CDX2*, respectively; suggesting that miRNAs may be an upstream regulator of lineage specification.

**Conclusion:** This comprehensive characterization of small ncRNA at single-cell and lineage specific resolution could aid in our understanding of what constitutes human preimplantation development and shed light on underlying mechanism(s) of cell fate specification. Understanding the biological processes during human preimplantation development is not only important for acquiring fundamental knowledge pertaining to human embryogenesis, but also for clinical application given the increased use of ART, stem cell biology and regenerative medicine.

#### O-046

**Screening Putative Haplo-Essential Translation Genes for Contribution to Early Reproductive Failure.** Luwam Ghidjei\*,<sup>1</sup> Denise Lanza,<sup>1</sup> Lauryl M. J. Nutter,<sup>2</sup> Pilar Cacheiro,<sup>3</sup> Violeta Munoz-Fuentes,<sup>4</sup> Jason Heaney.<sup>1</sup> <sup>1</sup>Baylor College of Medicine, Houston, TX, United States; <sup>2</sup>The Hospital for Sick Children, Toronto, ON, Canada; <sup>3</sup>Queen Mary University, London, United Kingdom; <sup>4</sup>Wellcome Genome Campus, Hinxton, United Kingdom.

**Introduction:** It is estimated that up to 3,000 human genes cannot tolerate loss-of-function (LoF) of one alleles (haploinsufficient) and that single-copy LoF of many of these genes is incompatible with life (haplo-essential). Thus, *de novo* LoF of haplo-essential genes in humans likely contributes to early reproductive failure. We hypothesized that targeted genome editing in mouse embryos followed by in vitro culture to blastocyst and embryo genotyping for LoF allele dosage could be used to experimentally assess genes for haplo-essentiality at early stages of development.

**Methods:** Putative haplo-essential genes were identified using DOMINO dominance predictions and data from the Genome Aggregation Database, Cancer Dependency Map Project, and the International Mouse Phenotyping Consortium. Included in the search were mouse genes that have a 1:1 human ortholog. Genecodis was used to assess the gene list for enrichment of biological processes. CRISPR-Cas9 was used to induce putative LoF frameshift mutations in 1-cell-stage mouse embryos. Embryos were cultured to blastocyst stage (120 hours), time-lapsed imaged on an Embryoscope+, and assessed for LoF allele dosage by Sanger sequencing. Fisher's exact tests were used to test for abnormal development and genotype-phenotype associations.

**Results:** Genes predicted to be haplo-essential enriched for ribosomal and mRNA translation initiation proteins. Given their critical role to basic cellular function, we predicted LoF of these genes would cause early lethality at, or before, the blastocyst stage. CRISPR-Cas9 editing resulted in a statistically significant enrichment of early embryonic lethality for *Rpl5*, *Rpl7a*, *Rpl31*, *Eif3d*, and *Eif4a3* when compared to control culture embryos. Although not significant, CRISPR-Cas9 editing of *Eif4a1* resulted in similar gross morphologic abnormalities in 56% of experimental embryos vs 25% control embryos. Preliminary data revealed an enrichment of early embryonic lethality for *Rpl5*, *Rpl7a* and *Eif3d* when  $\geq 25\%$  of the alleles detected in the embryo were predicted to be LoF frameshift mutations.

**Conclusion:** This pilot suggests that LoF mutations of genes predicted to be cell-essential enrich for early embryonic lethality phenotype. *De novo* mutations of these genes may explain early pregnancy failure. We

have demonstrated this pipeline as a successful screen. To circumvent issues with mosaic embryos, further studies can be performed using cell permeable Cre to conditionally knockout genes at the 1-cell stage as a high standard confirmatory process.

#### O-047

**Elucidating the Role of FOXO3 in Regulating Ovarian Reserve and Function in Humans.** Caterina Clementi, Karen Hunter Cohn, Genevieve Galarneau, Piraye Yurttas Beim\*. *Celmatix Inc., New York, NY, United States.*

**Introduction:** FOXO3 belongs to the forkhead family of transcription factors and has been implicated in regulating ovarian reserve in mice. Disruption of this gene in mice results in premature depletion of the follicular precursor pool, while a constitutively active transgene results in the maintenance of a larger follicle pool. In humans, FOXO3 has been linked to human longevity, but its role in reproductive longevity or ovarian function has not yet been elucidated.

**Methods:** Our Personalized Reproductive Medicine (PReM) Initiative cohort consists of a centralized repository of biological samples derived from patients undergoing treatment at multiple fertility centers in the United States, linked to detailed reproductive phenotype data. Samples underwent whole genome or exome sequencing. We used Ensemble Variant Effect Predictor to perform the functional annotation of variants with a minor allele frequency < 1% in gnomAD located within gene boundaries. For a subset of genes known to regulate ovarian function, we evaluated variants predicted to have high impact effects on protein function.

**Results:** We identified 14 fertility patients with different combinations of 7 unique, rare functional variants (RFV) in FOXO3. Two of these RFVs were within the forkhead domain and were predicted to have a negative impact on protein function by disrupting DNA binding and protein stability. Patients carrying both RFVs in the forkhead domain showed diminished ovarian reserve (DOR) and multiple IVF failures, indicative of severe folliculogenesis defects. Patients carrying only 1 RFV in the forkhead domain had increased miscarriage rates but showed normal ovarian reserve. Patients with RFVs outside of the forkhead domain of the protein were characterized by normal ovarian reserve and successful IVF on the first cycle. Interestingly, a rare variant predicted to impact a regulatory region of the protein was found in a patient with polycystic ovary syndrome (PCOS) with high ovarian reserve.

**Conclusion:** We identified a group of infertility patients carrying rare functional variants in FOXO3, a gene linked to longevity in humans and regulation of ovarian reserve in mice. When we evaluated the patient phenotypes and predicted functional impact of these variants, we identified a series of functional alleles with associated ovarian reserve phenotypes that are suggestive of a function-phenotype dose-response relationship. Mutations with a negative impact on protein function were found in DOR patients, while a predicted activating mutation was found in a patient with PCOS. These opposite effects on ovarian reserve observed in patients are consistent with phenotypes observed in *Foxo3* KO and transgenic mouse models.

#### O-048

**Loss of Ovarian Expression of the Noncoding RNA H19 Promotes Susceptibility to Doxorubicin-Induced DNA Damage.** Amanda N. Kallen\*,<sup>1</sup> Pingping Lu,<sup>2</sup> Jing Wang,<sup>3</sup> Joshua Johnson.<sup>4</sup> <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>Women's Hospital of Zhejiang University School of Medicine, Hangzhou, China; <sup>3</sup>Department of Oncology, Beijing Friendship Hospital, Capital Medical University, Beijing, China; <sup>4</sup>University of Colorado Denver, Aurora, CO, United States.

**Introduction:** Ovarian DNA damage occurs naturally with age and is accelerated by exposure to gonadotoxic agents such as chemotherapy. Emerging evidence suggests that noncoding RNAs (ncRNAs) may play important roles in regulating the ovarian DNA damage response to gonadotoxins. The ncRNA H19 plays essential roles in mammalian development, but little is known about its role in ovarian biology. We have previously shown that H19 null mice are more susceptible than their wild

type (WT) counterparts to chemotherapy-induced ovarian DNA damage. Specifically, *H19* null mice exhibit increased ovarian DNA damage and apoptosis, as well as complete fertility loss (while WT mice recover fertility), after treatment with doxorubicin (DXR), a chemotherapeutic agent used for cancer treatment in women. In this work, we sought to evaluate whether loss of *H19* directly impacts oocyte and embryo development at baseline and after gonadotoxin exposure.

**Methods:** We first induced DNA damage with doxorubicin (DXR). 5wk *H19* knockout (*H19* KO) and wild type (WT) mice (n=5) were injected with DXR or saline. 48 hours later, GV oocytes were collected for immunofluorescence to evaluate  $\psi$ H2AX, a marker of DNA breaks. Additional GV oocytes were cultured to evaluate *in vitro* maturation. For *in vivo*-matured MII oocyte retrieval, DXR-treated mice were superovulated, followed by hCG injection and collection of cumulus-oocyte complexes (COCs). Collected oocytes were subjected to *in vitro* fertilization in order to evaluate oocyte maturation, fertilization and embryo development after DXR. Stain positive follicles in *H19* KO and WT mouse ovaries, and fertilization and embryo development, were analyzed using one-way ANOVA.

**Results:** Pre-DXR, GV *H19* KO mouse oocytes exhibited decreased *in vitro* maturation (*H19* KO: 52.9±8.29 % maturation, versus WT 74.18±3.91 % maturation; p=0.002). Expression of  $\psi$ H2AX was higher in *H19* KO mouse oocytes as compared to WT. Post-DXR, *H19* KO oocytes exhibited significantly reduced fertilization rates (*H19* KO: 37.46±19.05 % fertilization, versus WT 69.8±2.11 % fertilization; p=0.04).

**Conclusion:** We observed decreased *in vitro* maturation and increased evidence of DNA breaks in *H19* KO mice as compared to WT. After DXR, *H19* KO oocytes exhibited reduced fertilization as compared to WT. This may represent a “two-hit” mechanism by which loss of *H19* reduces oocyte competence and increases oocyte DNA damage, and renders oocytes more susceptible to gonadotoxin-induced injury. Further mechanistic studies will determine the mechanism by which differential *H19* expression in the follicle microenvironment regulates oocyte sensitivity to gonadotoxins.

## O-049

**Novel Anti-Müllerian Hormone Receptor 2 Binding Peptide (AMHR2BP) Modulates Oocyte Function In Vivo.** Laura Detti\*,<sup>1</sup> Ghassan M Saed\*,<sup>2</sup> <sup>1</sup>Cleveland Clinic, Cleveland, OH, United States; <sup>2</sup>Wayne State University, Detroit, MI, United States.

**Introduction:** Anti-Müllerian hormone (AMH) inhibits hormone production, and ovarian cortex follicle development in *in vitro*, and *in vivo*, ovarian cortex, and in luteinized granulosa cells (GCs). We showed a novel binding peptide to AMH receptor 2 (AMHR2BP) to inhibit GCs replication and function, and to preserve the ovarian follicle number by minimizing the progression of follicular development, in addition to decreasing hormone production, cell replication and apoptosis, in a mouse model. We now sought to investigate whether AMHR2BP can modulate oocyte function by assessing the two oocyte-derived hormones that stimulate mitotic proliferation in granulosa cells, BMP15 and GDF9. Both, BMP15 and GDF9 act in an additive manner and have a critical role in granulosa cell and theca cell growth, as well as in differentiation and maturation of the oocyte.

**Methods:** This was a translational study where 24 18-weeks old C57BL female mice were equally divided and assigned to four treatments: Baseline (euthanized just prior to the experiment), AMHR2BP (AMHR2BP, 50  $\mu$ g /day), rAMH (recombinant AMH, 1.8  $\mu$ g /day), and placebo group (normal saline), via intraperitoneal pumps. Mice were euthanized 3 weeks after pump placement and the ovaries were explanted for real-time RT-PCR analysis. BMP15 and GDF9 expression was measured. We used Kruskal-Wallis's test for comparison of medians between groups (SPSS; p<0.05).

**Results:** Compared with Placebo, AMHR2BP and rAMH administration caused stalling of BMP15 and GDF9 expression and maintained baseline levels. The table reports the individual groups' data.

**Conclusion:** Oocyte-derived BMP15 and GDF9 stimulate granulosa cell replication and with this experiment we showed that AMHR2BP, as well as rAMH, could stall oocyte function for as long as they are administered. Although a direct effect on the oocyte could not be assessed with this

experiment, we confirmed an overall inhibitory effect on follicular function. Combined with our previous findings of direct inhibition of granulosa cells replication and *in vitro* and *in vivo* follicular development, we conclude that AMHR2BP could be used to inhibit time-linked loss of ovarian follicle reserve.

Ovarian cortex concentration of BMP15 and GDF9 in the mice groups, Baseline, Placebo, rAMH, AMHR2BP.					
Variable	Baseline Median (Q1, Q3)	Placebo Group Median (Q1, Q3)	rAMH Group Median (Q1, Q3)	AMHR2BP Group Median (Q1, Q3)	p-value
BMP15 (fg/ $\mu$ g RNA)	206.9 (153.8, 976.6)	4007.3 (2875.7, 5573.0)	116.1 (101.9, 800.3)	103.2 (102.0, 260.7)	0.005
GDF9 (fg/ $\mu$ g RNA)	49.1 (28.3, 61.1)	387.4 (355.2, 419.9)	21.5 (12.8, 33.1)	26.6 (11.3, 34.6)	0.013

## O-050

**Aneuploidy in Human Embryos May Elicit a Premature Differentiation Response in the Inner Cell Mass.** Angel Martín†,<sup>1</sup> Francisco Dominguez,<sup>1</sup> Alicia Quiñero,<sup>1</sup> Carmina Vidal,<sup>2</sup> Amparo Mercader,<sup>1,2</sup> Fernanda Insua,<sup>2</sup> Maria Jose De los Santos.<sup>1,2</sup> <sup>1</sup>IVI Foundation-IIS La Fe, Valencia, Spain; <sup>2</sup>IVI RMA, Valencia, Spain.

**Introduction:** Aneuploidy is among the major contributors to human infertility. However, the mechanisms by which aneuploid embryos fail to develop remain unclear. After implantation, morphogenetic remodeling leads to trophoblast differentiation and segregation of lineages within the inner cell mass (ICM) into the epiblast (EPI) and the primitive endoderm (PrE). Aneuploidy-induced overexpression of E-cadherin (E-cad) leads to premature differentiation of trophoblast cells. However, studies manipulating E-cad have failed to demonstrate a similar role in the EPI/PrE segregation. Here, we studied the transcriptional consequences of aneuploidy in the ICM of human blastocysts, and uncover manifestations of a premature state of lineage differentiation.

**Methods:** Prospective study comparing RNA-seq data of the ICM from day 5/6 blastocysts classified as euploid (n=5) and aneuploid (n=6) by preimplantation genetic testing for aneuploidy. Participants were recruited between October 2018 and November 2019 at IVI RMA Valencia. ICM fractions were separately collected and processed for RNA-seq. Differentially expressed genes (DEGs) were calculated with DESeq2 package [Benjamini-Hochberg (BH)-adjusted p<0.01 & abs(log2FoldChange)>2 significant]. Fgsea algorithm was used for enrichment analysis on Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms (BH-adjusted p<0.01 significant).

**Results:** 27 DEGs were identified. To address the functional implications of these differences, significantly deregulated pathways according to KEGG and GO categories were analyzed. ICM from aneuploid blastocysts displayed significant downregulation in 9 KEGG and GO processes involved in RNA metabolism, ribosomal biogenesis and mitochondrial function, which are known transcriptional hallmarks of aneuploidy. Moreover, the aneuploid group displayed a transcriptional phenotype reminiscent of a premature differentiation. This phenotype was characterized by significant upregulation of 27 GO processes including mesenchymal cell differentiation, axis specification, cell migration, and development of nervous and urogenital systems. Vimentin (VIM), a key marker of the epithelial to mesenchymal transition (EMT), was among the top upregulated DEGs. The GO term intercellular adhesion via plasma membrane was significantly upregulated, yet other members of the cadherin family rather than E-cad contributed significantly to enrichment.

**Conclusion:** EMT and regulation of cell adhesion may direct the segregation of human EPI and PrE lineages. An increase of cell adhesion properties in the ICM of aneuploid embryos may lead to premature differentiation. However, unlike in trophoblast cells, an E-cad-independent response may shape ICM identity and alter embryo developmental fate.

**O-051**

**Bromodomain Extraterminal (BET) Family Inhibitor JQ1 Inhibits Nuclear Maturation and Cytoplasmic Organization of Oocytes.** Keerthana Karunakar Poojary<sup>†</sup>, Sandhya Kumari<sup>†</sup>, Satish K Adiga, Guruprasad Kalthur\*. *Kasturba Medical College, Udupi, India.*

**Introduction:** JQ1 is a cell permeable potent inhibitor of Bromodomain and extraterminal (BET) proteins. It is used in the treating neurodegenerative diseases and cancer. JQ1 inhibits BET by competitively binding to the acetyl lysine pockets in the bromodomain protein resulting in the dissociation of the BET protein from the chromatin and inhibiting the cellular signalling pathways associated with it. Since BET proteins are necessary for the completion of oogenesis and play a significant role in oocyte maturation as well as embryo development, the present study was designed to understand the impact of JQ1 on the oocyte maturation and its cytoplasmic organization.

**Methods:** Germinal vesicle (GV) stage oocytes collected from 8-week old Swiss albino mice were randomly segregated into control, vehicle control (0.01% DMSO) and JQ1 groups (25, 50 and 100  $\mu$ M). The oocytes were subjected to in vitro maturation to assess the nuclear maturation. The MII oocytes were assessed for actin and spindle organization, chromosome alignment, acetylation level and intracellular reactive oxygen species (ROS). To understand the effect of JQ1 on embryo development, OCCs collected from superovulated mice were transferred to insemination droplet containing JQ1 (0 and 25  $\mu$ M in M16 media). Fertilization was checked after 12h of insemination and embryo development was monitored until the blastocyst stage.

**Results:** The maturation (MI) rate in JQ1 group was significantly ( $p < 0.001$ ) lower compared to control. In addition, non-significant increase in the fragmentation and degeneration of oocytes was observed in JQ1 group. MII oocytes from JQ1 group had higher percentage of aggregated F-actin distribution compared to control. Oocytes with abnormal spindle (25  $\mu$ M: 85.71% and 50  $\mu$ M: 100%) and misaligned chromosomes (25  $\mu$ M: 78.57 and 50  $\mu$ M: 83.33%) were significantly higher. JQ1 exposed oocytes had lower lysine acetylation and elevated intracellular ROS level. Further, to assess its effect on fertilization and embryo development, only lower concentration of JQ1 (25  $\mu$ M) was used for IVF experiment, since at 50 and 100  $\mu$ M concentrations oocytes had lower nuclear maturation rate. The fertilization rate in control and VC groups was 73.94 $\pm$ 4.01 and 75.34 $\pm$ 5.92% respectively, which was significantly lower in JQ1 group ( $p < 0.001$ ). Presence of JQ1 in the insemination droplet not only reduced the sperm motility, it decreased the lysine acetylation and increased the DNA damage in spermatozoa. There was 3-fold increase in the percentage of oocytes with abnormal fertilization (parthenogenic and triploid embryos) in JQ1 exposed oocytes ( $p < 0.05$ ) and lower blastocyst rate (37.55%) compared to control (51.35%).

**Conclusion:** BRDT inhibitor JQ1 significantly inhibits the oocyte maturation, affects the cytoplasmic organization of the oocyte and compromises the preimplantation embryo development.

**O-052**

**Opposite Effects of LH/PKA/MTOR and AMPK Signaling on Induction of Autophagy in Luteal Cells.** Emilia Przygodzka<sup>†</sup>, Michele R Plewes<sup>†</sup>, Guojuan Li, John S Davis\*. *University of Nebraska Medical Center, Omaha, NE, United States.*

**Introduction:** Autophagy is a self-degradative process important for balancing sources of cellular energy at critical times in development and in response to nutrient stress and can also lead to apoptosis. Several markers of autophagy increase in the corpus luteum (CL) during luteolysis. Mammalian target of rapamycin (MTOR) and 5' AMP-activated protein kinase (AMPK), key players in autophagy, are known to inhibit or activate autophagy, respectively. AMPK increases autophagy by inhibition of MTOR via phosphorylation of regulatory-associated protein of MTOR (RPTOR) at Ser792 and direct phosphorylation of ULK1 (Ser317) and Beclin (Ser93). MTOR inhibits autophagy by phosphorylation of ULK1 at Ser757, which disrupts the interaction between ULK1 and AMPK. Herein, we hypothesize that LH/protein kinase A (PKA)/MTOR signaling

and AMPK signaling exert opposite effects on autophagy by modulating the activity of proteins involved in autophagy induction (MTOR, RPTOR, ULK1) and autophagosome formation (ATG16L1; Beclin and LC3B).

**Methods:** To test this hypothesis, small luteal cells (SLC) isolated from the mature bovine CL ( $n=3-5$ ) were incubated with LH (1-100 ng/ml), AICAR (AMPK activator; 1 mM) or AICAR + LH (10 ng/ml). Additionally, SLC were incubated with forskolin (FSK; PKA activator; 10  $\mu$ M), H89 (PKA inhibitor; 30  $\mu$ M), rapamycin (MTOR inhibitor; 20 nM), chloroquine (autophagy activator; 10  $\mu$ g/ml), compound C (AMPK inhibitor; 50  $\mu$ M), or transfected with dominant negative AMPK $\alpha$ 1 (dn.AMPK $\alpha$ 1). Data were analyzed using one-way ANOVA.

**Results:** Treatment with LH or FSK decreased ( $p < 0.01$ ) phosphorylation of RPTOR (Ser792) and AMPK $\alpha$  (Thr172), and increased ( $p < 0.01$ ) phosphorylation of MTOR (Ser2448) and its substrates p70S6K (Thr389) and ULK1 (Ser757). Incubation in the presence of LH up to 4h decreased basal and chloroquine-stimulated content of LC3B, a marker of autophagy, by 46% and 52%, respectively. Pre-treatment with H89 or rapamycin abolished ( $p < 0.05$ ) LH-mediated effects on AMPK $\alpha$ , RPTOR, MTOR, p70S6K, ULK (Ser757) and ULK substrate ATG16L (Ser278). In contrast, the AMPK activator AICAR induced ( $p < 0.01$ ) phosphorylation of AMPK $\alpha$  (Thr172), RPTOR (Ser792), Beclin (Ser93), ULK1 (Ser317) and ATG16L1 (Ser278). The effects of AICAR on RPTOR, ULK1 (Ser317) and Beclin were abrogated after pretreatment with compound C or transfection with dn.AMPK $\alpha$ 1. Pretreatment with AICAR inhibited (75%,  $p < 0.01$ ) LH-stimulated progesterone production and inhibited LH-mediated effects on RPTOR. Confocal microscopy revealed that AICAR, but not LH, enhanced colocalization of autophagosomes and lysosomes in SLC.

**Conclusion:** Our results suggest LH/PKA/MTOR inhibits, while AMPK activates, key signaling pathways involved in luteal cell autophagy. Supported by NIFA USDA 2017-67015-26450, VA I01 BX004272 and NIH R01 HD092263.

**O-053**

**Epigenetic Effects of N-acetylcysteine on H<sub>2</sub>S-Mediated Protection Against Fetal Origins of Vascular Disease.** A. A. Paz<sup>†</sup>, T. A. Garrud<sup>†</sup>, E. Peñaloza<sup>†</sup>, F. Vega-Tapia<sup>†</sup>, S. G. Ford<sup>2</sup>, Y. Niu<sup>2,3,4</sup>, B. J. Krause\*, D. A. Giussani\*. <sup>2,3,4</sup> *Universidad de O'Higgins, Rancagua, Chile;* <sup>2</sup> *University of Cambridge, Cambridge, United Kingdom;* <sup>3</sup> *Centre for Trophoblast Research, University of Cambridge, Cambridge, United Kingdom;* <sup>4</sup> *Cambridge Cardiovascular Strategic Research Initiative, University of Cambridge, Cambridge, United Kingdom.*

**Introduction:** The search for therapy against cardiovascular dysfunction programmed developmentally is ongoing, as interventional mechanisms of human translational relevance remain uncertain. Chronic fetal hypoxia is a common outcome of complicated pregnancy in humans. Adopting a two-pronged approach by combining studies in humans and in the chicken embryo, we show that chronic hypoxia increases the vascular expression of the hydrogen sulphide (H<sub>2</sub>S) gene, cystathionine gamma-lyase (CTH) in both species. Further, the H<sub>2</sub>S precursor, N-acetylcysteine (NAC) protects against vascular dysfunction in hypoxic chicken embryos via epigenetic changes in the promoter region of CTH, enhancing H<sub>2</sub>S-dependent vascular relaxation.

**Methods:** Fertilized Bovans Brown eggs were incubated in normoxia or hypoxia (14% O<sub>2</sub>) from day 1 of incubation, term is 21 days). Half of the eggs were randomly assigned to daily NAC treatment (33  $\mu$ g/kg) starting at day 13 of incubation. At day 19, *ex vivo* femoral artery vascular responses were established by wire-myography and the aorta isolated to determine the expression of CTH by qPCR. The CTH gene promoter was analysed *in silico* with MathInspector and DNA methylation determined by Pyrosequencing. Human umbilical artery endothelial cells (HUAEC) from healthy pregnancies were exposed *in vitro* to hypoxia (48 h, 2% O<sub>2</sub>) and CTH expression evaluated.

**Results:** Hypoxic chicken embryos showed endothelial dysfunction, rescued by NAC via enhanced NO-independent mechanisms (Fig. 1 A,B). Embryos treated with NAC showed enhanced vascular relaxation to H<sub>2</sub>S (Fig. 1C). The vascular CTH expression was increased in hypoxic embryos and enhanced further by NAC treatment (Fig. 1D). The increased CTH

transcript was associated with decreased CpG methylation in a sense hypoxia-response element (HRE), along with increased CpG methylation in an antisense HRE (Fig. 1E). Sustained hypoxia *in vitro* induced CTH expression in HUAEC (Fig. 1F).

**Conclusion:** The gasotransmitter H<sub>2</sub>S appears an excellent candidate of human translational relevance for intervention against programmed cardiovascular disease.

*Supported by SRI, Fondecyt 1181341, The Wellcome Trust and The British Heart Foundation*

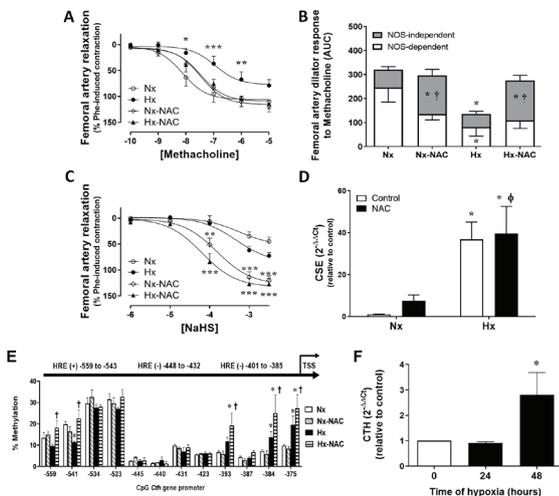


Figure 1. The data show mean±SEM for: (A) the concentration-response curves for methacholine; (B) the area under the curve (AUC) for the methacholine-dependent relaxation derived in the presence or absence of the NOS inhibitor L-NAME used to calculate the NOS-dependent vascular relaxation; (C) the concentration-response curves for the H<sub>2</sub>S donor NaHS in femoral arteries isolated from chicken embryos exposed to normoxic (Nx) or hypoxic (Hx) incubation with or without treatment with N-acetylcysteine (NAC); (D) for transcript levels and (E) promoter DNA methylation profile for the H<sub>2</sub>S synthesising enzyme CTH in aorta isolated from chicken embryos exposed to normoxic (Nx) or hypoxic (Hx) incubation with or without treatment with N-acetylcysteine (NAC); and for (F) transcript levels for CTH in human umbilical artery endothelial cells (HUAEC) exposed to hypoxia for 24 and 48 hours. Significant (P<0.05) differences are: \*p < 0.05, \*\*p < 0.01 & \*\*\*p < 0.001, vs. Nx-control; †p < 0.05 vs. Hx-control; ‡p < 0.05 vs. Nx-NAC (Two-Way RM ANOVA).

**Results:** Differentially expressed genes (DEG) in response to Jz-ICR1Δ were sexually dimorphic with only 25% of DEG shared between male and female fetuses (Fig1A). In Jz-ICR1Δ females, *G6pc* and *Rgs16* decreased and *Serpina7* increased in the fetal liver, effects that tended to remain in adulthood (Fig1B,C,D). Other fetal DEG analyzed were unaltered in Jz-ICR1Δ adults (not shown). Jz-ICR1Δ adult males and females had increased islet density (Fig1H,L). Jz-ICR1Δ males also displayed reduced pancreas islet area (Fig1E). Chow Jz-ICR1Δ males, but not females, had a greater proportion of small islets versus their diet controls (Fig1F,G,K). **Conclusion:** Placental endocrine malfunction induces sexually dimorphic changes in offspring hepatic transcriptome beginning *in utero* and adult pancreas morphology. These changes may explain the varied metabolic outcomes between male and female Jz-ICR1Δ offspring.

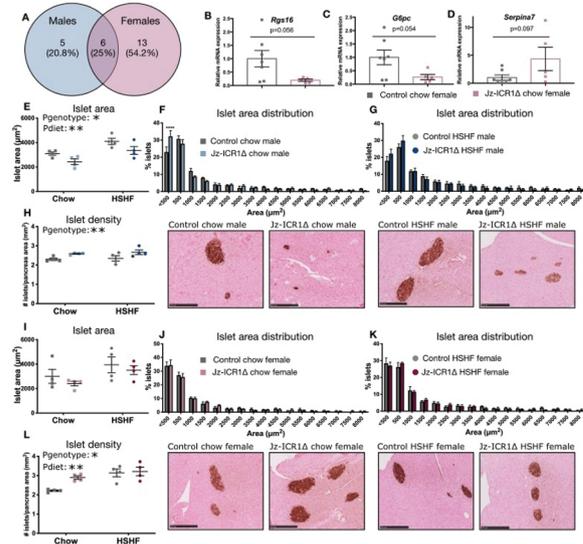


Figure 1. Jz-ICR1Δ and offspring hepatic transcriptome and pancreas morphology. (A) Venn diagram with number of differentially expressed genes in male and female fetuses in response to Jz-ICR1Δ. (B,C,D) Hepatic expression of select genes in chow-fed adult females. (E-L) Islet morphology in chow and high sugar high fat (HSHF) diet fed males and females in response to Jz-ICR1Δ. Data are mean±SEM and analyzed by two-way ANOVA/Sidak or t-test. n=3-7 per sex per group from 3-6 litters. Scale bars=250 μm. \*p<0.05.

O-054

**Placental Endocrine Malfunction Causes Sex Specific Changes in Metabolic Organs of Fetal and Adult Mouse Offspring.** Efthimia Christoforou†, Panayiotis Laouris†, Jorge Lopez-Tello†, Marta Ibanez Lligona†, Hannah Yong, Alison Forhead, Amanda Sferruzzi-Perri\*. *University of Cambridge, Cambridge, United Kingdom.*

**Introduction:** Little is known about the endocrine placenta in offspring programming. Recently we have shown placental endocrine malfunction in mice (induced by placental endocrine zone mis-expression of *Igf2-H19* genes; Jz-ICR1Δ) programmes sex-dependent changes in adult offspring insulin sensitivity and pancreatic insulin production, with only males being insulin resistant (*J DOHAD* 2019, 10:S109). However, the molecular and morphological changes underlying the sexually divergent outcomes remain unknown. Here, we show sex-specific alterations in the fetal and adult hepatic transcriptome, and adult pancreas morphology, in response to Jz-ICR1Δ.

**Methods:** Jz-ICR1Δ litters were generated by mating female *H19ICR1lox* and male *TpbaCre* mice. The reverse parental cross was used to generate control litters. At gestational day 19, liver transcriptomes of male and female fetuses were assessed by RNAseq. Separate litters that delivered and were standardized to 3 pups per sex, were fed either a chow or high sugar high fat (HSHF) diet from weaning. At 17 weeks, liver and pancreas were collected for hepatic gene expression analysis by qPCR and pancreas islet morphology by stereology. Data from 1-2 fetuses/offspring per litter (3-6 litters per group) were analyzed by two-way ANOVA (genotype, diet) or t-test (genotype), p<0.05. RNAseq, q<0.05.

O-055

**Maternal Obesity Upregulates Fetal Heart *Pparg* Expression with Consequences for Later Life Cardiac Metabolic and Contractile Function in Mice.** O. R. Vaughan,<sup>1</sup> J. Chan,<sup>2</sup> L. Cox,<sup>2</sup> V. Ferchaud-Roucher,<sup>1</sup> F. Rosario,<sup>1</sup> J. E.B. Reusch,<sup>1</sup> A. C. Keller,<sup>1</sup> T. L. Powell,<sup>1</sup> T. Jansson.<sup>1</sup> *<sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>Wake Forest School of Medicine, Winston-Salem, NC, United States.*

**Introduction:** Obesity in pregnant women causes fetal cardiac dysfunction and offspring cardiovascular disease risk. The mechanisms are poorly understood. Using a mouse model of maternal obesity with fetal overgrowth and increased placental glucose and fatty acid transport, we determined the role of myocardial metabolism in offspring cardiac dysfunction. We hypothesized that maternal obesity enhances offspring cardiac fatty acid metabolism.

**Methods:** Pregnant female C57 mice with pre-gestational, diet-induced obesity (Ob, n=15), or non-obese controls (Con, n=31), were euthanized on embryonic day (E) 18.5 or allowed to deliver (term~E20.5). E18.5 fetal hearts were dissected, weighed and analyzed by RNA-Seq. Offspring were weaned onto chow and, 6 months postnatally, cardiac diastolic function (echocardiography), glucose uptake (<sup>18</sup>F positron emission tomography) and fatty acid uptake (<sup>14</sup>C-oleic acid tracing) were assessed. Following necropsy, cardiac mitochondrial substrate respiration and fatty acid content were determined using respirometry and mass spectrometry. Gene expression and glucose transporter abundance were quantified by qPCR and western blot. Differences between Con and Ob offspring were determined by t-test, separately in males and females.

**Results:** Before birth, maternal obesity increased heart weight and expression of the master regulator of lipid metabolism, peroxisome

proliferator activated receptor  $\gamma$  (*Pparg*), in female and male fetuses (n=5 per sex, per group, P<0.05). Six months postnatally, cardiac *Pparg* expression remained higher in Ob than Con offspring (females +34% n=9-10; males +91% n=10-11, P<0.05). Diastolic function, assessed by the ratio of mitral inflow/wall displacement (E/E'), was lower in Ob than Con offspring and inversely correlated with cardiac *Pparg* expression in males (R=-0.82, P<0.01), but not females (P=0.61). Maternal obesity increased cardiac uncoupled fatty acid respiration in male offspring (+42%, P<0.05; females P>0.05) and decreased cardiac glucose uptake in female offspring (-23%, P<0.05; males P>0.05). Maternal obesity did not alter offspring cardiac fatty acid uptake or content, carbohydrate respiration, or glucose transporter abundance (P>0.05).

**Conclusion:** Increased cardiac fatty acid respiration is consistent with *Pparg* upregulation in male offspring of obese dams and may underpin the developmental programming of diastolic dysfunction. Decreased cardiac glucose uptake in female offspring of obese dams reflects the reported phenotype of people with diabetes. We speculate that impaired cardiac metabolic flexibility, driven by increased substrate availability *in utero*, contributes to long term cardiovascular risk in children of obese women.

### O-056

#### Defining the Role of the Hypothalamic Pituitary Adrenal Axis in the Relationship between Fetal Growth and Adult Cardiometabolic Outcomes.

Wriyu N Martin<sup>†</sup>,<sup>1,2</sup> Carol A Wang,<sup>1,3</sup> Stephen J Lye,<sup>4</sup> Rebecca M Reynolds,<sup>5</sup> Stephen G Matthews,<sup>4,6</sup> Carly E McLaughlin,<sup>7</sup> Christopher Oldmeadow,<sup>1,3</sup> Roger Smith,<sup>1,3</sup> Craig E Pennell\*.<sup>1,3</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter New England Local Health District, New South Wales, Australia; <sup>3</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>4</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>5</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>6</sup>University of Toronto, Toronto, ON, Canada; <sup>7</sup>Curtin University, Western Australia, Australia.

**Introduction:** Animal and human data demonstrate independent relationships between fetal growth, hypothalamic-pituitary-adrenal axis function (HPA-A) and adult cardiometabolic outcomes. While the association between fetal growth and adult cardiometabolic outcomes is well established, the role of the HPA-A in these relationships remains unclear. This study aims to determine whether HPA-A function mediates or moderates this relationship.

**Methods:** A total of 2900 pregnant mothers were recruited (1989-1991) in the Raine Study. Detailed anthropometric data was collected at birth (percent optimal birthweight [POBW] - is a measure of the fetus' fulfilment of its growth potential calculated using a formula which adjusts for fetal sex, maternal height, maternal parity and gestational age). The Trier Social Stress Test was administered at 18-years; HPA-A response profiles were determined (reactive responders [RR], anticipatory responders [AR] and non-responders [NR]). Adult cardiometabolic parameters (BMI, systolic BP [sBP] and LDL) were obtained at 20-years.

**Results:** Complete data was available on 703 Raine Study Gen2 participants; 404 were RR, 192 were AR and 107 were NR. Regression modelling demonstrated linear associations between POBW and BMI (p=0.001) and sBP (p=0.05); quadratic associations were observed for LDL (p=0.006). For every 10% increase in POBW, there was a 0.54 unit increase in BMI (standard error [SE] 0.15) and a 0.65 unit decrease in sBP (SE 0.34). Interaction analyses between HPA-A profile and POBW for BMI demonstrated the strongest effect in NR compared to AR and RR (p=0.10). Conversely, for sBP the strongest effect was seen in AR (p=0.03). No interactions were observed for LDL. Decomposition of the total effect revealed no evidence of mediation or moderation.

**Conclusion:** The relationship between fetal growth and adult cardiometabolic outcomes varies by HPA-A phenotype; this was not mediated through HPA-A function. Early prediction of adult HPA-A phenotypes may offer unique opportunities to develop early intervention strategies to prevent lifelong disease.

### O-057

#### Hypoxemia Prevents Insulin Mediated Suppression of Gluconeogenic Gene Expression in the Fetal Liver during Hypoglycemia. Priya Mukherjee<sup>†</sup>,<sup>1</sup> Amanda Jones,<sup>1</sup> Paul Rozance,<sup>1</sup> Brown Laura,<sup>1</sup> Sean Limesand,<sup>2</sup> Stephanie Wesolowski.<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University of Arizona, Tucson, AZ, United States.

**Introduction:** Placental insufficiency produces growth restricted (PI-IUGR) fetuses that are hypoxemic and hypoglycemic. These fetuses have increased gluconeogenic gene expression (*PCK1* and *G6PC*) and hepatic glucose production (HGP), which are absent in normal fetuses. Further, both of these features are resistant to suppression with insulin in PI-IUGR fetuses. Previous research has demonstrated that fetuses with experimental hypoglycemia (HG) have increased HGP and expression of *PCK1* and *G6PC* that is suppressible with insulin. In contrast, fetuses with experimental hypoxemia (HOX) have increased *PCK1* and *G6PC* expression without associated HGP. The objective of this study was to determine their combined effects and test the hypothesis that hypoxemia induces insulin resistance on suppression of gluconeogenic gene expression and HGP activated by hypoglycemia.

**Methods:** Late gestation sheep were made hypoglycemic (HG, n=11) via maternal insulin infusion or hypoglycemic and hypoxemic (HG+HOX, n=12) via nitrogen insufflation by tracheotomy. Fetal arterial blood samples were collected before (PRE) and after 4-6 days of treatment (POST). To measure HGP and test insulin sensitivity post treatment, metabolic studies were performed with U<sup>13</sup>C-glucose tracer to measure rates of glucose production (HGP) during basal (BASAL) and hyperinsulinemic-euglycemic clamp (HEC) periods. Fetal data were analyzed by 2-way ANOVA with main effects of treatment (TRT: HG, HG+HOX) and period (PRE vs POST; or BASAL vs HEC) and interaction (INT: treatment x period). Liver tissue samples were collected under HEC conditions to measure gene expression (*PCK1*, *G6PC*) and analyzed by MW-test.

**Results:** HG and HG+HOX fetuses had a 51% decrease in plasma glucose concentrations. HG+HOX fetuses had 25% lower arterial blood pO<sub>2</sub> compared to HG fetuses (INT: P<0.05), with no difference in fetal weight. During the BASAL period, HGP rates were active (P<0.05 vs zero, one-sided t-test) in HG (6.2  $\mu$ mol/min/kg) and HG+HOX (9.7  $\mu$ mol/min/kg) fetuses. HGP was higher in HG+HOX compared to HG fetuses regardless of period (TRT: P=0.07). In liver tissue collected under HEC conditions, expression of *PCK1* and *G6PC* was 13-fold and 5-fold higher, respectively, in HG+HOX group compared to HG fetuses (P<0.05).

**Conclusion:** In response to HEC conditions expression of the two main gluconeogenic genes was higher in the livers of HG+HOX compared to HG fetuses, while HGP remained 65% higher in HG+HOX compared to HG fetuses. Thus, hypoxemia may underlie molecular mechanisms that prevent insulin mediated suppression of *PCK1* and *G6PC*. These results provide new insight regarding the causative roles of reduced glucose and oxygen supply on the early activation of HGP and insulin resistance in fetuses with PI-IUGR.

### O-058

#### Maternal Obesity Induces Fetal Sheep Hepatic Oxidative Stress (OS) and Mitochondrial Damage Predominantly in the Right Lobe. Susana P Pereira<sup>†</sup>,<sup>1,2</sup> Luis F Grilo<sup>†</sup>,<sup>2</sup> Mariana S Diniz<sup>†</sup>,<sup>3</sup> Carolina Tocantins<sup>†</sup>,<sup>2</sup> João D Martins<sup>†</sup>,<sup>2</sup> Stephen Ford\*,<sup>4</sup> Peter W Nathanielsz\*,<sup>4</sup> Paulo J Oliveira\*.<sup>2</sup> <sup>1</sup>University of Porto, Porto, Portugal; <sup>2</sup>University of Coimbra, Coimbra, Portugal; <sup>3</sup>University of Coimbra, Porto, Portugal; <sup>4</sup>University of Wyoming, Laramie, WY, United States.

**Introduction:** Overweight prevalence among women of reproductive age is rising, leading to a pregnancy obesity phenotype. Maternal Obesity (MO) increases both fetal and maternal risk of pregnancy-associated adverse outcomes. Offspring can be programmed for metabolic diseases. MO can affect development of the fetal liver, an organ crucial for metabolic homeostasis. The fetal liver receives different quality blood in the right and left lobes, potentially altering programming. We investigated liver lobe-dependency of MO on oxidative stress (OS).

**Methods:** At 60 days before conception and throughout gestation, ewes consumed 150% (MO, n=8) or 100% (Control, C, n=10) of recommended

global nutrient intake. At 90% of gestation, we euthanized ewes under general anesthesia and fetal liver lobes were collected. Enzymatic activities, total antioxidant capacity (TAC) and protein carbonylation were measured spectrophotometrically. Data were compared between groups using an unpaired t-test, with  $p < 0.05$  considered statistically significant.

**Results:** MO decreased ewes fetal total (13.7%) and liver (22.4%) weight. MO increased fetal liver mitochondrial protein carbonylation ( $p = 0.04$ ), with more significant impact in the right lobe. Mitochondrial TAC was reduced in MO (-18.9%), consistent with lower mitochondrial free thiols concentration (-14.6%) and mitochondrial catalase activity (25.8%). Interestingly, hepatic superoxide dismutase and total catalase activities were unaffected. MO did not change fumarase activity; however, it decreased aconitase activity (-48.6%), a TCA cycle enzyme susceptible to oxidative stress. After chemical aconitase reactivation, a greater inhibition was observed due to MO (91%) compared with C (83.8%), again exacerbated in the right lobe.

**Conclusion:** MO affects prenatal hepatic function by inducing mitochondrial OS with potential modulation of fetal liver metabolism. All effects were exacerbated in the right lobe. These alterations likely represent fetal hepatic metabolic programming due to MO, with the potential of later-life compromise of hepatic mitochondrial function, predisposing offspring to metabolic disease. FEDER/COMPETE/FCT-Portugal: PTDC/DTP-DES/1082/2014 (POCI-01-0145-FEDER-016657) and UIDB/04539/2020; SFRH/BPD/116061/2016, 2020.05539.BD; and NIH: R01HD070096-01A1.

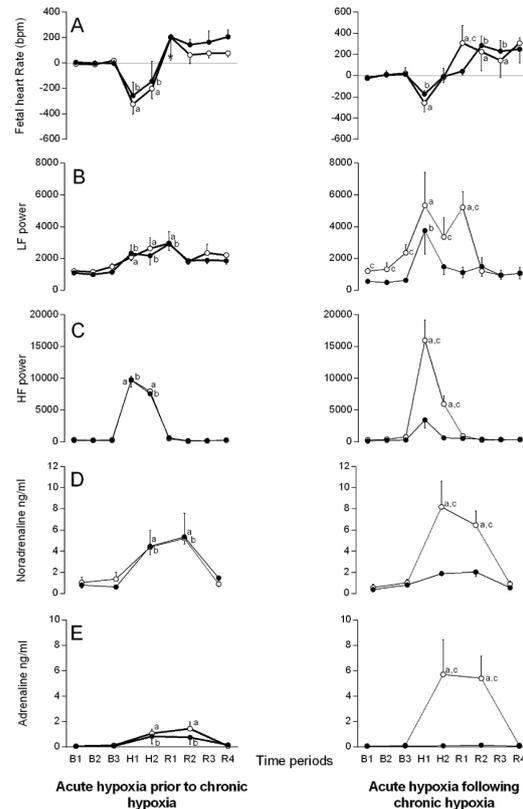
### O-059

**Impaired Autonomic Control of Heart Rate Variability during Acute Stress in the Chronically Hypoxic Fetus.** N. Hafiz<sup>†</sup>,<sup>1</sup> B. J Allison,<sup>2</sup> N. Itani,<sup>2</sup> K. J Botting,<sup>2</sup> Y. Niu,<sup>2</sup> C. C Lees,<sup>1</sup> C. J Shaw\*,<sup>1</sup> D. A. Giussani\*,<sup>2</sup> <sup>1</sup>Imperial College London, London, United Kingdom; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.

**Introduction:** Fetal heart rate variability (FHRV) is an excellent example of translating reproductive science to the bedside, as it is an established and powerful predictor of fetal wellbeing. The chronically hypoxic fetus is at a seven-fold risk of stillbirth or birth asphyxia (Gardosi et al. *BMJ* 346:f108, 2013), however underlying mechanisms remain unclear. Progress in this field has been hampered in part by the inability to record cardiovascular data from the chronically hypoxic fetus. We designed isolators able to maintain chronically instrumented pregnant sheep under controlled and significant hypoxic conditions for prolonged periods of gestation, coupled with a bespoke wireless data acquisition system (CamDAS) to record continuous *in vivo* fetal cardiovascular function. Here, we provide evidence of significant impairment in the autonomic control of FHRV during an episode of acute hypoxic stress in the chronically hypoxic fetus.

**Methods:** Eight pregnant sheep were anaesthetised at 117±2d gestational age (term ~147d) and the fetus surgically instrumented with vascular catheters. Five days postoperatively, the animals were subjected to acute hypoxia (fetal PaO<sub>2</sub> from 20±0.5 to 10±1 mmHg) followed by a 10-day exposure to normoxia (n=4) or chronic hypoxia (fetal PaO<sub>2</sub> 21±1 to 12±1 mmHg, n=4). Two days later, the pregnancy was exposed to second acute hypoxia of the same magnitude as the previous. FHR was sampled in 1-min blocks for 45 min prior to (baseline), during (30 min), and for 60 min after (recovery) the hypoxic episodes and power spectral indices calculated. Fetal blood was collected during all acute hypoxic episodes and processed for plasma catecholamines (ELISA). Data were analysed by Two-Way RM ANOVA. Significance was accepted when  $P < 0.05$ .

**Results:** There were no differences between groups prior to chronic hypoxia (Fig 1). However, the chronically hypoxic fetus showed markedly blunted values for rebound tachycardia (A), LF power (B, an index of sympathetic control), HF power (C, an index of parasympathetic control) and increments in plasma catecholamines (D and E) during subsequent acute hypoxia.



**Figure 1: Fetal heart rate and power spectral indices during acute hypoxia.** Values represent mean  $\pm$  SEM for the area under the curve over every 15 min during baseline (B), hypoxia (H) and recovery (R) for fetal heart rate, LF and HF power and the ratio of LF to HF power. Open circles (o, n=4) represent control fetuses; closed circles (•, n=4) represent fetuses exposed to chronic hypoxia. Significant differences: aP < 0.05 effect of time compared to baseline in control (normoxic) fetuses; bP < 0.05 effect of time compared to baseline in hypoxic fetuses; cP < 0.05 effect exposure to chronic hypoxia compared to control fetuses.

**Conclusion:** The chronically hypoxic fetus shows markedly impaired autonomic control of FHRV during acute stress, which may explain its much-increased vulnerability to stillbirth and birth asphyxia.

**Support:** The British Heart Foundation

### O-060

**RNAseq of Amniotic Fluid Reveals Activation of the Innate Immune Response and Differential Expression of Brain Transcripts in Fetuses with Cytomegalovirus Infection.** Lisa Hui\*,<sup>1,2,3,4</sup> Luc de Catte,<sup>5</sup> Neeta Vora,<sup>6</sup> Sally Beard,<sup>1</sup> Jovana Maksimovic,<sup>7</sup> Alicia Oshlack,<sup>7</sup> Susan P Walker,<sup>1,2</sup> Natalie Hannan\*. <sup>1</sup>University of Melbourne, Melbourne, Australia; <sup>2</sup>Mercy Hospital for Women, Heidelberg, Australia; <sup>3</sup>Murdoch Children's Research Institute, Parkville, Australia; <sup>4</sup>Northern Health, Epping, Australia; <sup>5</sup>Universitair Ziekenhuis, Leuven, Belgium; <sup>6</sup>University of North Carolina, Chapel Hill, NC, United States; <sup>7</sup>Peter MacCallum Cancer Centre, Parkville, Australia.

**Introduction:** Cell-free RNA in amniotic fluid is derived from multiple fetal organs and produces characteristic gene expression profiles during specific physiological states. Cytomegalovirus (CMV) is the most common congenital infection and is associated with neurological morbidity and perinatal mortality. We performed the first RNAseq study of live human fetuses with CMV to identify differentially-expressed genes (DEGs) associated with infection.

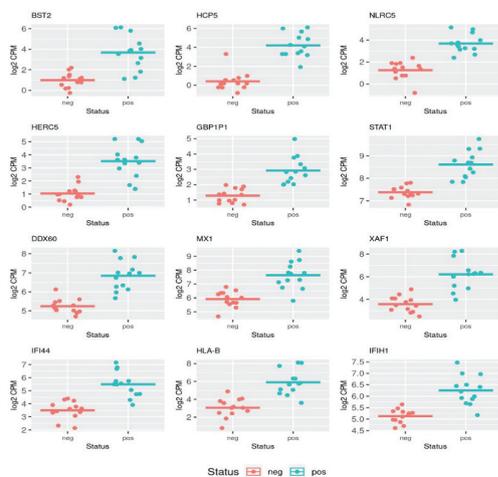
**Methods:** Amniotic fluid was collected from women at 18-22 weeks gestation undergoing amniocentesis for suspected CMV infection due to maternal seroconversion or ultrasound abnormality. CMV infection was diagnosed via viral PCR of amniotic fluid. Thirteen infected fetuses were paired to 13 non-infected controls, matched for gestational age (+/- 1 week) and fetal sex. Paired-end RNA sequencing was performed on

cell-free amniotic fluid RNA using the Novaseq 6000 at 30 million reads/sample. Following adapter trimming, reads were mapped using Star. Read counts were summarised across genes using featureCounts. Generalized linear models were fitted for each gene using edgeR, accounting for unwanted variation using RUVseq. DEGs were identified using quasi-likelihood tests. Genes with a false discovery rate <0.05 were considered statistically significant.

**Results:** There were 309 up-regulated and 32 down-regulated genes in the CMV-positive group, compared with the CMV negative group. Gene set enrichment analysis showed significant enrichment of multiple Gene Ontology categories involving the innate immune response to viral infection and interferon signaling. There were 32 down-regulated genes, including 8 neurodevelopmental genes known to be preferentially expressed by brain.

**Conclusion:** The fetal innate immune response to congenital CMV infection is detectable in second trimester amniotic fluid. We identified neurodevelopmental genes that are differentially expressed in infected fetuses. These mRNA transcripts have potential applications as biomarkers of neurodevelopmental outcome in congenital CMV.

Figure 1. Selected differentially-expressed genes in amniotic fluid of CMV negative (red) and CMV positive fetuses (green).



## O-061

**Uterine Fibroids Associated with Lower Fetal Fraction and Indeterminate NIPS Results in Non-Obese Subjects.** Teodora Kolarova†, Hayley MacKinnon†, Jaclynne Hedge, Christina Lockwood, Raj Shree\*. *University of Washington, Seattle, WA, United States.*

**Introduction:** Uterine fibroids are hypothesized to increase the risk of indeterminate non-invasive prenatal screening (NIPS) results due to low fetal fraction (FF). We sought to evaluate differences in first trimester NIPS FF and total cell free DNA (cfDNA) concentration in women with and without uterine fibroids.

**Methods:** We conducted a retrospective, single institution cohort study utilizing a previously validated in-house NIPS platform using whole genome sequencing on n=1112 independent samples. Fibroid size and number were obtained from first trimester ultrasound reports. Perinatal and delivery data were abstracted. First-trimester NIPS characteristics in those with fibroids (n=111) were compared to those without (n=1001) using univariate and bivariate analyses as appropriate. Logistic regression was used to determine the risk of an indeterminate result based on fibroid status, controlling for gestational age at NIPS, maternal age and fetal sex.

**Results:** Compared to controls, patients with fibroids were older and more likely to be non-white ( $p<0.05$ ). After excluding those with aneuploidy, autoimmune disease, or anticoagulation use, FF was significantly lower in those with fibroids compared to those without (9.8 vs. 11.5%;  $p<0.001$ ), however the rate of indeterminate results was statistically different only when evaluating non-obese (BMI<30 kg/m<sup>2</sup>) patients (8% vs 2%;  $p=0.009$ ). After controlling for gestational age at draw, maternal age,

and fetal sex, the presence of fibroids was significantly associated with indeterminate results in the non-obese cohort (OR 6.1; 95%CI [2.1,17.5];  $p=0.001$ ). Total cfDNA concentration was higher in those with fibroids ( $p=0.001$ ), suggesting possible dilution of the FF. Fibroid size did not affect rate of indeterminate results or total cfDNA concentration.

**Conclusion:** Uterine fibroids are associated with lower FF and, in non-obese gravidas, with a higher rate of indeterminate results. Fibroid size does not seem to impact these risks. Total cfDNA is higher in the setting of fibroids suggesting increased shedding of cfDNA from fibroids and a subsequent dilutional effect on FF.

Table 1

Demographics	(-) Fibroids (n=1001)	(+) Fibroids (n=111)	p-value
BMI at NIPS draw (kg/m <sup>2</sup> )	25 (22.3-29.3)	25.5 (22.6-29.5)	0.36
Maternal age at delivery (years)	35.5 ± 4.2	37.2 ± 3.8	<0.001
White race	673 (67.2)	55 (49.6)	<0.001
Gravidity	2 (1-4)	2 (1-4)	0.48
Autoimmune disorders	26 (2.6)	4 (3.6)	0.35
GA at NIPS draw (weeks)	12.1 ± 0.9	12.1 ± 0.8	0.73
Suspected aneuploidy	17 (1.7)	1 (0.9)	0.46
Anticoagulation use	19 (1.9)	3 (2.7)	0.38
GA at delivery (weeks)	38.2 ± 5.0	38.2 ± 4.0	0.47
Female fetal sex	397 (45.3)	39 (39)	0.45
<b>NIPS Results</b>			
Fetal fraction (%)	11.4 ± 5.0	9.7 ± 4.6	<0.001
Indeterminate result	44 (4.4)	9 (8.1)	0.1
cfDNA concentration (pg/uL)	84.1 (61.1-116.5)	98.6 (64.1-148)	0.01

Data represented as mean±SD, median(IQR) or n(%)

Table 2

Filtered cohort*	(-) Fibroids (n=932)	(+) Fibroids (n=100)	p-value
Fetal fraction (%)	11.5 ± 5.0	9.8 ± 4.5	<0.001
Indeterminate result	38 (4.1)	7 (7.0)	0.19
cfDNA concentration (pg/uL)	82.4 (60.5-112)	99.6 (69.1-147)	0.001
<b>Filtered cohort* &amp; BMI&lt;30</b>			
Fetal fraction (%)	12.2 ± 4.4	10.5 ± 4.5	0.003
Indeterminate result	14 (2)	6 (8)	0.009
cfDNA concentration (pg/uL)	79.8 (59.7-110)	101 (68.6-148)	0.002
<b>Filtered cohort* &amp; (+) Fibroids</b>			
	<b>Largest fibroid &lt;5 cm (n=72)</b>	<b>Largest fibroid ≥5cm (n=28)</b>	
Fetal fraction (%)	11.1 ± 4.6	9.3 ± 4.4	0.28
Indeterminate result	5 (6.9)	2 (7.1)	1.0
cfDNA concentration (pg/uL)	100.5 (67.7-149.5)	95.9 (78-144.5)	0.83

\*Filtered cohort excludes patients with suspected aneuploidy, autoimmune disease, anticoagulation use  
Data represented as mean±SD, median(IQR) or n(%)

## O-062

**Cervical Gene Delivery of the Antimicrobial Peptide Human  $\beta$  Defensin 3 (HBD3) Reduces Perinatal Neuroinflammation in a Mouse Model of Ascending Infection-Associated Preterm Birth.** Ashley K Boyle†, Natalie Suff,†, Simon N Waddington,†, Donald Peebles\*.<sup>1</sup> *University College London, London, United Kingdom;* <sup>2</sup>*King's College London, London, United Kingdom.*

**Introduction:** Preterm birth (delivery <37 weeks) is the leading cause of neonatal mortality worldwide. It is associated with high rates of adverse neurodevelopmental outcome and cerebral palsy. Approximately 40% of cases of spontaneous preterm birth have been associated with infection. Delaying preterm birth and improving the outcome of the premature babies has proved a great challenge and current therapies are ineffective. We have previously shown that cervical gene delivery of human  $\beta$  defensin 3 (HBD3) improves pup survival in a mouse model of ascending vaginal infection. Therefore, we hypothesised that this gene therapy would also reduce neuroinflammation in these pups.

**Methods:** An adeno-associated virus vector containing both the HBD3 gene and GFP transgene (AAV8-HBD3) was administered intravaginally into embryonic day (E)13.5 pregnant albino mice (C57BL/6 Tyr<sup>e-2j</sup>). A control vector containing only GFP was used throughout (AAV8-GFP). Ascending infection was induced on E16.5 by intravaginal administration of bioluminescent *Escherichia coli* (*E. coli* K1 A192PP-lux2, 20 $\mu$ l of 2x10<sup>2</sup> CFU). PBS (20 $\mu$ l) was delivered as a vehicle control. Treatment groups were as follows: AAV8-GFP + PBS, AAV8-GFP + K1 *E. coli* and AAV8-HBD3 + K1 *E. coli* (n=11-15 pups from 5 dams/group). Fetal tissues were harvested 48 hours after infection. Neuroinflammation was assessed by RT-qPCR.

**Results:** Cervical gene delivery of AAV8-HBD3 did not significantly impact the time to delivery of pups following infection with K1 *E. coli* compared to the AAV8-GFP + K1 *E. coli* positive control group ( $43.1 \pm 7$  hours vs  $44.5 \pm 6$  hours, respectively;  $p=0.9397$ ). However, AAV8-HBD3 gene delivery significantly increased the percentage of live born pups, in comparison to the AAV8-GFP + K1 *E. coli* group (82.9% vs 47.97%, respectively;  $p=0.0226$ ), confirming our previous results. The mRNA expression of the following inflammatory mediators were increased in perinatal brains from AAV8-GFP + K1 *E. coli* dams in comparison to AAV8-GFP + PBS control dams; *Tnfa* ( $p<0.0001$ ), *Il-1 $\beta$*  ( $p<0.0001$ ), *Il-6* ( $p<0.001$ ), as well as *Gfap* ( $p=0.0016$ ), which is associated with neuropathology. Maternal AAV8-HBD3 gene delivery significantly reduced *Tnfa* ( $p=0.0079$ ) and *Gfap* ( $p<0.0001$ ) mRNA expression in perinatal pup brains exposed to K1 *E. coli*, compared to control vector.

**Conclusion:** Cervical gene delivery of HBD3 significantly increased the percentage of live born pups in a mouse model of ascending infection-associated preterm birth, while reducing the mRNA expression of genes associated with inflammation and neuropathology in perinatal pup brains. Future studies will assess the impact of maternal HBD3 gene delivery on long term neurodevelopment in pups.

### O-063

**Functional Enrichment Analysis of Differentially Expressed Transcripts/Genes of Medium/Large STBEVs Identified Potentially Dysregulated Pathways in Early Onset Preeclampsia (EOPE).** [Toluwalase Awoyemi](#)<sup>†</sup>, Adam Cribbs, Wei Zhang, Chris Redman, Manu Vatish\*. *University of Oxford, Oxford, United Kingdom.*

**Introduction:** Early-onset preeclampsia is a multisystemic disease of pregnancy characterised by elevated blood pressure and end-organ dysfunction and diagnosed between 20 to 34 weeks gestation. Its exact mechanism remains elusive, but several competing pathological theories exist such as the role of a substance 'X' and the release of syncytiotrophoblast membrane extracellular vesicles (STBEV); small and medium/large STBEVs. Recently, STBEV's are being explored as carriers of this potential substance X(s) in their cargo. We hypothesise that the mRNA content of medium/large STBEVs differs substantially between EOPE and normal pregnancy (NP). The differentially expressed transcripts detected would highlight pathways that may be functionally important in EOPE.

**Methods:** STBMV's were generated by ex-vivo dual lobe placenta perfusion followed by proteomic analysis to identify DEPs between EOPE and NP STBMV. DEGs obtained were bioinformatically analysed to identify functionally enriched pathways in R with Cluster Profiler and SPIA. Enriched pathways were analysed in four categories GO: BP (biological process) GO: MF (molecular function) GO: CC (cellular component) and KEGG pathways while topographical analysis was done for KEGG.

**Results:** Two pathways were perturbed with topological analysis and activated: Focal adhesion, cytokine-cytokine receptor interaction. Eight B.P's were found to be perturbed, such as platelet degranulation and epithelial cell differentiation. Ten molecular functions were perturbed such as phosphatase regulator activity, phosphatidylcholine binding and quaternary ammonium group binding. Focal adhesion was the only overexpressed KEGG pathway.

**Conclusion:** A known complication of Preeclampsia is Eclampsia, a state of convulsion and coma in a woman diagnosed with preeclampsia. The finding of an activated neuroactive ligand-receptor pathway may explain the link between EOPE and neurological manifestations in the mother. The activated cytokine-cytokine receptor pathway may be the driver for the endothelial dysfunction and platelet activation characteristic of EOPE. In conclusion, EOPE is a disease with a complicated interacting mechanism, some of which may propagate disease or serve a self-protective role. Our analysis has bioinformatically teased out some of these pathways that may help identify mechanistic and therapeutic targets.

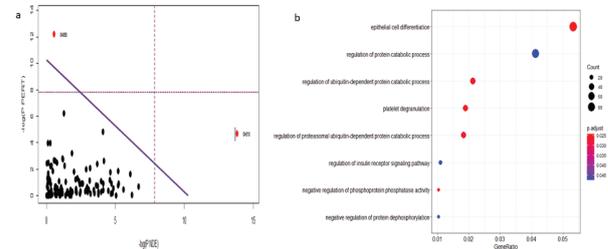


Figure showing (a) a two-way evidence SPIA plot showing the two significantly activated medium/large STBEV functional pathways (b) a dot plot of functionally enriched gene ontology biological processes. The colour represents the level of significance while the size of the dots represent the number of genes in our DEG list found in the pathways

### O-064

**Proinflammatory and Anti-Inflammatory Cytokine Responses by Decidua T Cells in Preeclampsia.** [Ai-ris Yonekura Collier](#), Dan H Barouch\*. *Beth Israel Deaconess Medical Center, Boston, MA, United States.*

**Introduction:** Regulatory T cells (Treg) are key mediators of immune tolerance. Both thymic-derived FoxP3<sup>+</sup> natural Treg (nTreg) and peripherally-induced PD-1<sup>hi</sup> Treg (iTreg) are found in human decidua. Decidua iTreg and nTreg suppress anti-fetal immune responses by inhibiting effector T cells (Teff) proliferation. iTreg produce anti- or proinflammatory cytokines and also induce cytokine production by Teff. We hypothesize that disruption in iTreg function is associated with preeclampsia (PE). Our objective is to compare the cytokine production by decidua T cells in PE to healthy pregnancies.

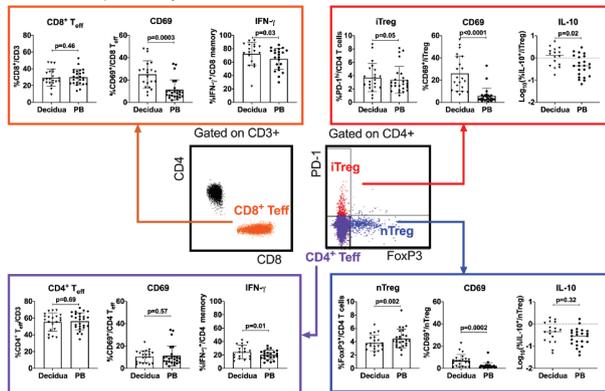
**Methods:** Maternal peripheral blood (PB) and decidua blood (from the surgical sponge at cesarean delivery) were collected from PE cases and controls. Isolated lymphocytes were stimulated *in vitro*, and stained with immunofluorescence antibodies for identification of FoxP3<sup>+</sup> nTreg, PD-1<sup>hi</sup> iTreg, CD4<sup>+</sup>, and CD8<sup>+</sup> effector T cell (Teff) subsets by flow cytometry. Production of anti-inflammatory IL-10, and proinflammatory cytokines IL-17, and IFN-g were detected using intracellular staining (Figure 1). Proportions were compared using two-sided tests with  $p<0.05$ .

**Results:** 61 participants were enrolled. The proportion of decidua Teff was similar to PB; however, decidua CD8<sup>+</sup> Teff expressed higher levels of activation marker CD69 and produced more IFN-g compared to PB (Fig. 1, left). Decidua contained greater iTreg and lower nTreg proportions compared to PB. Additionally, decidua iTreg exhibited more CD69 expression and IL-10 production (Fig. 1, right).

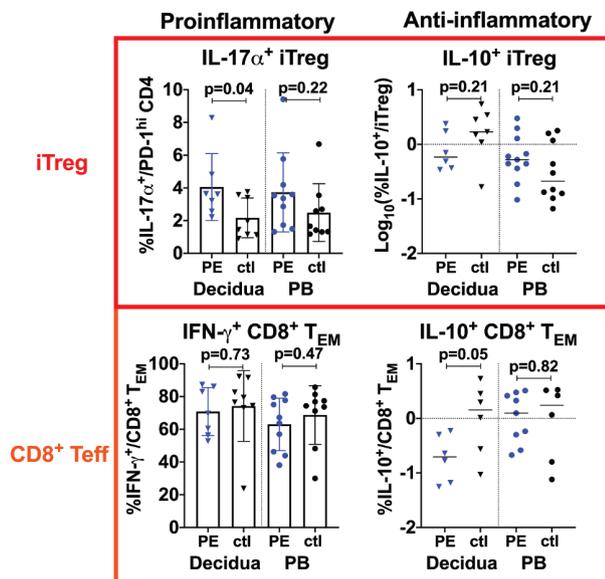
Decidua iTreg exhibited higher IL-17 production in PE. iTreg and memory CD8<sup>+</sup> Teff from decidua displayed lower IL-10 production in PE compared to controls but was not statistically significant (Fig. 2).

**Conclusion:** Human decidua harbors activated and cytokine-producing regulatory and effector T cells. Our data provide evidence for the maternal-fetal rejection phenotype in PE with an imbalance of proinflammatory cytokine production in PE due to enhanced IL-17 production by iTreg and decreased IL-10 production by decidua iTreg and CD8<sup>+</sup> Teff cells. Further elucidation of the immune mechanisms of PE will inform new therapeutic strategies.

**Figure 1.** Induced and natural regulatory T cells and CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells are activated and produce cytokines in human decidua.



**Figure 2.** Induced regulatory T cell and memory CD8<sup>+</sup> T cell production of proinflammatory cytokines IL-17 and IFN- $\gamma$  and anti-inflammatory IL-10 in preeclampsia.



### O-065

**Impact of Preeclampsia on Polarization and Functionality of Hofbauer Cells.** *Monika Horvat Mercnik<sup>†</sup>, Carolin Schlieffsteiner\*, Christian Wadsack\*. Medical University of Graz, Graz, Austria.*

**Introduction:** Hofbauer cells (HBCs) are resident macrophages of the feto-placental unit thereby regulating tissue homeostasis. HBCs are characterized by their plasticity and cells are believed to trigger inflammatory processes. Function of macrophages is reflected by variety of phenotypes, which depends on their polarization. HBCs of healthy placenta (CTR) mainly exhibit anti-inflammatory M2 phenotype. We hypothesize that under exaggerated inflammatory conditions, such as in preeclampsia (PE), HBCs lose plasticity thereby shifting to a pro-inflammatory M1 phenotype. Aim of this study is to investigate polarization and phenotype of PE HBCs and to determine possible alterations on HBCs functionality.

**Methods:** Primary HBCs were isolated from CTR (N=10) and PE term placentae (N=8). Specific polarization surface and intracellular markers, associated with respective phenotypes were analysed by FACS. To investigate secretion of cytokines, chemokines and tissue inhibitors of metalloproteinases (TIMPs), HBC culture supernatants were collected from CTR and PE and analysed with multiplex ELISA-on-beads assay.

Phagocytosis was determined with FACS and high content imaging. mRNA expression of metalloproteinase 9 (MMP9), TIMP1 and TIMP2 was detected with RT-qPCR. Activity of MMP-9 was determined with immunoblots and gelatine zymography. After normality testing, statistical significance was determined using t-test.

**Results:** Expression of M1 markers namely CD86, CD80 and CD40 ( $p=0,07$ ) is increased in PE HBCs, but only by trend. The M2 marker CD206 was increased in PE HBCs ( $p=0,01$ ). Both groups of HBCs were positive for CD68 (CTR  $84,7 \pm 8,4$ ; PE  $84,6 \pm 9,4\%$ ) and CD163 (CTR  $91,3 \pm 7,7\%$ ; PE  $90,8 \pm 10,5\%$ ), respectively. PE HBCs secrete higher levels of pro-inflammatory cytokines such as IL-6 ( $p \leq 0,05$ ), IL-8 ( $p \leq 0,01$ ), IL-12p70 ( $p \leq 0,05$ ), IL1 $\alpha$  ( $p=0,06$ ) and TNF $\alpha$  ( $p=0,06$ ). In the same samples also anti-inflammatory cytokines as IL-13 ( $p \leq 0,05$ ), IL-4 ( $p \leq 0,05$ ) and TGF $\beta$  ( $p \leq 0,05$ ) were elevated. Phagocytic activity was higher in PE HBCs ( $p \leq 0,05$ ). PE HBCs express higher MMP-9 levels ( $p \leq 0,05$ ) as CTR HBCs. Additionally, levels of IL-17 ( $p=0,06$ ), one of the regulators of MMPs were elevated. In line, release of TIMP1 was significantly decreased but secretion of TIMP2 was not affected by PE.

**Conclusion:** HBCs maintain anti-inflammatory phenotype and plasticity in placentas affected by PE. PE alters functionality of HBCs which is reflected by an increased phagocytosis. Of note, PE HBCs express higher levels of MMP9, which is tightly regulated by TIMP1 downregulation, indicating the significance of tissue remodelling in PE. Understanding the role of MMPs in HBCs and vascular remodeling could help design new approaches for management of preeclampsia.

### O-066

**CD4 T Cells Deficiency Enhances Internal Carotid Artery Constriction in Postpartum Mice: The Role of Nitric Oxide.** *Natalia I Gokina, Rebecca I Fairchild, Kirtika Prakash, Nicole M DeLance, Elizabeth A Bonney\*. University of Vermont, Burlington, VT, United States.*

**Introduction:** The risk of postpartum (PP) stroke is increased in pregnancies complicated by hypertension or preeclampsia. Deficiency in regulatory T cells, a subset of CD4 T cells, is associated with vascular disease and may contribute to PP health complications. Internal carotid artery (ICA) stenosis due to vasoconstriction or arterial dissection may lead to maternal ischemic stroke. In this study we (1) characterized function, mechanical behavior and structure of ICAs from C57BL/6 (WT) and CD4 deficient (CD4<sup>-/-</sup>) virgin and PP mice; (2) assessed the role of nitric oxide (NO) in the control of ICA function at pre-conception and PP. **Methods:** WT and CD4<sup>-/-</sup> mice were housed under pathogen-free conditions, mated to same-strain males and allowed to litter. At 3 days or 4 wks PP, mothers or virgin controls were euthanized. Vasoconstriction to phenylephrine (PE, 0.01 - 30  $\mu$ M) or to high K<sup>+</sup> (20 - 100 mM) and vasodilation to ACh (0.001 - 10  $\mu$ M) were assessed in pressurized at 80 mmHg ICAs. To evaluate the role of NO, PE, high K<sup>+</sup> and ACh were tested after NOS inhibition with L-NNA. To characterize ICA distensibility, passive lumen diameters were measured at 3-140 mmHg. The expression of eNOS in ICAs was evaluated by immunohistochemistry. Differences between sets of data were determined by t-tests or two-way RM ANOVA and were considered significant at  $P < 0.05$ .

**Results:** Constriction of WT ICAs to PE was not modified at any PP periods. In contrast, responses to PE were significantly increased in ICAs from 3 days and 4 wks PP CD4<sup>-/-</sup> mice ( $52.4 \pm 4.7\%$ , n=5 and  $52.3 \pm 4.5\%$  n=9) vs. CD4<sup>-/-</sup> virgins ( $38.4 \pm 4.7\%$ , n=10 at 10  $\mu$ M PE). Constriction to high K<sup>+</sup> was not enhanced. ICAs from WT and CD4<sup>-/-</sup> mice were highly sensitive to ACh with no significant difference in EC<sub>50</sub> values. L-NNA treatment resulted in a rightward shift of dose-response curves to ACh. NOS inhibition enhanced PE constriction of ICAs from WT virgin and PP mice. Although a similar effect was detected in ICAs of virgin CD4<sup>-/-</sup> mice ( $62.9 \pm 5.6\%$ , n=5 at 10  $\mu$ M PE), no such changes were observed in CD4<sup>-/-</sup> at 3 days ( $60.4 \pm 4.2\%$ , n=5) and 4 wks ( $55.6 \pm 3.9\%$ , n=6) PP. Passive distensibility was not modified at PP. ICA diameters increased from  $306 \pm 4 \mu$ m (WT, n=30) and  $305 \pm 3 \mu$ m (CD4<sup>-/-</sup>, n=24) at pre-conception to  $323 \pm 5 \mu$ m (n=24,  $P=0.008$ ) and  $318 \pm 3 \mu$ m (n=20,  $P=0.007$ ) at 4 wks PP, respectively. eNOS expression was significantly reduced in vessels from 4 wks PP CD4<sup>-/-</sup> mice but it was not different in ICAs from virgin vs. PP WT mice.

**Conclusion:** The PP in CD4<sup>-/-</sup> mice is characterized by decreased NO-dependent control of ICA constriction in part due to reduced expression of eNOS. CD4 immune deficiency in pregnancy may predispose the ICAs to vasospasm and increase the risk of maternal PP stroke.

## O-067

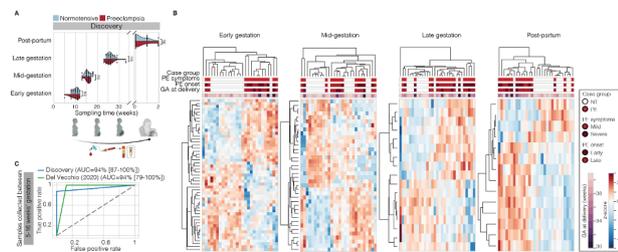
**Noninvasive Prediction of Preeclampsia in Pregnancy with Circulating RNA.** Mira N Moufarreġ<sup>†</sup>, Ronald J Wong,<sup>1</sup> Ana A Campos,<sup>1</sup> Cecele C Quaintance,<sup>1</sup> Rene V Sit,<sup>2</sup> Michelle Tan,<sup>2</sup> Norma F Neff,<sup>2</sup> Maurice L Druzin,<sup>1</sup> Virginia D Winn,<sup>1</sup> Gary M Shaw,<sup>1</sup> David K Stevenson,<sup>1</sup> Stephen R Quake\*,<sup>1,2</sup> <sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>Chan Zuckerberg Biohub, Stanford, CA, United States.

**Introduction:** No test exists to predict future onset of preeclampsia (PE) early in pregnancy. Liquid biopsies that measure circulating, cell-free RNA (cfRNA) offer an unprecedented opportunity to both noninvasively study the development of PE, the pathogenesis of which to date remains unclear, and to bridge gaps in clinical care like early diagnosis ( $\leq 16$  weeks of gestation) when such a test could guide the use of low-dose aspirin to reduce the risk of PE. Here, we sought to identify cfRNA transcriptomic changes across gestation and at post-partum that are associated with PE and to build a robust classifier that can predict risk of PE early in pregnancy.

**Methods:** To identify changes associated with PE well before clinical onset, we designed a prospective nested case control study, and sequenced cfRNA from 118 samples from 42 pregnant mothers (18 normotensive (NT), 24 PE). We then performed differential expression to identify gene changes associated with PE across pregnancy. Finally, we built and validated a robust classifier that can identify mothers at risk of PE at or before 16 weeks of gestation using the aforementioned dataset in training and an independent, previously published dataset (Del Vecchio et al 2020) for validation.

**Results:** cfRNA transcriptomic changes can distinguish between NT and PE pregnancies across early (5-12 weeks), mid (13-18 weeks), and late (23-33 weeks) gestation and even into the post-partum period (0-2 weeks after delivery) regardless of PE subtype. Interestingly, the majority of these cfRNA changes were most striking early in gestation suggesting that the identified cfRNA signal may correlate with PE pathogenesis, which is thought to also occur early in pregnancy. Indeed, gene ontology analysis identified pathways that reflect known PE biology. Finally, we identified and independently validated ( $n = 8$  NT, 8 PE/gestational hypertension) that 11 genes measured between 5-16 weeks of gestation can form a predictive PE signature with 88% [55-99%] specificity and 100% [74-100%] sensitivity in validation (AUC = 94%, [79-100%]) (All reported as value, [95% confidence interval]).

**Conclusion:** Our results show that cfRNA can form the basis for a robust predictor of PE well before its clinical development and that such measurements may provide a means by which to interrogate the pathogenesis of PE in real time.



**Figure 1.** Across gestation and prior to diagnosis, changes in the cfRNA transcriptome segregate PE and NT samples. (A) Matched sample collection time (weeks) across gestation and post-partum (ns = not significant). (B) In each sample collection period, a subset of differentially expressed genes can separate PE and NT samples despite differences in symptom severity, PE onset subtype, and GA at delivery. (C) Classifier performance for samples collected in early gestation between 5-16 weeks.

## O-068

**Circulating SIGLEC6 Is Deranged in Preeclampsia and May Be a Biomarker of Disease Severity.** Tu'uhevaha J Kaitu'u-Lino,<sup>1</sup> Susan P Walker,<sup>1</sup> Teresa M MacDonald,<sup>1</sup> Catherine Cluver,<sup>2</sup> Roxanne Hastie,<sup>1</sup> Lina Bergman,<sup>3</sup> Lesley McCowan,<sup>4</sup> Rennae Taylor,<sup>4</sup> Emerson Keenan,<sup>1</sup> Natalie J Hannan,<sup>1</sup> Ping Cannon,<sup>1</sup> Tuong-Vi Nguyen,<sup>1</sup> Manju Kandel,<sup>1</sup> Stephen Tong,<sup>1</sup> <sup>1</sup>University of Melbourne, Melbourne, Australia; <sup>2</sup>Stellenbosch University, Cape Town, South Africa; <sup>3</sup>Uppsala University, Uppsala, Sweden; <sup>4</sup>University of Auckland, Auckland, New Zealand.

**Introduction:** We assessed whether SIGLEC6, a primate-specific molecule, is dysregulated with preeclampsia (PE) and associated with disease severity.

**Methods:** SIGLEC6 was measured in the circulation and placentas of women with PE who delivered at  $<34$  weeks' gestation (43 with PE vs 33 controls). We prospectively collected blood in an unselected population at 36 weeks' gestation (FLAG cohort) and measured plasma SIGLEC6 in 41 women who later developed PE vs 950 controls. We then measured plasma SIGLEC6 at earlier gestations and compared levels among those who did and did not develop PE: at 28 weeks' (FLAG cohort, 93 PE, 190 controls); 20+1 weeks' (SCOPENZ 82 PE, 1863 controls) and 15+1 weeks' gestation (SCOPENZ 84 PE, 1923 controls). Finally, we measured SIGLEC6 and placental growth factor (PIGF) in samples from women with PE from South Africa (PROVE biobank) and grouped according to disease severity: 111 PE without severe features (mildest disease), 36 with eclampsia, 23 with PE + other organ involvement, 135 with PE + severe hypertension. We also exposed syncytialised 1<sup>st</sup> trimester cytotrophoblastic stem cells to hypoxia, TNF $\alpha$  or IL-6 and measured SIGLEC6 levels.

**Results:** In women with preterm PE, plasma ( $p=7.1 \times 10^{-11}$ ) and placental SIGLEC6 ( $p<0.0001$  for both mRNA and protein) were significantly elevated relative to controls. We next examined the predictive potential of SIGLEC6. Relative to controls, SIGLEC6 was significantly increased preceding PE diagnosis: at 36 weeks ( $p=2.9 \times 10^{-4}$ ), 28 weeks ( $p=4.3 \times 10^{-3}$ ) and 20 weeks ( $p=3.9 \times 10^{-4}$ ); and a non-significant increase at 15 weeks' gestation ( $p=0.058$ ). Assessing the PROVE cohort, compared to women with PE without severe features (mildest disease variant), we observed a 2.5 fold increase (95% CI 1.7-3.6,  $p<0.001$ ) in SIGLEC6 in women with eclampsia, a 2 fold increase (95% CI 1.3-3.1,  $p=0.002$ ) in those with PE + other organ involvement, and a 1.6 fold increase (95% CI 1.2-2,  $p<0.001$ ) in those with PE + severe hypertension. Interestingly the fold changes were more marked when expressed as SIGLEC6/PIGF (fold changes as high as 11 in eclampsia). SIGLEC6 expression and secretion in syncytialised 1<sup>st</sup> trimester cytotrophoblastic stem cells were increased by hypoxia and inflammatory cytokines.

**Conclusion:** Circulating SIGLEC6 is consistently elevated preceding PE diagnosis in several large cohorts and its secretion may be stimulated by placental hypoxia or inflammation. Importantly, it is strongly associated with disease severity. Alone, or as a ratio with PIGF, SIGLEC6 is a promising new biomarker for PE, including for disease severity.

## O-069

**Pregnancy Outcomes Following Routine Early Provision of IUD after First Trimester Induced Abortion - 5-year Follow-Up of a Randomized Controlled Trial.** Oskari M. Heikinheimo\*,<sup>1</sup> Elina Pohjoranta<sup>†</sup>,<sup>1</sup> Satu Suhonen\*,<sup>2</sup> Maarit Mentula\*,<sup>1</sup> Mika Gissler,<sup>3,4</sup> <sup>1</sup>Helsinki University Hospital, Helsinki, Finland; <sup>2</sup>Centralized Family Planning, Helsinki, Finland; <sup>3</sup>National Institute for Health and Welfare, Helsinki, Finland; <sup>4</sup>Karolinska Institute, Stockholm, Sweden.

**Introduction:** In cohort studies, post-abortion contraception with long acting reversible contraceptives (LARC), i.e. contraceptive implants and intrauterine devices (IUD) is effective in reducing the need of subsequent termination of pregnancy (TOP). We have carried out a randomized controlled trial assessing the need of subsequent TOP following routine provision of intrauterine contraception shortly (1-4 weeks) following first trimester TOP by the same unit responsible for abortion care vs. initiating oral contraceptives and directing women to primary health care for further contraceptive provision. In the present study we analyzed the effects of this intervention on all pregnancies during a five-year follow-up.

**Methods:** Women in the intervention group (n=375) were offered an IUD (LNG-IUS [91%] or Cu-IUD [9%]) either during surgical TOP (n=69) or at a follow-up visit 1-4 after medical TOP (n=306). The control group (n=373) was provided with oral contraceptives and directed to primary health care for IUD provision. By 3 months 92.5% women in the intervention and 20.4% in the control group had received an IUD. Data on all deliveries, miscarriages and TOPs during the follow-up were obtained from Finnish national registries maintained by the National Institute for Health and Welfare, and analyzed according to randomization groups.

**Results:** Altogether 135 women gave birth to one or several children during follow-up, and there was no significant difference between the intervention (n=58 [15.5%]) and the control group (n=77 [20.6%]), HR 1.42 [CI95% 0.98-2.07]  $p=0.066$ . The mean time interval between the index abortion and the first delivery was 1073 days (SD 402) in the intervention and 1014 (SD 456,  $p=0.438$ ) in the control group. Thirty-eight women had  $\geq 1$  miscarriage(s) during the follow-up, 16 (4.3%) in the intervention and 22 (5.9%) in the control group (HR 1.41 [CI95% 0.73-2.72],  $p=0.312$ ). The mean time interval between the index abortion and the first miscarriage was 824 (SD 629) days in the intervention and 794 (573) in the control group ( $p=0.872$ ). Altogether 40 (10.7%) women in the intervention and 63 (16.9%) in the control group underwent  $\geq 1$  subsequent TOP(s) (HR 1.67 [CI 95% 1.13 to 2.49],  $p=0.011$ ). The mean time interval between the index and the first subsequent TOP was 973 days (SD 494 days) in the intervention, and 742 days (SD 455 days) in the control group ( $p=0.013$ ).

**Conclusion:** Routine provision of IUD to women undergoing TOP is an effective means to reduce the need of subsequent TOP. Also the time to next TOP was prolonged. However, the number of women delivering or being diagnosed with miscarriage(s), nor their timing were not significantly affected.

#### O-070

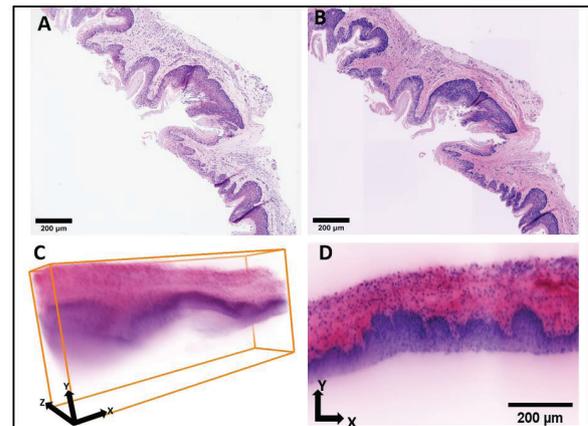
**Towards 3-D Imaging of the Murine Vagina.** Diego R Gatica†, Li Guang†, J. Quincy Brown, Kristin S Miller. Tulane University, New Orleans, LA, United States.

**Introduction:** Pelvic organ prolapse (POP) is the descent of the pelvic organs. 12% of women require surgical correction for POP and ~30% of surgeries fail due to insufficient strength or mismatches in graft and native tissue properties (1). Quantifying composition in 3-D as opposed to traditional 2-D histologic analysis may reveal new information on vaginal microstructure to inform diagnostics and design criteria to improve interventions. Dual-view inverted selective plane illumination microscopy (diSPIM) images a tissue in 3-D using fluorescent stains (3). The fluorescent stain TO-PRO-3 Iodide and Eosin (T&E) is a potential analog to Hematoxylin and Eosin (H&E) that can be used to acquire 3-D images (2). Therefore, the objective of this study was to validate T&E as an H&E fluorescent analog and then to acquire 3-D images of the murine vagina.

**Methods:** To validate T&E, vaginas (fibulin-5  $+/+$ , fibulin-5  $+/-$ , and fibulin 5  $-/-$ , n=3/genotype) were extracted from C57BL6 X 129 SvEv female mice at 3-6 months at estrus and snap frozen (IACUC approved). Samples were formalin-fixed, sectioned and randomly allocated for either H&E or T&E staining. Sections stained with H&E were imaged using bright-field optical microscopy at 10X objective magnification, and T&E imaged using a fluorescent slide scanner at 20X objective magnification and then pseudo-colored to resemble H&E (4). The epithelial layer and extracellular matrix area fractions were quantified and evaluated for equivalence between stains using Bland-Altman. For the diSPIM imaging of murine vaginas, vaginas were extracted from CD1 mice (nonparous, day 18 pregnant, and postpartum (n=1/timepoint)) (IACUC approved). Samples were formalin-fixed, stained with TO-PRO-3, dehydrated, stained with eosin, and optically cleared with Ethyl Cinnamate. Next, samples were imaged with diSPIM reconstructed with MATLAB and visualized using Amira (3).

**Results:** Bland-Altman analysis showed similarity between the T&E and H&E stains, validating T&E as an analog to H&E in the murine vagina. 3-D images of the pregnancy samples demonstrated the capability of diSPIM to acquire images across the entire vaginal volume (Fig 1).

**Conclusion:** These findings suggest that T&E is a valid analog to H&E in vaginal tissue, and that it can be used with diSPIM to observe vaginal microstructure in 3D. T&E and diSPIM can be used together to reveal novel information on vaginal anatomy and potentially aid in understanding fundamental changes with POP. [1] Barski et al., Biomed Res Int, 2015. [2] Elfer et al., PLoS One, 2016. [3] Hu et al., Biomedical Optics Express, 2019. [4] Li et al., bioRxiv 2020.



**Figure 1:** A: Fibulin-5  $+/+$  H&E-stained sample B: Fibulin-5  $+/+$  T&E-stained sample of similar region to H&E. Similarities in stained structures can be observed. C: Amira 3-D rendering of a region of the postpartum vaginal sample, allowing shape of the sample to be visualized D: slice extracted on the XY plane from 3-D rendering in C.

#### O-071

**A Meta-Analysis Investigation of Anti-Mullerian Hormone Trends in Survivors of Childhood Cancer.** Allison Kumnick†, Veronica Gomez-Lobo, Ninet Sinaii, Jacqueline Maher\*. National Institutes of Health, Bethesda, MD, United States.

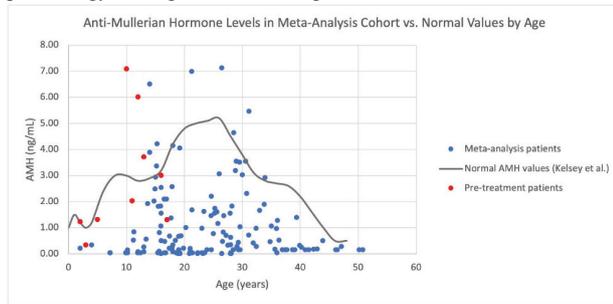
**Introduction:** There are an estimated 400,000 female childhood cancer survivors (CCSs) of childbearing age. Many chemotherapy and radiation therapies are gonadotoxic, increasing the likelihood of premature ovarian insufficiency and infertility. Anti-Mullerian hormone (AMH), one of the biomarkers used to assess ovarian reserve, may have a role in the care of CCSs. This study aims to investigate the association between AMH levels after treatment and their relationship with reproductive outcomes.

**Methods:** We conducted a literature review and meta-analysis of 509 studies pertaining to AMH values in CCSs. Nine studies containing individual patient data were analyzed. Data are described as median (IQR) or mean (SD). Spearman rho and mixed models were used for analysis.

**Results:** A total of 161 patients were represented with median age of 21.3 (16.0-29.3) years at the time of AMH measurement (Figure 1). Median age at diagnosis was 12.7 (7.0-15.7) years with follow-up time of 11.7 (6.0-18.5) years. Controlling for age at study, AMH values [median 0.47 (0.15-1.66) ng/mL] were positively correlated with age at diagnosis ( $r_s=0.29$ ,  $p<0.001$ ), representing their baseline levels; as well as inversely correlated with duration of follow-up ( $r_s=-0.20$ ,  $p=0.013$ ). Compared to AMH at baseline 2.89 ng/mL, levels were 1.66 ng/mL up to two years, 0.51 ng/mL from 2-10 years, and 1.01 ng/mL at 10+ years after diagnosis ( $p=0.20$ ,  $p<0.001$ , and  $p<0.01$ , respectively). From the top 3 most common diagnoses, AMH levels were 1.44 (SD 1.59) ng/mL in lymphoma, 0.72 (SD 0.94) ng/mL in leukemia, and 0.14 (SD 0.25) ng/mL in neuroblastoma. Compared to lymphoma, patients with leukemia and neuroblastoma had significantly lower age-controlled AMH values ( $p<0.001$  for each). For women 21+ years old, AMH was not different by history of pregnancy versus never pregnant [1.02 (1.33) vs. 1.43 (1.75) ng/mL;  $p=0.052$ ]. An inverse correlation was noted between AMH and FSH ( $r_s=-0.35$ ,  $p<0.001$ ), as expected.

**Conclusion:** This meta-analysis of 9 studies show AMH values continue to decline from baseline to 10 years post-diagnosis and then increase again, which contrasts past studies that have shown some AMH recovery and plateau at 2-3 years. There were significant differences between cancer diagnoses. These results highlight both the utility and the limitations of

AMH levels in children and adolescent girls with a history of cancer treatment that are useful for practicing reproductive endocrinologists, pediatric gynecologists, and oncologists.



### O-072

**Euploid Miscarriage Is Associated with *Lactobacillus* spp. Deplete Vaginal Microbial Composition and Local Inflammation.** Karen Grewal†, Yun S Lee, Ann Smith, Jan J Brosens, Tom Bourne, Maya Al-Memar, Samit Kundu, David A MacIntyre, Phillip Bennett. <sup>1</sup>Imperial College London, London, United Kingdom; <sup>2</sup>University West of England, Bristol, United Kingdom; <sup>3</sup>University of Warwick, Warwick, United Kingdom.

**Introduction:** Emerging evidence supports the role of the vaginal microbiota in adverse pregnancy outcome, but the underlying mechanisms are poorly understood. We have previously shown that miscarriage is associated with vaginal dysbiosis but without knowledge of the cytogenetic status of those miscarriages. We aim to investigate the vaginal microbial composition and the local immune response in chromosomally normal and abnormal miscarriages and compare this to uncomplicated pregnancies delivering at term.

**Methods:** We used 16S rRNA gene based metataxonomics to interrogate the vaginal microbiota in a cohort of 167 women, 93 miscarriage patients (54 euploid and 39 aneuploid using molecular cytogenetics) and 74 women who delivered at term and correlate this with the aneuploidy status of the miscarriages. We also measured the concentrations of IL-2, IL-4, IL-6, IL-8, TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-18 and IL-10 in cervical vaginal fluid.

**Results:** We show that euploid miscarriage is associated with a significantly higher prevalence of *Lactobacillus* spp. deplete vaginal microbial communities compared to aneuploid miscarriage ( $P=0.008$ ). In women having *Lactobacillus* spp. deplete vaginal microbial communities, euploid miscarriage associates with higher concentrations of pro-inflammatory cytokines IL-1 $\beta$ , IL-8, IL-6 ( $P<0.001$ ,  $P=0.01$  and  $P<0.001$  respectively) and lower concentrations of anti-inflammatory cytokines IL10 ( $P<0.001$ ) when compared to viable term pregnancy. We identified *Prevotella bivia* and *Streptococcus* as particularly common in euploid miscarriage and as drivers of pro-inflammatory cytokines (IL-1 $\beta$ , IL-6 and TNF- $\alpha$ ). Co-occurrence network analyses revealed low levels of co-occurrence between *Lactobacillus crispatus* and all other organisms and strong co-occurrence between Streptococcal species. Our data show that an adverse vaginal microbiota combined with a heightened cytokine response influences early pregnancy outcome. Although this may be a reflection of intrinsic maternal immune response, it appears that the cytokine response is largely driven by the bacterial taxa present in the vagina, which presents an opportunity for specific, directed intervention. The negative co-occurrence between *L. crispatus* and all other organisms suggests a possible therapeutic role for probiotics containing this organism. The influence of Streptococci also suggests a potential benefit of targeted antibiotics with probiotics for some patients.

**Conclusion:** These findings support the hypothesis that the vaginal microbiota plays an important aetiological role in euploid miscarriage and may represent a target to modify the risk of pregnancy loss.

### O-073

**Anemia and Abnormal Uterine Bleeding Prevalence in the Environment, Leiomyomas, Latina and Adiposity Study (ELLAS).** Torie C Plowden, Anne Waldo, Anita Malone, Felix M Valbuena, Charo Ledon, Donna D Baird, Erica E Marsh\*. <sup>1</sup>Womack Army Medical Center, Fort Bragg, NC, United States; <sup>2</sup>University of Michigan, Ann Arbor, MI, United States; <sup>3</sup>(CHASS) Center, Detroit, MI, United States; <sup>4</sup>Buenos Vecinos, Ann Arbor, MI, United States; <sup>5</sup>NIEHS, Research Triangle Park, NC, United States.

**Introduction:** Abnormal uterine bleeding of the heavy menstrual bleeding subtype (HMB) is a common gynecologic concern in reproductive age women and a leading cause of clinic based, emergency department, and surgical care. Prolonged HMB can lead to significant anemia (Hb <12.3 g/dL or Hct <35.9%), adversely affecting quality of life and causing substantial morbidity. The objective of this study was to determine the prevalence of HMB and laboratory-confirmed anemia in a cohort of reproductive age Latinx (LX) females.

**Methods:** ELLAS is a longitudinal prospective cohort study of 21-50 year old LX females. Participants completed questionnaires that collected demographic, medical and reproductive health data. In addition, menorrhagia severity was determined using the validated 15-item Aberdeen Menorrhagia Severity Score (AMSS) (possible score ranges from 0-100). Whole blood was collected and Complete Blood Count analysis was performed. Chi-squared tests for categorical variables and t-tests for continuous variables were used for statistical analysis.

**Results:** 549 participants have completed the first study visit and had lab values. Mean age of participants was 37.6  $\pm$  6.92 yrs. Mean Hg levels were 12.7  $\pm$  1.37 g/dL and mean Hct levels were 38.3  $\pm$  3.3% across the cohort. 145 (26.4%) had a low Hg or Hct. AMSS scores ranged from 2-76. Mean AMSS in participants with normal/high Hg or Hct was 20.1  $\pm$  10.9 vs 25.5  $\pm$  12.7 in participants with low Hg or Hct ( $p<0.001$ ), and 13.4% had a score  $\geq 30$  in the normal/high group vs 25.2% in the low Hg or Hct group ( $p<0.001$ ). 25.5% of participants with normal/high Hg or Hct levels categorized their periods as heavy/extremely heavy versus 47.4% in the low Hg or Hct group ( $p<0.001$ ). Of the 154 participants reporting heavy/extremely heavy periods, 42.9% had low Hg or Hct levels vs 22.3% of the 327 participants reporting low/moderate periods ( $p<0.001$ ). Increasing age and food insecurity were associated with anemia ( $p=0.04$  and  $p=0.02$  respectively).

**Conclusion:** We found that anemia was highly prevalent in this population - significantly higher than previously reported in a large national study. Many women who perceived their bleeding as heavy were subsequently found to have anemia. Given the high prevalence of anemia in this population, there may be a role for community level education to increase awareness of what constitutes normal menses and of HMB as a cause of anemia.

### O-074

**Route of Myomectomy and Probability of Pregnancy or Live Birth.** Sophia Anderson†, Laine Thomas, Lauren Wise, Elizabeth A Stewart\*. <sup>1</sup>COMPARE-UF Team. <sup>1</sup>DCRI, Durham, NC, United States; <sup>2</sup>Boston University, Boston, MA, United States; <sup>3</sup>Mayo Clinic, Rochester, MN, United States.

**Introduction:** Uterine fibroids (UF) are a major cause of morbidity in reproductive-aged women. Studies have shown that myomectomy (Myo) for UF is associated with improved fertility, but less is known about the extent to which route of Myo (abdominal=AM, hysteroscopic=HM or laparoscopic=LM) influences fertility. Existing studies are limited by small sample size, retrospective design, or analysis of only one surgical route.

**Methods:** The Comparing Options for Management: Patient-centered Results for Uterine Fibroids (COMPARE-UF) registry enrolled reproductive-aged women undergoing surgical UF treatments to assess comparative effectiveness. We assessed prospectively the association between Myo route (AM, LM or HM), baseline characteristics and self-reported pregnancy (PG) and livebirth (LB) at 12, 24, and 36 months' follow-up. We used life-table methods to estimate cumulative probabilities and 95% confidence intervals (CI) of PG and LB in each time interval, using propensity score weighting to create comparable cohorts by

procedure. Analyses were repeated in subgroups defined by pregnancy intent (intending PG within the next 2 years or actively trying). We also used logistic regression with repeated intervals, restricted to women actively trying or intending to conceive within the next 2 years to estimate odds ratios (ORs) and CIs.

**Results:** Among 1095 women who underwent Myo, 202 reported PG and 91 reported LB during 36 months. There was no appreciable difference in the probability of PG by route of Myo overall, among women intending PG within 2 years, or among women actively trying to conceive (Table 1). Similar patterns were observed for LB (Table 2). After further control for age, compared with AM, ORs for PG were 1.2 (CI: 0.7-2.2) for HM and 1.3 (CI: 0.8-2.2) for LM, and 1.2 (CI: 0.6-2.2) for HM and 1.4 (CI: 0.8-2.3) for LM among women intending PG. With respect to LB, there was only sufficient data among those intending PG, ORs were 1.5 (CI: 0.7-3.3) for HM and 1.6 (CI: 0.8-3.3) for LM.

Myomectomy Route	Population	Number (%) of Subjects with Pregnancy	Probability (95% Confidence Limits) of 1st Pregnancy by		
			12 months	24 months	36 months
Abdominal	All	69/388 (17.8%)	0.13 (0.08, 0.14)	0.20 (0.16, 0.25)	0.24 (0.19, 0.30)
	Intending Within 2 Years	64/238 (26.9%)	0.25 (0.17, 0.34)	0.37 (0.27, 0.48)	0.45 (0.33, 0.59)
	Actively Trying	36/113 (31.9%)	0.28 (0.18, 0.42)	0.41 (0.28, 0.57)	0.47 (0.30, 0.66)
Hysteroscopic	All	37/273 (13.6%)	0.16 (0.11, 0.22)	0.24 (0.17, 0.32)	0.33 (0.23, 0.45)
	Intending Within 2 Years	34/94 (36.2%)	0.26 (0.17, 0.38)	0.41 (0.29, 0.55)	0.56 (0.40, 0.74)
	Actively Trying	22/53 (41.5%)	0.36 (0.24, 0.53)	0.48 (0.33, 0.66)	0.63 (0.42, 0.83)
Laparoscopic	All	96/434 (22.1%)	0.16 (0.13, 0.19)	0.24 (0.20, 0.29)	0.27 (0.23, 0.34)
	Intending Within 2 Years	88/241 (36.5%)	0.28 (0.21, 0.35)	0.40 (0.32, 0.49)	0.50 (0.38, 0.62)
	Actively Trying	60/125 (48.0%)	0.40 (0.30, 0.52)	0.54 (0.42, 0.67)	0.67 (0.50, 0.83)

Myomectomy Route	Population	Number (%) of Subjects with Live Birth	Probability (95% Confidence Limits) of 1st Live Birth by		
			12 months	24 months	36 months
Abdominal	All	28/388 (7.2%)	0.01 (0.00, 0.05)	0.10 (0.06, 0.17)	0.10 (0.06, 0.17)
	Intended Within 2 Years	26/238 (10.9%)	0.01 (0.00, 0.07)	0.20 (0.12, 0.32)	0.20 (0.12, 0.32)
	Actively Trying	16/113 (14.2%)	0.02 (0.00, 0.12)	0.25 (0.14, 0.43)	0.25 (0.14, 0.43)
Hysteroscopic	All	19/273 (7.0%)	0.04 (0.02, 0.08)	0.13 (0.08, 0.21)	0.19 (0.12, 0.30)
	Intended Within 2 Years	17/94 (18.1%)	0.05 (0.02, 0.14)	0.21 (0.12, 0.35)	0.31 (0.18, 0.50)
	Actively Trying	10/53 (18.9%)	0.06 (0.02, 0.19)	0.27 (0.14, 0.48)	0.30 (0.16, 0.52)
Laparoscopic	All	44/434 (10.1%)	0.02 (0.01, 0.05)	0.12 (0.08, 0.17)	0.14 (0.10, 0.21)
	Intended Within 2 Years	39/241 (16.2%)	0.03 (0.01, 0.08)	0.20 (0.13, 0.29)	0.25 (0.16, 0.37)
	Actively Trying	22/125 (17.6%)	0.03 (0.01, 0.10)	0.26 (0.15, 0.41)	NA (no data)

**Conclusion:** There was little association between myomectomy route and fertility after control for potential confounders.

## O-075

**Cell Free Fetal DNA (cffDNA) from Human Amnion Epithelial Cells Increase Inflammatory Load in Human Fetal Membrane Tissue.** Chelsea Saito Reis, Samantha Oetjen, Claire Kendal-Wright. *Chaminade University of Honolulu, Honolulu, HI, United States.*

**Introduction:** Inflammation is critical for parturition initiation where proinflammatory cytokines and matrix metalloproteinases (MMP) aide in fetal membrane (FM) weakening. The danger associated molecular pattern (DAMP) such as unmethylated DNA activates toll like receptor 9 (TLR9) to promote inflammation. Data from our lab suggest cffDNA treatment increases IL6 production. Studies show fetal sex may promote inflammation by increased cytokine production during pregnancy. The study objectives were to determine if fetal sex specific cffDNA promotes inflammation within FM. We hypothesized that cffDNA sex contributes to the activation of TLR9 mediated inflammation in human FM leading to parturition.

**Methods:** Human FM were collected from term repeat Cesarean sections at Kapi'olani Medical Center for Women and Children with IRB approval. FM explants were placed in a transwell to create a two-compartment system. The amnion side of the explant system was treated with cffDNA for 24hrs (n=5). IL-6 (R&D Systems), lactate dehydrogenase (LDH) and MMP levels were assessed (ELISA, activity assay and zymography respectively), from conditioned media from both compartments and normalized to sample total protein. Fetal sex was determined by SRY and ALT1 expression and methylation status by a DNA methylation ELISA (Epigentek).

**Results:** Increased IL6 production was measured in the top and bottom compartments upon cffDNA treatment after 24hrs. Male cffDNA increased IL6 production in fetal (10.14 fold, p=0.02) and decidual (1.49 fold, p=0.02) compartment. Female cffDNA increased IL6 production in fetal

compartment (n.s.) only. Zymography data shows male cfDNA increased MMP2 production in decidual (1.179 fold,  $p=0.0149$ ) and female cfDNA increased MMP9 production in fetal (n.s.) compartment. We assessed if DNA methylation status could contribute to increased IL6 and MMP production. Pearson correlation test indicate increased IL6 is correlated with DNA methylation status in both fetal ( $r=0.94$ ) and decidual ( $r=0.98$ ) compartments regardless of sex. Further analysis show male cfDNA methylation status correlates with MMP2 production ( $r=0.99$ ). No difference measured with cell stress LDH levels of conditioned medium from each compartment.

**Conclusion:** These data suggest that cfDNA treatment contributes to the activation of proinflammatory signals in the FM as measured by increased IL-6 and MMP2 production. It also suggest Male cfDNA showed a more robust response in the FM. DNA methylation level clearly correlated with IL6 and MMP2 production. Together these data suggest that cfDNA may contribute to the signal needed to activate parturition. On going studies will test if TLR9 initiates the signal detected by increased IL-6 production upon cfDNA treatment.

### O-076

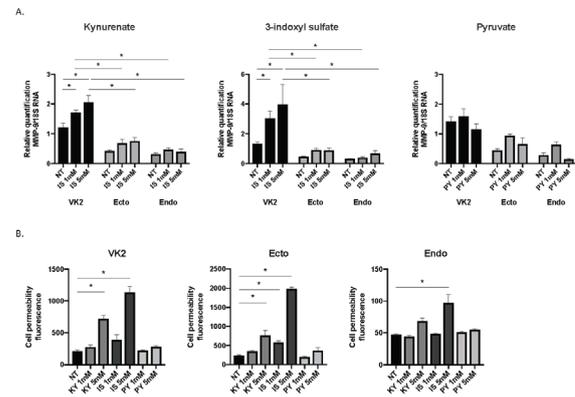
**Microbial Metabolites Compromise Epithelial Barrier Integrity: Potential Mechanisms by Which Non-Optimal Cervicovaginal Microbiota Lead to Preterm Birth.** Kristin D Gerson<sup>†</sup>, Yusra Gimie, Lauren Anton, Michal A Elovitz\*. *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*

**Introduction:** Supernatants from non-optimal microbiota linked to spontaneous preterm birth (sPTB) can induce cervicovaginal (CV) epithelial disruption. In other biologic systems, microbial metabolites serve as regulators of epithelial compromise. We recently found that CV metabolomic output, including an increase in tryptophan (Trp) catabolites, among women with non-optimal microbiota can distinguish those who have sPTB. No studies have examined mechanisms by which microbial metabolites may disrupt the CV epithelial barrier, potentially triggering premature cervical remodeling and sPTB.

**Methods:** Vaginal (VK2), ectocervical (ecto), and endocervical (endo) cells were cultured in monolayer. Trp metabolites kynurenate (KY) and 3-indoxyl sulfate (IS) were chosen as disruptive metabolites and pyruvate (PY) served as a negative control. Metabolites were reconstituted in media at 1mM and 5mM. Cells were treated for 24 hours. qPCR quantified RNA abundance of epithelial-mesenchymal transition (EMT) markers. Permeability was measured by FITC-Dextran movement across collagen-coated membranes. Two-way ANOVAs established significance ( $p<0.05$ ).

**Results:** IS and KY induced SNAI1, ZEB1, and ZEB2 in VK2 cells, while IS induced SNAI1 in ecto cells and ZEB1 in ecto and endo cells ( $p<0.05$  for all). IS induced MMP-9, FSP1, and  $\alpha$ SMA in VK2 cells ( $p<0.05$  for all) but not ecto or endo cells. KY induced MMP-9 in VK2 cells ( $p<0.05$ ) but had no effect on ecto or endo cells. KY and IS increased cell permeability in all cell lines, and most robustly in VK2 and ecto cells ( $p<0.0001$ ). Interrogation of the aryl hydrocarbon receptor (AhR) pathway, induced by select Trp metabolites, revealed upregulation of AhR, ARNT, and AHRR by IS ( $p<0.05$ ) in all cell lines. IL-1B, a known AhR target and activator of MMP-9, was increased by IS in all cell lines ( $p<0.05$ ).

**Conclusion:** Select Trp catabolites induce features of EMT in vaginal epithelial cells, including induction of a protease that may degrade the cervical matrix. Our data highlight a role for these metabolites in regulating mucosal barrier integrity in the CV space. Concomitant upregulation of AhR signaling suggests a potential pathway through which these metabolites may exert their effects. While our findings begin to ascribe a biologic link between non-optimal CV microbiota and cervical remodeling, further studies are warranted to determine the efficacy of targeting these metabolites in sPTB prevention. PENN MOD PRC



**Figure 1. Tryptophan metabolites induce features of EMT**  
A. Relative mRNA expression of MMP-9 by qPCR after treatment with kynurenate (KY), 3-indoxyl sulfate (IS), and control metabolite pyruvate (PY) in vaginal (VK2), ectocervical (ecto), and endocervical (endo) cells.  
B. Cell permeability following treatment with KY, IS, and PY in VK2, Ecto, and Endo cells.

### O-077

**Single Cell Transcriptomics Identify Distinct Epithelial Populations in Early versus Late Pregnancy.** Shanmugapriya Madhukaran<sup>†</sup>, Anne Cooley, Gary Hon\*, Mala Mahendroo\*. *UTSW Medical Center, Dallas, TX, United States.*

**Introduction:** Through pregnancy, the cervix must remain closed while concurrently remodel to prepare for parturition. Understanding molecular changes in the cervix across gestation is required to identify cervical contributions to premature birth, in particular the protective mechanisms that limit ascending infection. The cervical epithelia serve as the first line of defense providing physical and immune barrier. Dynamic changes in cervical epithelial morphology and proliferation are documented yet the transcriptional programs that drive epithelial functions during pregnancy are unclear. Here we address the hypothesis that cervical epithelia have unique properties and functions during pregnancy and labor distinct from the non-pregnant (NP) cervix.

**Methods:** Single cell libraries were made from mouse cervix from NP, early pregnancy (day 6 and 12); late (day 15 and 18) and in labor (IL) using the 10x Genomics Single cell platform. A range of 5000-10,000 cells per library was sequenced. After normalization, cell clustering was performed using Seurat software.

**Results:** Cluster analysis for epithelial cells indicate the transcriptome profile of NP and IL are closely related and distinct from the gestational days 6, 12, 15, 18. Differential expression and comparison with known cell markers led to the identification of two epithelial cell types, basal (TP63 and KRT5) and luminal (DMKN and TPRG) across all libraries. Multiple clusters emerged within each of the epithelial cell types with distinct gene signatures. The pattern of keratin (Krt) expression (intermediate filament proteins) highlight epithelial subtypes. First, Krt expression variations between clusters discriminate between basal, intermediate and terminal luminal populations. Second, NP, early, late and IL expressed distinct Krt genes, with a reduction in keratin gene expression during pregnancy. Within the terminal luminal epithelial clusters, cells were identified with gene signatures of goblet cells (e.g., SPDEF and mucin genes). Transcriptome profiles of goblet cells in NP suggest they are functionally distinct from pregnancy. In the NP cervix, goblet cells express transcripts encoding antiviral defense, innate immune response and antimicrobial and protease inhibitor genes that are distinct from pregnancy. In contrast to NP goblet cells, mucosal immunity genes are upregulated during pregnancy. Further there is a spatially distinct pattern of goblet cell organization between the endocervical canal and exocervix. Collectively these studies identify functionally and spatially distinct goblet cell populations between NP and pregnant cervix. We propose these cells provide a customized physical and immune barrier to ensure cervical function in NP and pregnancy

**Conclusion:** Understanding epithelial cellular heterogeneity in normal pregnancy with aid in understanding pathogenesis in ascending infection mediated PTB.

### O-078

**Essential Roles of Uterine Peristalsis by Cav1.2 in Reproduction and Adenomyosis in Mice.** Mingzi Qu<sup>†</sup>, Ping Lu, Christina Baer, Lawrence Lifshitz, Ronghua ZhuGe\*. *University of Massachusetts Medical School, Worcester, MA, United States.*

**Introduction:** Uterine peristalsis has been inferred to facilitate embryo implantation, and be involved in the etiology of adenomyosis. However, the molecular basis of uterine peristalsis remains unclear, which in turn makes it a challenge to elucidate its role in reproduction and adenomyosis at the molecular level. We recently show that voltage-gated Ca<sup>2+</sup> channel Cav1.2 is a key ion channel for the generation of uterine peristalsis in mice. Here we investigate the roles of Cav1.2 in uterine peristalsis, reproduction and adenomyosis using Cav1.2 transgenic mice and a mouse model of adenomyosis.

**Methods:** A precision-cut uterine slice preparation (250 µm thick) that preserves the cell communication and organization present *in vivo* was used 1). to study Ca<sup>2+</sup> signaling and cell shortening under a 2-photon microscope and 2). to measure the changes in slice lumen under a wide-field microscope. Embryo implantation and pregnancy were assessed by Chicago Sky Blue 6B stains and an infrared video system respectively, after intraperitoneal injection of a Cav1.2 blocker and in heterologous Cav1.2 knockout mice. Adenomyosis was induced by the stomach administration of tamoxifen to neonatal CD-1 mice. Gene expression was quantified by qPCR.

**Results:** 2-photon imaging detected clusters of myometrial cells consistently generating spontaneous synchronized Ca<sup>2+</sup> oscillations that cause cell shortening. The Ca<sup>2+</sup> oscillations were abolished when extracellular Ca<sup>2+</sup> was removed and by Cav1.2 blocker nifedipine. These treatments also abolished peristalsis, as assessed by changes in uterine slice lumen under the wide-field microscope. Nifedipine (35 mg/kg body weight) reduced the mouse embryo implantation rate by 80% compared to solvent control. Heterologous Cav1.2 knockout (i.e., Cav1.2<sup>fl/fl</sup>;SMA-Cre) CD-1 mice produced litters with smaller size (5.5± 1.9 pups in the KO vs 9.8±1.6 pups in the control (Cav1.2<sup>fl/fl</sup> mice) and delayed in labor (19.3±0.2 days in the KO vs 18.7±0.2 days in the control when the time of detecting vaginal plug is considered day 0.5 of pregnancy). Tamoxifen treatment (1 mg/kg on days 1-5 after birth) caused endometrial glands to migrate into endometrium, a hallmark of adenomyosis, in CD-1 mice (45-60 days old). Moreover, nine out of 14 mice treated with tamoxifen failed to implant, and the remaining 5 mice gave birth with a litter size of 2.2±1.2 pups. Of 10 control mice, nine gave birth with a litter size of 14.7±1.8 pups. Uteri from 45-60 days old mice treated with tamoxifen showed significantly weaker spontaneous Ca<sup>2+</sup> signals and agonist-induced contractions, and mRNA for Cav1.2 in the myometrium was decreased by (74.7±4.6)% compared to control mice.

**Conclusion:** Cav1.2-mediated uterine peristalsis plays a critical in embryo implantation, and a dysfunction of this peristalsis could contribute to the pathogenesis of adenomyosis and the impaired reproduction seen in this disorder.

### O-079

**Differential Regulation of SOX Family Transcription Factors in the Pregnant and Labouring Mouse Myometrium.** Nawrah Khader<sup>†</sup>,<sup>1</sup> Virlana Shchuka<sup>†</sup>,<sup>1</sup> Anna Dorogin,<sup>2</sup> Oksana Shynlova\*,<sup>2</sup> Jennifer Mitchell\*.<sup>1</sup> *<sup>1</sup>University of Toronto, Toronto, ON, Canada; <sup>2</sup>Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada.*

**Introduction:** Preterm birth, defined as birth prior to 37 weeks of gestation, is a major contributor to neonatal mortality and increased morbidity, worldwide. There is a lack of effective preventative treatments due to the understudied molecular mechanisms causing the myometrium to switch from a quiescent to a contractile phenotype at labour. Previous research has identified transcription factors such as activator protein-1 and progesterone receptors that regulate the expression of contraction-associated genes such as Gjal during active labour.

**Methods:** We investigated the differentially expressed transcription factors, using RNA-seq, during mouse gestation to better understand how gene expression is activated in the labouring myometrium. This revealed the SOX family of transcription factors are differentially regulated during pregnancy in the myometrium. To further investigate the role of these SOX factors in mouse gestation, their expression was investigated by RT-qPCR during normal gestation, RU486-induced preterm labour, and an LPS inflammation model of preterm labour. Additionally, chromatin accessibility was assessed at various timepoints throughout normal mouse gestation using an Assay for Transposase Accessible Chromatin paired with Sequencing (ATAC-seq). These data were subjected to differential peak analysis and subsequently used for motif analysis to uncover the transcription factor binding sequences.

**Results:** RNA-Seq analysis revealed upregulation of Sox4, Sox7, and Sox9 during labour. Conversely, Sox8 was expressed at Day 15 and downregulated during active labour (p<0.01). RT-qPCR confirmed the expression level changes for all transcription factors during normal gestation. Interestingly Sox4 was also confirmed to increase in both pre-term labor models whereas Sox7 and Sox8 were only differentially regulated in RU486-induced preterm labor. Accessible chromatin regions identified by ATAC-seq were evaluated revealing an enrichment of SOX factor binding motifs (SOX4, SOX7, and SOX9) at term-not-in-labour and term labour compared to Day 15 of gestation.

**Conclusion:** As Sox4 was expressed in term and both pre-term labour models this transcription factor may be critical in modulating gene expression changes required for labour onset. Results from RU486-induced pre-term labour suggest that Sox7 and Sox8 might be inhibited or activated, respectively, by progesterone signaling but are less responsive to infection induced signals. Together these data suggest that the SOX factors expressed at term in the myometrium cause changes in the accessible chromatin landscape, consistent with their roles as regulatory transcription factors in the pregnant myometrium.

### O-080

**Intrauterine Infection Induced Preterm Labor Is Associated with Site-Specific Phosphorylation of Progesterone Receptor A in Myometrial Cells.** Rachel A Wilson<sup>†</sup>,<sup>1</sup> Pietro Presicce,<sup>2</sup> Monica Cappelletti,<sup>2</sup> Alan Jobe,<sup>3</sup> Senad Divanovic,<sup>3</sup> Sing Sing Way,<sup>3</sup> Claire Chougnat,<sup>3</sup> Sam Mesiano,<sup>1</sup> Suhas Kallapur.<sup>2</sup> *<sup>1</sup>Case Western Reserve University, Cleveland, OH, United States; <sup>2</sup>University of California, Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States.*

**Introduction:** Progesterone (P4), via the P4 receptor (PR) isoforms, PR-A and PR-B, maintains uterine quiescence by inhibiting the response of myometrial cells to pro-labor inflammatory stimuli. We hypothesize that intrauterine infection (IUI) induces labor by inducing phosphorylation of PR-A at serine-344/345 (pSer<sup>344/345</sup>-PRA) that inhibits P4/PR-B anti-inflammatory activity in myometrial cells. Preterm labor (PTL) can be triggered by IUI but not invariably so. To test this, we determined the effect of IUI induced by lipopolysaccharide (LPS; does not trigger PTL) or live E. coli (triggers PTL) on myometrial pSer<sup>344/345</sup>-PRA and tissue inflammatory state in our established rhesus macaque model.

**Methods:** Rhesus macaques at 80% gestation were treated with either intra-amniotic injection of LPS (derived from E. coli O55:B5, 1mg; n = 8), live E. coli (10<sup>6</sup> CFU; n = 5), live E. coli + antibiotics (ABs; n = 6) or vehicle (PBS; n = 6). Animals were delivered by c-section 48h-72h later and uterine tissue was collected and subjected to protein and mRNA analyses.

**Results:** E. coli ± ABs induced active labor within 48h, whereas animals treated with PBS or LPS did not exhibit signs of active labor. pSer<sup>344/345</sup>-PRA was localized to the nucleus of myometrial cells and its abundance was increased markedly by E. coli ± ABs and only slightly by LPS compared to PBS. The increase in pSer<sup>344/345</sup>-PRA was associated with increased abundance and activation of the ERK1/2 and SAPK/JNK mitogen activated protein kinases (MAPKs). Compared to PBS, LPS and E. coli ± ABs equivalently increased expression of inflammatory genes *IL8*, *CCL2*, and *IL1β* (each P<0.05). However, E. coli increased

expression of key labor associated genes *PTGS2* and *IL6* (each  $P < 0.05$  compared to PBS), while LPS variably and non-significantly increased expression of *PTGS2* and *IL6*.

**Conclusion:** Intrauterine infection increases pSer<sup>344/345</sup>-PRA generation in myometrial cells by activating specific MAPKs. Live *E. coli* appeared to impart a higher inflammatory load than LPS as indicated by a broader increase in the expression of inflammatory-response genes and increased levels of pSer<sup>344/345</sup>-PRA. We propose that an inflammatory load threshold exists above which inflammatory stimuli via MAPKs induce the generation of pSer<sup>344/345</sup>-PRA in myometrial cells and expression of pro-inflammatory pro-labor genes. Increased pSer<sup>344/345</sup>-PRA may inhibit the P4/PR-B anti-inflammatory activity leading to a tissue level positive-feedback inflammatory state that induces labor.

## O-081

**Cumulus Cells Transcriptomic Biomarkers of Euploid Human Embryo Implantation.** Cynthia Scott†,<sup>1</sup> Shiny Titus†,<sup>2</sup> Emre Seli\*.<sup>3</sup> <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>The Foundation for Embryonic Competence, Basking Ridge, NJ, United States; <sup>3</sup>Yale School of Medicine, New Haven, CT, United States.

**Introduction:** Approximately 35% of transferred euploid embryos fail to implant in women undergoing infertility treatment with in vitro fertilization (IVF). While a number of factors have been implicated as causative factors, the biological explanation for the failure of euploid embryo implantation remains unknown. Bi-directional communication between the oocyte and surrounding cumulus cells (CC) play a key role in healthy follicle development and oocyte cytoplasmic and nuclear maturation, suggesting a role for CC in determining pregnancy outcome. In this study, we hypothesized that factors associated with CC could play a role in determining the implantation potential of euploid embryos. We used in-depth transcriptomic analysis to compare CC from euploid embryos that resulted in a live birth to CC from euploid embryos that failed to implant. **Methods:** Written consent was obtained from patients undergoing IVF treatment. CC associated with individual oocytes were collected and stored. Following IVF and ICSI, embryos were individually cultured, blastocysts were biopsied for preimplantation genetic testing for aneuploidy (PGT-A) and cryopreserved. Frozen embryo transfer was performed in a subsequent cycle. CC were processed (n=20; 10 implanted and delivered, 10 non-implanted) for RNA sequencing and then for pathway analysis. Real-time PCR analysis was performed in independent samples (n=10; 5 implanted and delivered, 5 non-implanted) to verify the gene expression levels in both sets.

**Results:** All samples subjected to RNA-seq showed homology (70-80%) to the human reference genome. Approximately 15,000 genes showed differential expression between the two groups. Using Gene Ontology Enrichment analysis, it was observed that approximately 60 differentially expressed genes were involved in the biological function of fertilization ( $p < 0.05$ ), and about 120 genes were involved in the molecular function of guanyl nucleotide exchange factor activity ( $p < 0.05$ ). KEGG enrichment pathway analysis showed that about 100 differentially expressed genes were involved in the calcium signaling pathway and about 50 genes were a part of the cell adhesion molecules ( $p < 0.05$ ). Real-time PCR for few genes from the top 20 genes viz. SLC30A2 and SLC6A19 were performed with CC from the two groups to validate RNA sequencing data. PCR results were consistent with RNA sequencing analysis, demonstrating a significant increase in the expression of SLC30A2 ( $p = 0.003$ ) and increased expression approaching significance in SLC6A19 ( $p = 0.17$ ).

**Conclusion:** Our study reveals significant differences in CC transcriptome associated with embryos that result in a live birth compared to those that fail to implant. We also identify SLC30A2 and SLC6A19 as potential biomarkers. The clinical relevance of our findings will need to be validated in future studies.

## O-082

**Rapid Aneuploidy Testing in Reproduction Using Nanopore-Based Sequencing.** Shan Wei,<sup>1</sup> Alexandre Djandji,<sup>1</sup> Nataly Hoffman,<sup>1</sup> Claudia Cujar,<sup>1</sup> Refik Kayali,<sup>2</sup> Cegniz Cinnioglu,<sup>3</sup> Ronald Wapner,<sup>1</sup> Mary D'Alton,<sup>1</sup> Brynn Levy\*,<sup>1</sup> Zev Williams\*.<sup>1</sup> <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>IGenomix Los Angeles, Torrance, CA, United States; <sup>3</sup>NextGen Genetics, Santa Clara, CA, United States.

**Introduction:** Aneuploidy is the leading cause of miscarriage, congenital defects and a major impediment to fertility, making diagnostic testing an important component of prenatal and fertility care. However, current methods for comprehensive aneuploidy testing are slow, costly, and limited in their clinical applications. Here, we compare traditional clinical testing with Short-read Transposome Rapid Karyotyping (STORK), a rapid, low-cost, point-of-care test for aneuploidy we developed using nanopore-based whole genome sequencing and data analysis.

**Methods:** Using STORK, we tested 218 blinded, sequential, remnant, reproductive specimens comprised of products of conception (POC) following spontaneous losses (n=61), chorionic villus sampling (CVS; n=52), amniocentesis (n=53), and trophoctoderm biopsies of embryos undergoing preimplantation genetic testing for aneuploidy (PGT-A; n=52). Results from standard clinical testing were then unblinded and compared with the results from STORK.

**Results:** STORK sequencing was successfully completed in all cases. The test sensitivity and specificity were 100% for POC, CVS and amniocentesis samples. Ten POC results were initially discordant but subsequent testing validated the results from STORK. One miscarriage sample did not have a clinical testing result due to culture failure but was successfully sequenced using STORK. For PGT-A samples, the test sensitivity and specificity were 94.7% and 100%, respectively. For all specimen types, the sequencing time and cost ranged from 10 min and \$200 per sample for testing a single sample to 2 hours and <\$50 per sample when ten samples were multiplexed and sequenced simultaneously. **Conclusion:** STORK enables accurate, low-cost, same-day, point-of-care aneuploidy testing across all reproductive specimen types.

Table 1. Performance characteristics of STORK

Sample type	Sample size	Results with STORK (n)	Results with standard testing (n)	Cases of abnormality*	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV (%)	NPV (%)	FDR (%)	FNR (%)
CVS	52	52	52	10	100 (69.2-100)	100 (89.1-100)	100 (69.2-100)	100 (89.1-100)	0	0
Amnio	53	53	53	6	100 (54.1-100)	100 (92.5-100)	100 (54.1-100)	100 (92.5-100)	0	0
POC	61	61	60**	28	100 (87.2-100)	100 (89.1-100)	100 (87.7-100)	100 (89.1-100)	0	0
PGT-A	52	52	52	19	94.7 (74.0-99.9)	100 (89.4 - 100)	100 (81.5-100)	97.1 ( 84.7 - 99.9)	0	2.9
Total	218	218	217	63	98.4 (91.5-100)	100 (97.6-100.0)	100 (94.2-100)	99.6 (96.5-100)	0	0.6

\*Includes aneuploidy, large CNV, and high-level mosaicism.

\*\*One sample has no G-band result due to culture failure.

PPV: Positive predictive value; NPV: Negative predictive value; FDR: False discovery rate; FNR: False negative rate.

## O-083

**Characterization of the Non-Classical Progesterone Receptor Membrane Component 2 (PGRMC2) during the Human Menstrual Cycle and In Vitro Decidualization.** Yassmin Medina-Laver†,<sup>1</sup> Indra Diaz-Hernandez†,<sup>2</sup> Pilar Alama\*,<sup>1</sup> Roberto Gonzalez-Martin†,<sup>1</sup> Andrea Palomar†,<sup>2</sup> Alicia Quiñero\*,<sup>1</sup> Francisco Dominguez\*,<sup>1,2</sup> <sup>1</sup>IVI Foundation-RMA Global, Valencia, Spain; <sup>2</sup>IIS La Fe, Valencia, Spain.

**Introduction:** Progesterone (P4) endometrial response is regulated by classical (PGR) and non-classical P4 receptors (ncPGR). Our previous studies (Garrido-Gomez *et al.*, 2014; Salsano *et al.*, 2017; Salsano *et al.*, 2019) demonstrate that one of these ncPGR, P4 receptor membrane component 1 (PGRMC1), was downregulated along the secretory phase and its overexpression inhibits decidualization. PGRMC2, another ncPGR with structural similarity to PGRMC1, has not been studied yet in humans. Thus, the main objective of this research was to characterize PGRMC2 along the human menstrual cycle and assess its behaviour during the *in vitro* decidualization process, comparing them with PGRMC1 and PGR. **Methods:** To describe *in vivo* PGRMC2 expression along human menstrual cycle and correlate it with PGRMC1 and PGR expression patterns, immunohistochemistry (IHC; n=14), quantitative PCR (qPCR; n=24) and Western Blot (WB; n=16) were used in natural menstrual cycle endometrial biopsies from egg donors distributed in five groups: EP= Early proliferative, LP= Late proliferative, ES= Early secretory, MS= Mid secretory and LS= Late secretory. Regarding decidualization

studies, we induced it *in vitro* in primary endometrial stroma cells (ESC) to compare PGRMC2 in non-decidualized (ndESC) and decidualized (dESC) stromal cells by qPCR (n=15), WB (n=15) and immunofluorescence (n=8), correlating it with PGRMC1 and PGR. Decidualization was checked measuring secreted prolactin by ELISA (Novus Biologicals) and cytoskeleton reorganization by F-actin staining.

**Results:** *In vivo* IHC analysis showed a strong PGRMC2 protein increase during ES and MS in endometrial epithelial glands and a rise during MS in ESC and endometrial luminal epithelial cells. qPCR and WB results support this pattern showing an increase of PGRMC2 mRNA and protein expression during MS, oppositely to PGRMC1 and PGR expression. Regarding *in vitro* decidualization analysis, PGRMC2 mRNA (p<0.0001) and protein (p<0.05) were significantly higher in dESC compared to ndESC, in contrast to PGRMC1, which mRNA expression lightly decreased in dESC. Finally, we detected a greater amount of ncPGR mRNA expression compared to PGR along the menstrual cycle and in *in vitro* (p<0.0001) experiments.

**Conclusion:** Our data support that PGRMC2 increases during the human MS phase and in dESC, contrary to what we observed in PGRMC1 and PGR. In addition, the higher expression of ncPGR compared to PGR observed during decidualization and in *in vivo* endometrium suggest an important role of PGRMC2 in the implantation process. However, further studies are needed to clarify the functional role of PGRMC2 in P4 signalling during the embryo-implantation.

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#### O-084

**Human Placenta Mesenchymal Stem Cells Derived Exosomes Successfully Reverse Infertility in Chemotherapy Induced Premature Ovarian Insufficiency Mouse Model.** *Esra Cetin*, Hang-soo Park, Hiba Sibli, Ayman Al-Hendy. *University of Chicago, Chicago, IL, United States.*

**Introduction:** Premature ovarian insufficiency (POI) is a condition characterized by amenorrhea and reduced follicle counts leading to infertility under the age of 40. Our research previously demonstrated the therapeutic effects of human mesenchymal stem cells conditioned media (hMSC-CM) in POI. After treatment with hMSC-CM, our *in vitro* cell model showed a significant increase in cell proliferation and decreased apoptosis of human granulosa cells. The hMSC-CM contains extracellular vesicles such as microvesicles, exosomes, and membrane particles. To increase the efficacy, we aimed to use purified placental human mesenchymal stem cell-derived exosomes (hMSC exosomes) in this study. We hypothesize that hMSC exosome treatment rejuvenates ovary functions in a POI mouse model and increases human granulosa cells' viability in an *in vitro* cell model.

**Methods:** After treating cyclophosphamide treated HGrC1 human nonluteinized granulosa cell line with hMSC-CM and hMSC exosome for 24 hours, we analyzed cell morphology, cell count to show cell proliferation, and steroid sex hormone genes by real-time RT-PCR. For the *in vivo* part, we established POI in C57/BL6 mice by using a standard cyclophosphamide chemotherapy protocol (CTX). We injected hMSC exosomes by retro-orbital intravenous injection to 3 groups (n=16) on the 14th day after the CTX induction. The groups were separated into two groups: a breeding group (n=6) and an experimental group (n=10). On the 17th day, breeding was started, and the experimental group used for estrus cycle follow-up and sacrificed 2 weeks after the treatment for tissue collection.

**Results:** Our *in vitro* cell model showed a significant increase in proliferation of the hMSC exosomes treated HGrC1 cells. (1.29±0.11-fold, p<0.05) We found that the hMSC exosome upregulates CYP 19 and STAR genes. (respectively, 3.19 ± 0.61-fold, p<0.05, 2.58 ± 0.53-fold, p<0.05) There was a significant difference in the CYP 19 gene between the two treatment groups. (p<0.05) As a result of our *in vivo* work, before the treatment, vaginal cytology showed all CTX treated mice significantly increased the metestrus-diestrus (M-D) phase while there was no significant difference between the proestrus-estrus phase and M-D phase in the healthy control. In the treatment group, the extended M-D

phase was decreased after the hMSC exosome injection. The size of the ovary showed a significant difference between the hMSC exosome treated group and the POI group. (1.268 mm<sup>3</sup>±0.54 p<0.05)

**Conclusion:** Our preliminary data confirms the effect of exosomes by demonstrating restoration of ovarian functions and increasing the viability of HGrC1 cells. This approach could be a promising novel treatment approach for POI; however, additional studies are needed to discover the effects of exosomes further.

#### O-085

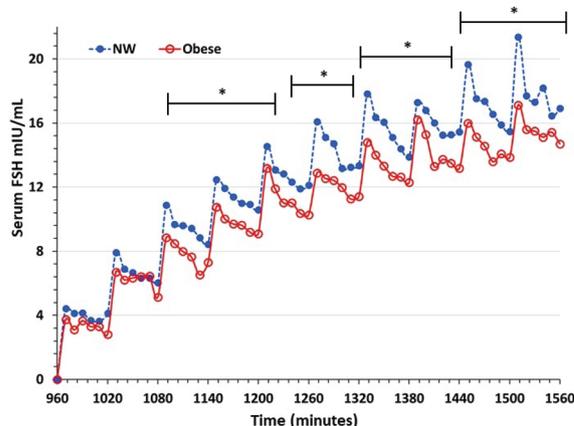
**An In Vivo Model of Human FSH Dysregulation in Obesity: Obese Women Exhibit Lower Serum FSH Levels in Response to Intravenous Recombinant FSH after GnRH Suppression.** *Katherine Kuhn\**,<sup>1</sup> Thanh-Ha Luu<sup>†</sup>,<sup>1</sup> Andrew P Bradford,<sup>1</sup> Luke Wittenberg,<sup>2</sup> Nne-Omoji Nwobodo,<sup>3</sup> Polotsky J Alex\*,<sup>1</sup> Michael Wemple.<sup>4</sup> <sup>1</sup>U. Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>2</sup>U. California Davis, Davis, CA, United States; <sup>3</sup>UCHealth, Aurora, CO, United States; <sup>4</sup>University of Colorado, Aurora, CO, United States.

**Introduction:** Women with obesity exhibit reduced fertility and lower live birth rates with assisted reproductive technology (ART) compared to normal weight (NW) women. ART employs GnRH suppression and FSH stimulation to induce follicular development. Obese women are known to require higher FSH doses during ART. It remains unclear whether this higher exogenous FSH requirement for ART is related to abnormal hypothalamic-pituitary dynamics, abnormal ovarian environment, or pharmacodynamics/ pharmacokinetics. Thus, the need for higher FSH levels in obese women undergoing ART remains unexplained. We have developed a model to examine IV recombinant (r)FSH levels after GnRH antagonist suppression.

**Methods:** 27 NW and 25 obese women, with regular menstrual cycles, underwent early follicular phase frequent blood sampling (q10min) for 10hrs. At 10hrs post study initiation, a GnRH antagonist, cetrorelix (3 mg), was given followed by a second dose (0.25mg) 6hrs later. Hourly IV rFSH (30 IU) was initiated at this time and continued for 10hrs. LH and FSH were measured by immunoassay (Centaur XP, Siemens). Serum FSH values were adjusted for baseline and used in non-compartmental analysis (WinNonlin) to assess clearance (CL<sub>ss</sub>), steady state volume of distribution (V<sub>ss</sub>) and total FSH exposure. Serum cetrorelix levels were determined by mass spectrometry. Pharmacokinetics were compared by two-tailed, unpaired t test.

**Results:** Baseline FSH levels were significantly lower in obese compared to NW subjects (p=?). Cetrorelix suppressed FSH by 30% in both groups with no significant differences in cetrorelix levels. The rate of FSH accumulation in serum and total overall FSH exposure was significantly lower in obese women (p=0.01, for both) even after baseline correction. No differences in CL<sub>ss</sub> or V<sub>ss</sub> were observed.

#### Lower Serum FSH Levels in Women with Obesity



Serum FSH levels post GnRH suppression in NW and obese subjects in response to recombinant FSH (rFSH). rFSH was administered via IV hourly starting at 960 min (30 IU) for 10 hours (300IU total). NW serum FSH levels increase as a faster rate and achieve higher total levels as compared to their obese counterparts. Asterisks indicate a statistical difference (p<0.05) between NW and Obese.

**Conclusion:** Sequential IV boluses of rFSH, after GnRH suppression, mimic endogenous FSH secretion but yield significantly lower serum FSH levels in women with obesity. FSH pharmacokinetics and serum cetrorelix levels were similar in both groups, indicating that another factor underlies the observed significant reduction in serum FSH in obese subjects. This model of pulsatile, IV rFSH reveals a novel and clinically relevant difference in pharmacodynamics between NW and obese women, which has not been previously observed.

#### O-086

**Different Immunoregulatory Components at the Decidua Basalis of Oocyte Donation Pregnancies.** Kim van Bentem<sup>†</sup>, Manon Bos, Carin van der Keur, Hanneke Kapsenberg, Lisa Lashley\*, Michael Eikmans\*, Marie-Louise van der Hoorn\*. *Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** Regulatory T cells (Tregs) and related immunoregulatory cytokines, such as interleukins (IL), transforming growth factor- $\beta$  (TGF- $\beta$ ) and galectin-1 (gal-1), play a key role in maintaining tolerance at the decidua basalis in human pregnancy. Previous studies observed decreased numbers of decidual Tregs in miscarriage and preeclamptic pregnancies. These complications occur in higher frequencies in oocyte donation (OD) pregnancies, characterized by more fetal-maternal human leukocyte antigen (HLA) mismatches compared with naturally conceived (NC) and non-donor in vitro fertilization (IVF) pregnancies, as the fetus obtains paternal and donor-derived HLA genes. Consequently, the maternal immune system has to cope with greater immunogenetic dissimilarity. Involved immunoregulatory mechanisms however remain poorly understood. Therefore, we examined whether the number of Tregs and immunoregulatory cytokines in decidua basalis of OD pregnancies differs from NC and IVF pregnancies.

**Methods:** This case-control study included 27 OD, 11 IVF and 16 NC placentas of uncomplicated pregnancies, collected after delivery at term between 2005 and 2013. Clinical data, maternal peripheral blood and umbilical cord blood were collected. Decidua basalis was dissected from the placentas and processed to formalin-fixed, paraffin-embedded slices (4  $\mu$ m). Immunohistochemical staining for FOXP3, IL-10, IL-6, gal-1, TGF- $\beta$  and Flt-1 was performed. Semi-quantitative (FOXP3+ Tregs) and computerized analysis (cytokines), using Image-J software, were executed. Maternal peripheral blood and fetal umbilical cord blood were typed for HLA class I and II, using Sequence Specific Oligonucleotides PCR, to calculate the number of fetal-maternal HLA mismatches.

**Results:** No significant differences were found when comparing the three groups for the mean number of FOXP3+ Tregs. However, when the amount of fetal-maternal HLA mismatches was related to the percentage of Tregs, the Tregs were significantly higher in pregnancies with 4-6 HLA class I mismatches ( $n = 16$ ), than in those with 0-3 HLA class I mismatches ( $n = 38$ ;  $p = 0.029$ ). Furthermore, OD pregnancies express less IL-10, IL-6, gal-1 and Flt-1 in decidua basalis compared to NC pregnancies. Moreover, the amount of IL-10 was significantly lower with 3-4 fetal-maternal HLA class II mismatches ( $p = 0.032$ ).

**Conclusion:** This study suggests that the immunoregulation at the fetal-maternal interface in OD pregnancies with a higher amount of fetal-maternal HLA mismatches appears to be altered. Unravelling mechanisms of immunomodulation during OD pregnancy, reflected by high level of fetal-maternal dissimilarity, could help to reach the ultimate goal in transplantation; induction of donor-specific tolerance. In addition, it might help to understand the development of complications in OD pregnancy.

#### O-087

**Associations of Maternal Urinary Bisphenol and Phthalate Urine Concentrations in Pregnancy with Offspring Pubertal Development.** Sophia Blaauwendraad<sup>†</sup>, Romy Gaillard\*, Vincent Jaddoe\*. *Erasmus Medical Center Rotterdam, Rotterdam, Netherlands.*

**Introduction:** Fetal exposure to bisphenols and phthalates influences the steroid-dependent development and maturation of the reproductive system and might subsequently influence pubertal development. We examined the associations of maternal urinary bisphenols and phthalates concentrations in pregnancy with offspring pubertal development.

**Methods:** In a population-based, prospective cohort study among 1059 mother-child pairs, we measured maternal urinary bisphenol and phthalate concentrations in first, second and third trimester. We measured child's Tanner stage and age of first menstruation at the mean age of 13.5 years by questionnaire and ovarian and testicular volume at the mean age of 9.7 years using MRI. Analyses were performed for boys and girls separately.

**Results:** In boys, higher average, and especially second trimester, maternal urinary phthalic acid concentrations were associated with lower risks of delayed childhood genital pubertal development (Odds Ratio (OR) 0.63 (95% CI 0.40, 0.99) per interquartile range increase in phthalic acid, and OR 0.53 (95% CI 0.23, 0.85)), but not with changes in pubic hair development. Higher average, and especially first trimester, total bisphenol were only associated with lower risks of delayed pubic hair development (OR 0.54 (95% CI 0.36, 0.82) and OR 0.65 (95% CI 0.42, 1.00)). Higher maternal average high-molecular weight phthalate and, especially in second trimester, total bisphenol were associated with higher childhood testicular volume (all  $p$ -values  $< 0.05$ ). In girls, higher maternal average, and especially first trimester, high-molecular weight phthalate were associated with lower risks of delayed pubic hair development (OR 0.19 (95% CI 0.28, 0.87) and OR 0.59 (95% CI 0.35, 0.98)), but not with breast development. Higher maternal second trimester high-molecular weight phthalates and third trimester phthalic acid were associated with an earlier and later age at first menstruation, respectively ( $p$ -values  $< 0.05$ ). No associations for low-molecular weight phthalates and total bisphenol with Tanner Stage, age at first menstruation or ovarian volume were present.

**Conclusion:** Our results suggest sex-dependent associations of maternal urinary phthalate and bisphenol concentrations with offspring pubertal development. This study needs to be replicated among larger and more diverse populations, possibly with repeated measurement of pubertal characteristics, stratification for overweight children, and with exploration of the long-term consequences of altered pubertal development.

#### O-088

**Maternal Levels of Perfluoroalkyl Substances (PFAS) during Early Pregnancy in Relation to Preeclampsia Subtypes and Biomarkers of Preeclampsia Risk.** Paige A Bommarito<sup>†</sup>,<sup>1</sup> Kelly K Ferguson,<sup>1</sup> John D Meeker,<sup>2</sup> Thomas F McElrath,<sup>3</sup> David E Cantonwine.<sup>3</sup> <sup>1</sup>*National Institute of Environmental Health Sciences, Durham, NC, United States;* <sup>2</sup>*University of Michigan School of Public Health, Ann Arbor, MI, United States;* <sup>3</sup>*Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States.*

**Introduction:** Prenatal exposure to perfluoroalkyl substances (PFAS) has been previously associated with preeclampsia, although findings are mixed. Despite the heterogeneity of preeclampsia, no studies have examined associations between PFAS and preeclampsia subtypes. Given that preeclampsia subtypes may have distinct etiologies, we examined associations between PFAS and preeclampsia, including individual preeclampsia subtypes (i.e. early- and late-onset). In addition, we estimated the associations between PFAS and angiogenic biomarkers of preeclampsia risk (i.e. soluble fms-like tyrosine kinase-1 [sFlt-1] and placental growth factor [PlGF]).

**Methods:** This case-control study ( $n = 75$  cases,  $n = 75$  controls) was sampled from the LIFECODES birth cohort. Nine legacy PFAS were quantified in maternal plasma from early pregnancy (median 10 weeks) and angiogenic biomarkers were quantified in maternal plasma from four study visits (median 10, 18, 26, and 35 weeks). Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (95% CI) of the association between an interquartile range (IQR)-increase in PFAS and preeclampsia. Linear regression was used to estimate associations between an IQR-increase in PFAS and angiogenic biomarkers. Lastly, as a mixtures-based approach, quantile g-computation was used to estimate the joint associations of PFAS with both preeclampsia and angiogenic biomarkers.

**Results:** Both perfluorodecanoic acid (OR: 1.76, 95% CI: 1.07, 2.91) and perfluorooctanesulfonic acid (OR: 2.29, 95% CI: 1.21, 4.35) were associated with higher odds of late-onset preeclampsia, though associations were null for overall and early-onset preeclampsia. Using quantile g-computation, a simultaneous one-quartile increase in PFAS was

associated with higher odds of late-onset preeclampsia (OR: 2.28, 95% CI: 1.12, 4.64). Few associations were noted with PFAS and angiogenic biomarkers.

**Conclusion:** Maternal PFAS concentrations were associated with odds of late-onset preeclampsia, though associations were null for overall and early-onset preeclampsia. Heterogeneity of preeclampsia should be considered in future studies as populations may have different distributions of disease subtypes.

## O-089

**Maternal Preconception Platelet Activation and Pregnancy Outcomes.** Ashley Shea<sup>†</sup>,<sup>1,2</sup> Lauren Theilen\*,<sup>1,2</sup> Heather Campbell,<sup>1,2</sup> Erica Johnstone,<sup>1</sup> Meredith Humphreys,<sup>1</sup> Sunni Mumford,<sup>3</sup> Alexandra Purdue-Smithe,<sup>3</sup> Lindsey Sjaarda,<sup>3</sup> Neil Perkins,<sup>3</sup> Robert Silver,<sup>1,2</sup> Enrique Schisterman.<sup>3</sup> <sup>1</sup>University of Utah, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States; <sup>3</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States.

**Introduction:** Platelet activation may affect fecundability via inflammation and decreased perfusion of reproductive organs. We aimed to determine: 1) whether women with high plasma platelet factor 4 (PF4) concentrations, a biomarker of platelet activation, are less likely to conceive and have a live birth; 2) whether treatment with low-dose aspirin (LDA) mitigates these risks.

**Methods:** This was an ancillary analysis of the Effects of Aspirin in Gestation and Reproduction trial, which randomized women with a history of pregnancy loss who were attempting conception to LDA or placebo. We included 1,185 women with available pre-pregnancy plasma PF4 concentrations. Outcomes of interest were fecundability, pregnancy loss, and live birth. We used discrete-time Cox proportional hazards models to estimate fecundability odds ratios, and we used log-binomial regression to estimate relative risks for pregnancy loss and live birth by PF4 tertile. Multivariable models included age, BMI, smoking, education, income, and previous live birth. Analyses were conducted for the overall population and stratified by treatment group.

**Results:** During follow up, 775 (65.4%) women had a positive pregnancy test within 6 menstrual cycles. High pre-pregnancy PF4 was associated with improved fecundability overall, especially among women randomized to LDA (Table 1). There was no association between pre-pregnancy PF4 and fecundability among women randomized to placebo. Pre-pregnancy PF4 was not associated with pregnancy loss or live birth, overall or stratified by treatment group (Table 2).

**Conclusion:** High pre-pregnancy plasma PF4 concentration was positively associated with fecundability, especially among participants randomized to treatment with LDA. Inhibition of platelet activation via LDA may be beneficial to achieving pregnancy among women with a history of pregnancy loss.

**Table 1.** Fecundability odds ratios (FOR) for the association between pre-conception PF4 and time to pregnancy, overall and stratified by treatment group (aspirin versus placebo).

Overall	PF4 tertile			p
	1	2	3	
Unadjusted FOR (95% CI)	1.00 (ref)	0.94 (0.77, 1.15)	1.24 (1.02, 1.52)	0.015
Adjusted FOR (95% CI)*	1.00 (ref)	0.92 (0.75, 1.14)	1.25 (1.02, 1.54)	0.009
<b>Aspirin</b>				
Unadjusted FOR (95% CI)	1.00 (ref)	0.96 (0.72, 1.28)	1.45 (1.09, 1.93)	0.005
Adjusted FOR (95% CI)*	1.00 (ref)	0.95 (0.71, 1.28)	1.46 (1.09, 1.96)	0.006
<b>Placebo</b>				
Unadjusted FOR (95% CI)	1.00 (ref)	0.93 (0.7, 1.25)	1.07 (0.8, 1.42)	0.665
Adjusted FOR (95% CI)*	1.00 (ref)	0.9 (0.66, 1.22)	1.07 (0.79, 1.43)	0.532

\*Adjusted for age, BMI, smoking, education, household income, and previous live birth.

**Table 2.** Relative risk of pregnancy loss and live birth among women who conceived according to tertile of preconception PF4, overall and stratified by treatment group (aspirin versus placebo).

	Overall				Aspirin group				Placebo group			
	PF4 tertile 1	PF4 tertile 2	PF4 tertile 3	p	PF4 tertile 1	PF4 tertile 2	PF4 tertile 3	p	PF4 tertile 1	PF4 tertile 2	PF4 tertile 3	p
<b>Pregnancy loss (among pregnancies, weighted*)</b>												
Unadjusted RR (95% CI)	1.00 (ref)	0.93 (0.68, 1.27)	0.9 (0.66, 1.21)	0.7684	1.00 (ref)	0.93 (0.61, 1.42)	0.68 (0.43, 1.07)	0.225	1.00 (ref)	0.92 (0.58, 1.46)	1.15 (0.78, 1.75)	0.590
Adjusted RR (95% CI)†	1.00 (ref)	0.99 (0.73, 1.36)	0.94 (0.69, 1.28)	0.918	1.00 (ref)	0.99 (0.65, 1.51)	0.71 (0.45, 1.13)	0.266	1.00 (ref)	0.96 (0.6, 1.53)	1.17 (0.78, 1.79)	0.658
<b>Live birth (overall)</b>												
Unadjusted RR (95% CI)	1.00 (ref)	1.01 (0.92, 1.12)	1.03 (0.93, 1.13)	0.8565	1.00 (ref)	1.02 (0.98, 1.19)	1.11 (0.97, 1.27)	0.250	1.00 (ref)	1.01 (0.88, 1.16)	0.95 (0.82, 1.09)	0.626
Adjusted RR (95% CI)†	1.00 (ref)	1 (0.91, 1.11)	1.03 (0.93, 1.13)	0.817	1.00 (ref)	1.01 (0.87, 1.17)	1.13 (0.98, 1.29)	0.115	1.00 (ref)	0.98 (0.85, 1.14)	0.92 (0.8, 1.05)	0.519

\*Adjusted for age, BMI, smoking, education, household income, and previous live birth.

†Models utilize inverse probability of pregnancy weights accounting for age, BMI, smoking, education, income, previous live birth

## O-090

**The Association between Living in a Food Desert and the Likelihood of Initiating Breastfeeding.** Adriana Campos<sup>†</sup>, Jean Paul Tanner,<sup>1</sup> Ronee E Wilson,<sup>1</sup> Jason L Salemi,<sup>2</sup> Peeraya Sawangkum,<sup>1</sup> Kimberly Fryer,<sup>1</sup> Adetola Louis-Jacques\*,<sup>1</sup> University of South Florida, Tampa, FL, United States;

<sup>2</sup>Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Breastfeeding is associated with positive maternal and infant health outcomes, as well as with reductions in overall infant mortality. However, there are many reasons mothers do not initiate breastfeeding. Prepregnancy obesity and other indicators of poor maternal nutritional status have been shown to decrease likelihood of breastfeeding initiation (BFI). Persons living in food deserts, areas of limited access to healthy food, are less likely to be able to adhere to healthy nutritional guidelines and more likely to encounter poor health outcomes. Minimal research has been done examining the association between food access and breastfeeding initiation. This study explores the relationship between living in a food desert and BFI.

**Methods:** Data from the Florida Community Health Assessment Research Tool Set, including demographics and BFI (whether or not the infant was breastfed during the birth hospitalization), for births occurring between 2008 and 2018 were linked, at the census tract-level, to food access data from the United States Department of Agriculture (USDA) Food Access Research Atlas. A food desert, or low-income, low-access tract (LILA), was defined as a census tract with 20% of the population having income less than 80% of the statewide median family income and with at least 500 people, or 33% of the population, living more than 1 mile (urban areas) or more than 10 miles (rural areas) from the nearest supermarket. Risk ratios (RR) and 95% confidence intervals (CI) were calculated using modified Poisson regression models.

**Results:** Between 2008 and 2018 there were 2,422,995 total live births in Florida, of which 2,368,114 (97.7%) had information on BFI and food access designation. Seventeen percent (n= 404,577) of live births resided within a food desert. Breastfeeding was not initiated during the birth hospitalization by 17.4% of mothers. Women who lived within a food desert were more likely to *not* breastfeed before hospital discharge (Adjusted RR = 1.37 [95% CI: 1.36 - 1.38]). When stratified by race/ethnicity, the association between living in a food desert and BFI was highest among White non-Hispanic mothers (White non-Hispanic: RR = 1.46 [95% CI: 1.45 - 1.48]; Black non-Hispanic: RR = 1.29 [95% CI: 1.28 - 1.31]); Hispanic: (RR = 1.34 [95% CI: 1.32 - 1.36]).

**Conclusion:** Maternal food desert residence is associated with decreased BFI; however, the specific pathway(s) through which this social determinant of health imposes its effect needs further elucidation. Focusing breastfeeding initiation programs in hospitals and prenatal care facilities serving populations living in food deserts may help increase overall breastfeeding initiation rates.

**O-091**

**How Late Is Too Late to Reverse the Effects of the Developmental Origins of Health and Disease?** Craig E Pennell,<sup>1,2</sup> Carol A Wang,<sup>1,2</sup> Wendy H Oddy,<sup>3</sup> Claire E Meyerkort,<sup>4</sup> Stephen G Matthews,<sup>5,6</sup> Stephen J Lye.<sup>5</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>University of Tasmania, Hobart, Australia; <sup>4</sup>Sir Charles Gairdner Hospital, Western Australia, Australia; <sup>5</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>6</sup>University of Toronto, Toronto, ON, Canada.

**Introduction:** It is well established that adverse antenatal and postnatal environments increase the risk of adult disease; however, not all individuals exposed develop poor outcomes suggesting a potential role for genetics. **Aim:** To evaluate the potential for nutritional intervention in the first three years to reduce the risk of adult disease in those at increased genetic risk.

**Methods:** A polygenic score was developed based on the results of a birthweight and adult disease GWAS performed in the EGG consortium (n=153K). Raine Study Gen 2 participants were genotyped (n=1494), and nutritional assessment was performed during the first, second and third year of life. Adult cardiometabolic outcomes were assessed at age 20 and 22 years (BMI, DEXA, BP, lipids, fasting glucose & insulin). Multivariable analyses were performed to evaluate the impact of nutrition on the relationship between polygenic score and adverse adult health outcomes. The reference for comparisons was polygenic score between the 20th and 80th percentile.

**Results:** During the first year of life, healthy nutrition profiles were associated with reduced risk of obesity, reduced levels of fasting insulin, reduced systolic BP and increased HDL, independent of the polygenic score. By the third year of life, the only association that remained significant was with the risk of adult obesity. Significant interactions were identified between polygenic score, duration of breastfeeding and cardiometabolic outcomes which persisted after adjustment for diet quality during years 1 and 3. Breastfeeding reduced the relative risk of obesity and high fasting insulin (to reference levels) in those with high (80-100th percentile) and low (0-20th percentile) polygenic scores. For high systolic BP and low HDL, these results were only replicated for those with high polygenic scores.

**Conclusion:** Good nutrition in the first year of life offers the greatest potential to reduce the risk of adverse adult health outcomes in those at increased genetic risk.

**O-092**

**Predictors of Teenage Pregnancy in Zambia between 2007 and 2018.** Claire H Packer†,<sup>1,2,3</sup> Nelly-Claire Muntalima,<sup>2</sup> Ana M Langer,<sup>1</sup> Michael T Mbizvo\*,<sup>2</sup> <sup>1</sup>Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>2</sup>Population Council, Lusaka, Zambia; <sup>3</sup>Oregon Health & Science University, Portland, OR, United States.

**Introduction:** In Zambia, 29% of adolescents aged 10-19 have begun childbearing. Early childbearing has been shown to have detrimental social and medical outcomes for young adolescents. As far as we are aware, there has been no study in Zambia investigating the predictors of teenage pregnancies over a 10-year period. Using DHS data, we sought to determine predictors of teenage pregnancies among teenagers in Zambia between the years of 2007 and 2018.

**Methods:** We conducted a study of survey data of 11,194 adolescents. This study used data from the 2007, 2014 and 2018 DHS nationally representative survey of adolescents age 15-19. The surveys adopted a cross sectional study design which is purely quantitative. Analyses were conducted with Stata/SE software and we examined rates of teenage pregnancy based on multiple predictors of interest. Chi-square tests and multivariable logistic regression models were employed for statistical comparison using a p-value of 0.05 to indicate statistical significance.

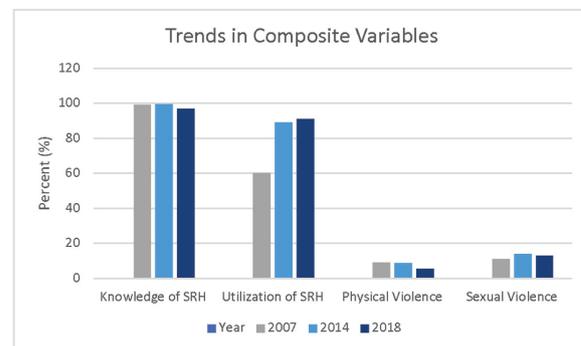
**Results:** After exclusions, 11,194 adolescents under the age of 19 were included in our analysis, with a 29% prevalence of teenage pregnancy. Teenagers were more likely to become pregnant if they were married, had younger sexual debut, had less education, were illiterate, lived in rural areas, or were of the poorest wealth index than if they were not. Among adolescents with teenage pregnancies, utilization of sexual and

reproductive health services among teen mothers significantly increased between 2007 and 2018. On multivariate analysis, teenage pregnancy was found to be significantly different given predictors when compared to the reference group, with significant effect modification due to marital status. **Conclusion:** Our study shows that there are significant demographic, intrapersonal, and socioeconomic factors that impact rates of teenage pregnancy in Zambia over the past 10 years. Understanding these predictors will inform programmatic interventions targeting reduction of teenage pregnancies.

Table 1. Analysis of intrapersonal, institutional and policy drivers with teenage pregnancy (N=11,194)

Variable	Characteristics	No Teenage Pregnancy (%)	Teenage Pregnancy (%)	X <sup>2</sup>	P-value
Marital status	0 = never married	7718 (85)	1562 (17)	4222.74	<0.001
	1 = married or cohabitating	171 (97)	1596 (90)		
	2 = divorced or separated	18 (12)	128 (80)		
Age at first cohabitation	1 = <16 years	6.2 (20)	94 (32)	13.6015	0.03
	2 = 16-19 years	32 (80)	88 (68)		
	3 = ≥16 years	1055 (40)	1613 (60)		
Age at first sex	1 = <16 years	1055 (40)	1613 (60)	15.21	0.004
	2 = 16-19 years	1125 (48)	1390 (55)		
	3 = ≥16 years	174 (58)	134 (47)		
Highest education level	0 = No education	3007 (66)	1715 (66)	286.83	<0.001
	1 = Primary	4660 (77)	1413 (23)		
	2 = Secondary	35 (88)	4.7 (12)		
	3 = Higher than secondary	6039 (75)	2270 (25)		
Employment status	0 = Not currently working	1050 (51)	1002 (49)	475.11	<0.001
	1 = Currently working	4116 (68)	1628 (20)		
Place of residence	1 = urban	3791 (63)	2259 (37)	414.99	<0.001
	2 = rural	743 (69)	333 (31)		
Region	1 = Central	150 (81)	978 (19)	1395.64	<0.001
	2 = Copperbelt	913 (62)	409 (58)		
	3 = Eastern	561 (70)	244 (30)		
	4 = Lusaka	1583 (81)	376 (19)		
	5 = Southern	547 (71)	236 (29)		
	6 = North-west	596 (71)	240 (37)		
	7 = Northern	446 (68)	256 (41)		
	8 = Southern	700 (59)	487 (41)		
	9 = Western	337 (37)	230 (43)		
	10 = Other	1365 (53)	1210 (47)		
Literacy	0 = Cannot read	1073 (71)	440 (29)	542.76	<0.001
	1 = Able to read parts of text	5425 (77)	1612 (23)		
	2 = Able to read whole text	976 (58)	805 (45)		
Wealth index	1 = Poorest	1182 (62)	797 (38)	911.66	<0.001
	2 = Poorer	1338 (63)	769 (37)		
	3 = Middle	1799 (75)	696 (38)		
	4 = Richer	2611 (90)	279 (9.7)		
	5 = Richest	5719 (70)	2483 (30)		
Sex of household head	1 = Male	2184 (73)	805 (27)	12.76	0.012
	2 = Female	590 (66)	309 (34)		
Age of most recent partner	1 = <15 years	667 (31)	1472 (69)	474.32	<0.001
	2 = 15-19 years	137 (22)	477 (78)		
	3 = 20-24 years	13 (10)	130 (90)		
	4 = 25-29 years	201 (48)	221 (52)		
	5 = 30-49 years	160 (76)	53 (24)		
Knowledge of Sexual and Reproductive Health Services (SRH)	0 = No	7238 (71)	3323 (29)	2.74	0.26
	1 = Yes	115 (10)	1248 (92)		
Physical violence	0 = No	114 (8.2)	1167 (91)	0.32	0.72
	1 = Yes	12 (6.2)	179 (94)		
Sexual violence	0 = No	4561 (91)	446 (8.9)	1896.37	<0.001
	1 = Yes	3327 (54)	2840 (46)		

Figure 1. Trends In Composite Variables



**O-093**

**Maternal Exposure to Δ9-Tetrahydrocannabinol Results in Symmetrical IUGR Associated with Cardiac Dysfunction in Postnatal Life.** Kendrick Lee†,<sup>1,2</sup> Kristian McCarthy,<sup>1</sup> Steven R Laviolette,<sup>1</sup> Qingping Feng,<sup>1,3</sup> Daniel B Hardy\*,<sup>1,2</sup> <sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>Children's Health Research Institute, London, ON, Canada; <sup>3</sup>Children's Health Research Institute, London, ON, United States.

**Introduction:** Approximately ~20% of pregnant women (18-24 years) continue to use cannabis in pregnancy. Clinical studies suggest that cannabis use in pregnancy leads to impaired fetal growth, but the long-term effects on cardiac function in the offspring are unknown despite the fact that fetal growth deficits are associated with an increased risk

of developing cardiovascular disease in postnatal life. While animal studies have shown that maternal exposure to  $\Delta 9$ -tetrahydrocannabinol ( $\Delta 9$ -THC, the major psychoactive ingredient in cannabis) can decrease fetal growth, to date little is known about its effects on cardiac function in the offspring. Therefore, we hypothesize that maternal exposure to  $\Delta 9$ -THC during pregnancy will impair fetal development resulting in cardiac dysfunction in postnatal life.

**Methods:** Pregnant Wistar rats were exposed to 3 mg/kg  $\Delta 9$ -THC *i.p.* daily during gestation (gd 6-22) followed by echocardiogram analysis of cardiac function at postnatal day 1 and 21. Heart tissue was collected at both time points for molecular assessment of cardiac remodelling.

**Results:** Exposure to  $\Delta 9$ -THC during pregnancy led to fetal growth restriction with a significant ( $p < 0.05$ ) decrease in heart:body weight ratios. This was accompanied by significantly higher neonatal heart rate and lower stroke volume at birth. By three weeks, pups exhibited catch-up growth along with significantly greater left ventricle anterior wall (LVAW) thickness (at systole) with decreased fractional shortening, stroke volume, and cardiac output. Moreover, these  $\Delta 9$ -THC exposed offspring exhibited increased expression of collagen type I and III, along with  $\beta$ -MHC, associated with cardiac remodelling.

**Conclusion:** These data suggests that maternal exposure to  $\Delta 9$ -THC during pregnancy impedes fetal growth resulting in impaired postnatal heart function in early life. Furthermore, the increased thickness in the left ventricle along with an increase in markers of cardiac hypertrophy and fibrosis may underlie the adverse physiological changes observed in the heart at 3 weeks. Further studies are warranted to address whether the cardiac deficits in these  $\Delta 9$ -THC exposed offspring persist into adulthood. Given the high rate of maternal cannabis consumption coupled with its legalization in North America, understanding the long-term detrimental effects of  $\Delta 9$ -THC on offspring health is of great importance. This is especially concerning given the concentration of  $\Delta 9$ -THC has drastically increased over the past two decades.

#### O-094

**Under-Expression of Placental Endocrine-Specific *Igf2* Programmes Cardiovascular Disease in the Adult Male Offspring.** W. Ching†, Y. Niu, T. A. Garrud†, H. Yong, E. R. Christoforou†, J. Lopez-Tello, D. A. Giussani\*, A. N. Sferruzzi-Perri\*. *University of Cambridge, Cambridge, United Kingdom.*

**Introduction:** The role of the placenta in developmental programming has been comparatively ignored. We have previously induced placental endocrine-specific malfunction in mice by placental endocrine zone deletion of the paternally-expressed imprinted *Igf2* gene (UE). While offspring exposed to UE are growth-restricted *in utero* (Placenta 2018; 69:e60-61), the impact of UE on the cardiovascular physiology of the offspring is completely unknown. Here, we show that adult male offspring exposed to UE have an increased programmed risk of cardiovascular dysfunction.

**Methods:** UE litters were generated by crossing *TpbaCre* females with *Igf2*-floxed males. Offspring of the reverse genetic cross, with normal placental endocrine function, served as controls. After natural delivery, litters were standardised to 3 female and 3 male pups. At 12-13 weeks of age, 1-2 males per litter (from 5-6 litters per group) were killed and hearts and second-order femoral arteries were isolated and mounted on a Langendorff and wire myography preparation, respectively. Significance was set at  $p < 0.05$  and determined by Two-way ANOVA followed by Tukey test or the Student's *t* test for unpaired data, as appropriate.

**Results:** Compared with controls, male UE offspring showed cardiac systolic (Fig. A and B) and diastolic (C and D) dysfunction, increased cardiac sympathetic dominance (E), without a change in coronary flow rate (F). Moreover, isolated femoral arteries from male UE offspring showed a blunted contractile response to phenylephrine (G and H) and a greater relaxant response to acetylcholine (I and J).

**Conclusion:** Placental *Igf2* endocrine-specific loss programmes defects in the cardiovascular function of adult male offspring. The increased dilator while reduced constrictor phenotype of femoral arteries may reflect compensation in the peripheral circulation to offset cardiac load in these offspring.

Supported by The Royal Society, The Lister Institute and The British Heart Foundation.

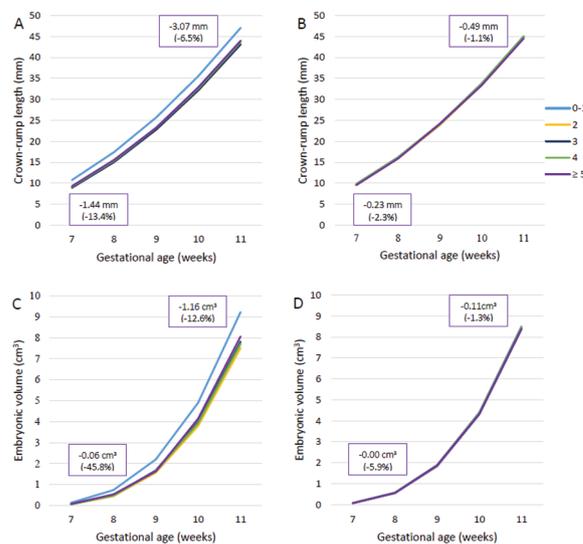
#### O-095

**Accumulation of Suboptimal Periconceptional Social, Lifestyle and Medical Exposures as Markers for the Maternal Vulnerable Condition and Impairment of Embryonic Growth: The Rotterdam Periconceptional Cohort (Predict Study).** Sofie K.M. van Zundert†, Lenie van Rossem\*, Sten P. Willemsen\*, Lindsey van der Meer†, Hiske E. Ernst-Smelt\*, Régine P.M. Steegers-Theunissen\*. *Erasmus MC, University Medical Center, Rotterdam, Netherlands.*

**Introduction:** Suboptimal periconceptional social, lifestyle and medical exposures, defined as vulnerability markers, are associated with poor maternal health and pregnancy outcomes. Evidence is rising that a smaller embryo is associated with impaired fetal growth, adverse birth outcomes and increased risks of non-communicable diseases later in life. So far, it is unknown whether an accumulation of vulnerability markers has a detrimental effect on embryonic growth. The aim of this study is to investigate the association between the degree of the maternal vulnerable condition, determined by an accumulation of vulnerability markers, and embryonic growth depicted by longitudinal crown-rump length (CRL) and embryonic volume (EV) measurements.

**Methods:** We included 555 ongoing singleton pregnancies from The Rotterdam Periconceptional Cohort (Predict Study), comprising 324 natural and 231 in vitro fertilization/intra-cytoplasmic sperm injection (IVF/ICSI) pregnancies. Data on vulnerability markers were collected through questionnaires. Researchers performed standardized CRL and EV measurements at 7, 9 and 11 weeks of gestation using three-dimensional ultrasound scans and virtual reality techniques. We used linear mixed models to investigate the associations in natural and IVF/ICSI pregnancies.

**Results:** Exposure to two or more vulnerability markers was associated with a smaller embryo in natural pregnancies (Figure I). For example, the CRL and EV of embryos of women exposed to five or more vulnerability markers were 1.44 mm (13.4%) and 0.06 cm<sup>3</sup> (45.8%) smaller at 7 weeks of gestation than those of embryos of women exposed to zero or one vulnerability marker. At 11 weeks of gestation, the CRL and EV of these embryos were 3.07 mm (6.5%) and 1.16 cm<sup>3</sup> (12.6%) smaller than those of embryos of women exposed to zero or one vulnerability marker. In IVF/ICSI pregnancies, no associations between the degree of the maternal vulnerable condition and embryonic growth emerged.



**Figure I.** Longitudinal CRL and EV measurements in natural (A and C) and IVF/ICSI pregnancies (B and D) for each vulnerability category.

**Conclusion:** A higher degree of the maternal vulnerable condition is associated with reduced embryonic growth in natural pregnancies. This

finding highlights the importance of identifying vulnerable women as early as possible to optimize modifiable periconceptional social, lifestyle and medical conditions, and subsequent embryonic growth.

### O-096

#### Maladaptive Cardiomyocyte Calcium Handling in Adult Offspring of Hypoxic Pregnancy: Protection by Antenatal Maternal Melatonin.

Mitchell C Lock†, Kerri LM Smith,<sup>1</sup> Youguo Niu,<sup>2</sup> Olga V Patey,<sup>2</sup> Sage G Ford,<sup>2</sup> Andrew W Trafford,<sup>1</sup> Dino A Giussani\*,<sup>2</sup> Gina LJ Galli\*.<sup>1</sup> <sup>1</sup>The University of Manchester, Manchester, United Kingdom; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.

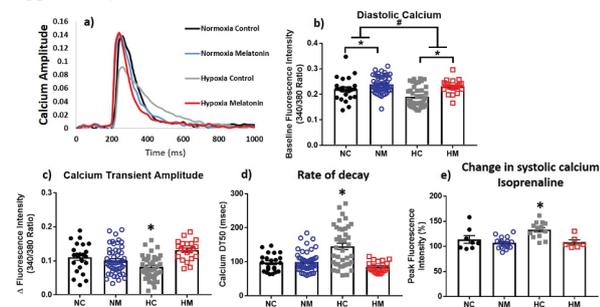
**Introduction:** Developmental hypoxia can programme cardiac abnormalities in the offspring and maternal antioxidant treatment appears protective (Giussani & Davidge. *J DOHaD* 4(5):328, 2013). However, underlying mechanisms at the level of calcium handling within individual cardiomyocytes are completely unknown. Therefore, we investigated cardiomyocyte calcium homeostasis in adult offspring of hypoxic pregnancy and the potential of melatonin as antenatal antioxidant therapy in rats.

**Methods:** Time-mated female Wistar rats were randomly assigned to Normoxia (21% O<sub>2</sub>) or Hypoxia (13% O<sub>2</sub>) with or without Melatonin in drinking water (5µg/ml). Chronic hypoxia (isobaric chamber) and melatonin treatment was from GD6-20 (term =23d). Offspring were culled to 4 males and 4 females per pregnancy and raised until 4 months of adulthood. At 4 months, offspring were humanely killed (CO<sub>2</sub> inhalation) and cardiomyocytes isolated by Langendorff perfusion with a collagenase/protease solution. Cells were loaded with the calcium sensitive dye Fura-2 and excited at 340/380nm and field stimulated at 1Hz. Comparisons for statistical significance were performed using a nested two-way ANOVA.

**Results:** Cardiomyocytes from adult offspring of hypoxic pregnancy showed: 1) a decrease in the calcium transient amplitude, 2) slower reuptake of systolic calcium and 3) a greater response to isoprenaline indicating higher sensitivity to β-adrenergic stimulation, all of which were ameliorated by maternal antenatal melatonin treatment (Fig. 1a-e). However, antenatal melatonin treatment alone increased cardiomyocyte diastolic calcium, indicating reduced cardiac buffering power (Fig. 1b).

**Conclusion:** Cardiomyocytes of offspring of hypoxic pregnancy show smaller calcium transients with delayed cytoplasmic calcium removal, providing subcellular mechanisms underlying programmed systolic and diastolic dysfunction. Melatonin may prevent programmed cardiac dysfunction programmed by gestational hypoxia. However, the data also suggest antenatal melatonin may alter cardiomyocyte calcium dynamics independently, indicating that it should only be administered to pregnancies diagnosed with chronic fetal hypoxia, rather than given prophylactically to all pregnancies.

Supported by The British Heart Foundation.



**Figure 1: Calcium homeostasis in isolated cardiomyocytes of hypoxic pregnancies.** a) Example calcium transient, b) diastolic level of calcium measured as base fluorescence intensity, c) calcium transient amplitude measured as difference from baseline to peak fluorescence intensity, d) time from peak to 50% calcium transient decay, e) percent change in systolic calcium level from normal tyrodes solution to isoprenaline treatment. NC; Normoxia Control, NM; Normoxia Melatonin, HC; Hypoxia Control, HM; Hypoxia Control. Data represented as mean±SEM. Each dot plot represents a single cell (b, c, d; n=137 cells, 20 animals, e; n=45 cells, 14 animals).

### O-097

#### Vascular Disorders of Pregnancy Increase Susceptibility to Neonatal and Infantile Pulmonary Hypertension in High-Altitude Populations.

Alexandra Heath,<sup>1</sup> Colleen G Julian\*,<sup>2</sup> Lilian Toledo-Jaldin,<sup>3</sup> Inge von Alvensleben,<sup>1</sup> Litzo Lazo Vega,<sup>3</sup> Hussna Yasini,<sup>2</sup> Jesus Dorado Madera,<sup>2</sup> Margaret Stalker,<sup>2</sup> Julie A. Houck,<sup>2</sup> Lorna G. Moore.<sup>2</sup> <sup>1</sup>Kardiozentrum, La Paz, Bolivia, Plurinational State of; <sup>2</sup>University of Colorado School of Medicine, Aurora, CO, United States; <sup>3</sup>Hospital Materno-Infantil, La Paz, Bolivia, Plurinational State of.

**Introduction:** Perinatal exposures exert a profound influence on physiological function, including developmental processes vital for efficient pulmonary gas transfer throughout the lifespan. Prior work suggests that preeclampsia (PreE) increases an offspring's risk of developing pulmonary hypertension (PHTN) in adulthood. In this prospective cohort study, our objective was to establish whether this effect of PreE is apparent during infancy and, if so, whether such effects are compounded by fetal growth restriction and/or hypoxemia.

**Methods:** Subjects were 79 maternal-infant pairs (39 PreE, 40 controls) in La Paz-El Alto, Bolivia (3,600-4,100m). Umbilical artery and venous blood gases, erythropoietin (Epo) and hemoglobin (Hb) were measured as indices of fetal/newborn hypoxia. Postnatal echocardiography studies were conducted at 1-week and 6-to-8 months of age to assess pulmonary hemodynamics and indices of PHTN. PHTN was defined as an estimated right ventricular systolic pressure (RVSP) >40 mmHg, RVSP/systemic systolic pressure >0.5, any cardiac shunt with bidirectional or right-to-left flow, or ventricular septal wall flattening. The influence of small-for-gestational age (SGA) vs. appropriate-for-gestational age (AGA) status was also considered.

**Results:** Compared to controls, PreE infants weighed 494 g less at birth and were more often SGA (43% [28, 59] vs. 6% [6,19]). PreE newborns were comparatively hypoxemic as indicated by lower umbilical venous pO<sub>2</sub> (P = 0.028), trends toward higher pCO<sub>2</sub> (P = 0.069) and lower pH (P = 0.087), as well as elevated Epo (P < 0.01) and Hb (P < 0.05). While elevated RVSP:SYS ratios at 1-week of age were modestly associated with PreE (P = 0.06), when PreE cases were complicated by SGA, 40% of newborns had elevated RVSP:SYS ratios compared to 0% of normotensive AGA controls (P = 0.019). By 6-months of age, no infants had an elevated RVSP but 43% of PreE infants showed evidence of septal flattening compared to only 8% of controls (P < 0.05). Septal flattening at 6 months of age also tended to be associated with lower umbilical venous pH and elevated Hb (P = 0.053 and P = 0.067, respectively).

**Conclusion:** PreE, particularly when accompanied by SGA, is associated with abnormalities in the pulmonary circulation during the neonatal period that extend into infancy at high altitude. Consideration of such developmental effects of PreE and SGA can aid in the identification of at-risk individuals and important maturational windows for the prevention of pulmonary vascular disease among babies born to highland residents or those with exaggerated hypoxia in utero or newborn life.

### O-098

#### Reduced TGFβ Responsiveness in Skeletal Muscle Satellite Cells from Lambs with Fetal Growth Restriction.

Rosa I Luna Ramirez†, Miranda J Anderson, Ravi Goyal, Sean W Limesand\*. *The University of Arizona, Tucson, AZ, United States.*

**Introduction:** Placental insufficiency causes fetal growth restriction (FGR) and compromises skeletal muscle growth. As a consequence, infants with FGR exhibit reduced muscle mass throughout life, increasing their risk for metabolic health problems. Postnatal muscle growth is regulated by endocrine factors, including transforming growth factor beta (TGFβ), which regulates gene expression to prevent proliferation and differentiation of satellite cells. Activation of the TGFβ receptors promotes the translocation of SMAD complexes into the nucleus to stimulate transcription. Activation of I-SMAD proteins (SMAD-6 or 7) antagonized TGFβ signaling as part of a negative feedback loop. Crosstalk with other non-SMAD pathways, such as insulin-like growth factor-1 (IGF-1) signaling, also contribute to the regulation of muscle

growth and TGF $\beta$  responsiveness. We tested the hypothesis that TGF $\beta$  responsiveness is reduced in satellite cells from lambs with FGR due to an enhancement in IGF-1 signaling

**Methods:** Lambs with placental insufficiency-induced FGR were generated by exposing pregnant ewes to environmental hyperthermia during mid-gestation. FGR lambs were compared to control lambs from thermoneutral ewes (n=6/group). Satellite cells were isolated from 30-day old lambs. Cells were differentiated with low serum in the presence of increasing TGF $\beta$ 1 concentrations (0-3ng/ml), and myogenin was measured as a proxy of differentiation. Differences were determined with a t-test. TGF $\beta$ -induced gene expression (3 versus 0 ng/ml) was determined in differentiating satellite cells with high-throughput RNA sequencing. Differentially expressed genes were identified with DESeq2 (FDR<0.05) and modeled into functional pathways.

**Results:** Dose-response experiments for TGF $\beta$  showed 52% higher (P<0.05) maximum rate of differentiation in FGR cells, which also required a greater concentration of TGF $\beta$  to inhibit differentiation (IC50= 237 $\pm$ 61 vs. 103 $\pm$ 24 pg/ml; P<0.05). After confirming that TGF $\beta$  responsiveness was reduced in FGR cells, we examined their transcriptome to define the molecular mechanisms that impair TGF $\beta$  activity. TGF $\beta$  caused differential expression of 1033 genes in control cells and differential expression of 1163 genes in FGR cells. 555 DE genes were unique in FGR cells compared to controls. TGF $\beta$ , PI3K/Akt, and MAPK signaling pathways were enriched and SMAD-6 & 7 levels were upregulated to a greater extent with TGF $\beta$  in FGR cells.

**Conclusion:** These findings show that TGF $\beta$  responsiveness is lower in satellite cells from FGR lambs due to two potential mechanisms. First, higher concentrations of SMAD-6 and 7 indicate that TGF $\beta$  signaling is antagonized. Second, PI3K/Akt signaling downregulates TGF $\beta$ -related genes to promote differentiation. Therefore, developmental programming during FGR disrupts TGF $\beta$  responsiveness, which will restrict satellite cell expansion and muscle growth.

#### O-099

**Polycomb Repressive Complex 2 Antagonizes Wound Healing Responses in the Decidual Stroma by Modulating the Transforming Growth Factor Beta Pathway.** Ivan Osokine<sup>†</sup>, Damon Rideaux, Tara McIntyre, Johan Siewiera, Adrian Erlebacher\*. *University of California, San Francisco, San Francisco, CA, United States.*

**Introduction:** The decidual stroma is generated from the endometrium upon embryo implantation and provides support for the developing fetus. Recent work from our lab has uncovered that decidualization triggers an epigenetic silencing program within decidual stromal cells (DSCs) that transcriptionally silences a diverse set of ~800 genes, including genes associated with type 1 immunity, as well as fibroblast activation and wound healing. This program is generated by PRC2 (Polycomb Repressive Complex 2), whose primary catalytic subunit is the histone methyltransferase EZH2. Given its vast scope, we elected to explore how this program affects decidual biology and impacts pregnancy.

**Methods:** Employing Ezh2 conditional uterine knockout (cKO) mice (*Pgr<sup>Cre/+</sup> Ezh2<sup>fl/fl</sup>*), we performed immunohistochemistry, transcriptional analyses (RNA-seq, qRT-PCR), and western blotting on whole tissues and isolated DSCs of early pregnancy to identify altered biological pathways. To assess leukocyte responses, we treated mice with anti-CD40 and Poly(I:C) on E6.5 followed by flow cytometric analysis. To determine whether Ezh2 cKO decidua was more sensitive wounding signals, we performed RNA-seq on TGF- $\beta$ 1-treated wild type (WT) and Ezh2 cKO DSCs, and performed surgical wounding of implantation sites *in vivo*. To determine whether Ezh2 cKO decidual abnormalities mediated by TGF- $\beta$  *in vivo*, we treated Ezh2 cKO mice with TGF $\beta$ R-I inhibitor LY364947.

**Results:** The decidua of Ezh2 cKO mice were smaller compared to wild type and showed accumulations of myofibroblasts, deposition of type I collagen around the embryo, and fewer uterine NK cells (p<0.05). After inflammatory stimulation, antigen presenting leukocytes could traffic out of Ezh2 cKO but not WT decidua. Transcriptomic analyses of Ezh2 cKO decidua and DSCs revealed increased expression of numerous genes associated with wound healing and the TGF- $\beta$  pathway, and Ezh2 cKO DSCs significantly induced ~3-fold more genes than WT DSCs after

culture with TGF- $\beta$ 1. *In vivo* inhibition of the TGF- $\beta$  signaling pathway in Ezh2 cKO mice significantly (p<0.05) increased decidual size and reduced myofibroblast formation and collagen levels. Direct surgical wounding induced myofibroblasts and TGF- $\beta$  target proteins in Ezh2 cKO but not wild type decidua.

**Conclusion:** The decidua employs PRC2-mediated gene silencing to tightly control local immune regulation and pro-fibrotic signals. This may serve to prevent the decidua from assuming a chronically wounded state in response to invasion and growth of fetal tissues. TGF- $\beta$  signaling was central to the observed defects, and *in-vivo* blockade of TGF- $\beta$  signaling partially rescued the dysregulation caused by PRC2 deficiency. **Funding:** NIH R01AI143187 / March of Dimes #6-FY18-798 / Cancer Research Institute Postdoctoral Fellowship

#### O-100

**Proteomic Analysis of Extracellular Vesicles Secreted by Primary Endometrial Epithelial Cells from Fertile Women Reveals Functions Related to Embryo Implantation Not Present in an Endometrial Epithelial Cell Line.** Marina Segura-Benítez<sup>†,1,2</sup>, María Cristina Carballo-García<sup>†,1,2</sup>, Ana Corachán<sup>†,1,2</sup>, Amparo Faus,<sup>1</sup> Antonio Pellicer\*,<sup>1,3</sup> Hortensia Ferrero\*.<sup>1</sup> *IVI Foundation - IIS La Fe, Valencia, Spain;* <sup>2</sup>*University of Valencia, Valencia, Spain;* <sup>3</sup>*IVIRMA Rome, Rome, Italy.*

**Introduction:** The role of extracellular vesicles (EVs) in embryo-maternal communication during implantation has been suggested, although it is not well understood yet. The aim of this study was to describe the protein content and related function of EVs secreted by primary endometrial epithelial cells (pEECs) and Ishikawa endometrial epithelial cell line (ICL) to define the best model to study the role of these EVs in embryo implantation.

**Methods:** ICL (n=3 replicates/experiment) and pEECs obtained from fertile women (n=24) were hormonally treated and cultured *in vitro*. Ultracentrifugation (UC), ExoQuick-TC reagent (EXOQ) and Norgen Purification Kit (NOR) were used for ICL EVs isolation; pEECs EVs were isolated by the most efficient one. EVs were characterized by Nanoparticle Tracking Analysis (NTA), Western Blot (WB) and Transmission Electron Microscopy (TEM). Student t-test was calculated to analyse data. Proteomic content of EVs was analysed by liquid chromatography-mass spectrometry and GO enrichment analysis by PANTHER.

**Results:** NTA revealed an ICL EVs size within 50-200 nm, and a lower concentration in NOR samples compared to EXOQ and UC (6.8E+10; 4.7E+11; 5.9E+11 particles/mL). Higher expression of EVs markers HSP70, TSG101, CD9 and CD81 was observed in UC EVs compared to NOR and EXOQ EVs, being significant in HSP70 and TSG101 (P<0.05). Characterization of pEECs EVs isolated by UC revealed presence of small (<200 nm) and medium/large EVs (>200 nm), obtaining a mean size of 275.1 nm, and expression of all EVs protein markers evaluated. EVs morphology and size range was corroborated by TEM in all cases. Proteomic analysis of EVs content found 26% of the proteins in common between pEECs and ICL, some of them involved in embryo implantation as ANXA2, PFN1 and LAMC1. Enrichment analysis in both pEECs and ICL showed significantly enriched GO Biological Process terms related to cell adhesion, migration and extracellular matrix organization, which are essential for embryo implantation. However, functions related to embryo development, angiogenesis, differentiation and cell communication were only significantly enriched in pEECs EVs protein cargo.

**Conclusion:** EVs secreted by pEECs from fertile women cultured *in vitro* can be efficiently isolated by UC, and their protein cargo reveals their involvement in biological processes related to embryo implantation, which are not present in EVs isolated from ICL. Based on this, pEECs would be a more accurate *in vitro* model to study the communication system via EVs between the embryo and the endometrium, which could be altered in infertile women. Support: FPU18/03735; ACIF/2019/139; APOSTD/2020/123; CP20/00120

**O-101**

**Jagged1 Regulates Endometrial Receptivity in Both Humans and Mice.** Wei Zhou, Ellen Menkhorst, Evdokia Dimitriadis. *University of Melbourne, Melbourne, Australia.*

**Introduction:** Human endometrium is only receptive to an implanting blastocyst within a narrow window of 2-4 days in the mid-secretory phase. Recent single cell sequencing of human endometrium across the menstrual cycle has identified an abrupt and discontinuous transcriptomic activation in the epithelia in the mid-secretory phase. Such transcriptomic and accordingly functional changes require delicate interplay between a diversity of factors including cytokines and signaling pathways. The Notch signaling pathway members are expressed in human endometrium. The Notch ligand Jagged1 (JAG1) localizes in the endometrial luminal epithelium (LE) and is abnormally reduced in infertile women. However, the functional consequences of reduced JAG1 production on endometrial receptivity and embryo implantation is unknown. This study aimed to determine the role of JAG1 in regulating endometrial receptivity in humans and mice.

**Methods:** Primary human endometrial epithelial cells (HEECs) and Ishikawa cells were used to determine the effect of *JAG1* knockdown on cell adhesion (via a spheroid adhesion assay and xCELLigence). In mice Ago*Jag1* siRNA or scrambled control was injected into each uterine horn. Uteri were collected at E4 and E4.5 (day of plug=E0) and implantation sites counted and fixed. LE was isolated enzymatically for E4 uteri. *Jag1* knockdown in human cells and mouse LE was measured by qPCR and immunoblotting. Changes in genes and proteins in response to *JAG1* knockdown were assessed via a customized RT<sup>2</sup> Profiler PCR array, qPCR, immunoblotting and immunohistochemistry. Paired student's t-test or repeated measures ANOVA were used as appropriate for statistical analysis with a significance threshold of  $P < 0.05$ .

**Results:** Knockdown of *JAG1* in both HEECs and Ishikawa cells significantly reduced their adhesive capacity ( $P < 0.05$ ), compared to control. We confirmed that in human endometrial epithelial cells, JAG1 interacted with NOTCH3 and knockdown of *JAG1* significantly reduced the expression of Notch signaling downstream target *HEY1* and classical receptivity markers (*LIFR*, *SPP1*, *ITGB3*, *MAOA* and *IGFBP1*). Knockdown of *Jag1* in mouse LE significantly impaired embryo implantation compared to control ( $P < 0.05$ ). We identified ten genes (related to tight junction, infertility and cell adhesion) that were differentially expressed by *Jag1* knockdown in the LE in mice. Further analysis of the tight junction family members in both human and mouse models revealed that JAG1 altered the expression of tight junction components *Cldn4*, *Cldn11* and *Ocln* in mice.

**Conclusion:** Our data demonstrate that JAG1 alters endometrial epithelial cell adhesive capacity and regulates endometrial receptivity in both humans and mice.

**O-102**

**Antiphospholipid Antibodies Accelerate Endometrial Stromal Cell Decidualization and Senescence and Induce Inflammation via TLR4, p38 MAPK and ROS Signaling.** Mancy Tong†, Teimur Kayani†, Deidre M Jones, Lawrence W Chamley, Vikki M Abrahams\*. <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>The University of Auckland, Auckland, New Zealand.

**Introduction:** Antiphospholipid autoantibodies (aPL) are a major risk factor for recurrent pregnancy loss by targeting the maternal-fetal interface. While aPL is known to induce placental inflammation via Toll-like receptor 4 (TLR4), less is known about how aPL affect endometrial stromal cells (EnSCs). EnSCs are the major cell type of the endometrium and undergo decidualization monthly to prime the uterus for implantation. Thus, appropriate EnSC function is key for pregnancy success. We hypothesized that aPL accelerate EnSC decidualization, premature senescence, and inflammation through TLR4 activation.

**Methods:** aPL effects were studied using a telomerase-immortalized EnSC line (n=8-10) and validated in primary EnSCs (n=3). EnSCs were exposed to decidualizing conditions (10nM estradiol, 1µM MPA and 0.5mM cAMP in 2% FBS), in the presence or absence of a mouse monoclonal aPL targeting human β<sub>2</sub>-glycoprotein I or control IgG (20µg/

ml). For mechanistic studies on the EnSC line, a TLR4 antagonist (LPS-RS, 10µg/ml); a p38 MAPK inhibitor (SB203580, 10µM); or a ROS inhibitor (DPI, 5µM) was added. At 48h, secretion of decidualization markers IGFBP-1 and prolactin (PRL); and inflammatory IL-8 were quantified by ELISA. Senescence was evaluated by senescence-associated β-galactosidase activity (SA-β-gal). In a mouse model of decidualization, 1mg aPL or control IgG was injected ip, and 6h later, uterine expression of decidualization markers *Bmp2* and *PRP*; and inflammatory *IL6* were quantified by qRT-PCR (n=9).

**Results:** aPL, but not control IgG, augmented EnSC secretion of IGFBP-1 (76.7±2.3-fold), PRL (2.3±0.2-fold), and IL-8 (3.6±1.0-fold) ( $p < 0.05$ ); and increased SA-β-gal. This was confirmed in primary EnSCs. LPS-RS reduced aPL-induced EnSC IGFBP-1 (19.4±5.8%) and IL-8 (20.2±4.4%) ( $p < 0.05$ ) without affecting PRL or SA-β-gal. SB203580 reduced aPL-induced EnSC IGFBP-1 (62.9±11.5%); PRL (48.3±10.9%); and IL-8 (41.9±15.8%) ( $p < 0.05$ ) without affecting SA-β-gal. DPI reduced aPL-induced EnSC IGFBP-1 (46.0±11.2%); PRL (50.3±8.4%); and SA-β-gal without affecting IL-8. *In vivo*, aPL augmented uterine *Bmp2* (8.5±5.3-fold); *PRP* (47.7±38.2-fold); and *IL6* (12.9±4.5-fold) expression compared to control IgG ( $p < 0.05$ ).

**Conclusion:** aPL augmented EnSC decidualization via TLR4, p38 MAPK and ROS signaling. aPL induced EnSC inflammation via TLR4 and p38 MAPK signaling, but independently of ROS. Finally, aPL increased EnSC senescence via ROS signaling independently of TLR4 and p38 MAPK. Thus, this work demonstrates that aPL can directly and deleteriously affect EnSC function which may contribute to early pregnancy loss in this high risk population.

**O-103**

**The Long-Term Impact of Selective Progesterone Receptor Modulator (SPRM) Ulipristal Acetate (UPA) on the Cell Cycle and Cell Proliferation in Human Endometrium as Assessed by RNA Sequencing.** Aleksandra Tsoolova†, Rohan Chodankar, Alison Murray, Lucy Whitaker, Moira Nicol, Alistair Williams, Hilary Critchley. *University of Edinburgh, Edinburgh, United Kingdom.*

**Introduction:** The SPRM UPA controls symptoms of fibroid associated heavy menstrual bleeding (HMB) in up to 90% of users, often with rapid onset of amenorrhoea (median ten days). The endometrial mechanism of action (MoA) of UPA is unknown. In the endometrium, it leads to PAEC (Progesterone Receptor Modulator Associated Endometrial Changes), a poorly understood morphological class effect that occurs with all SPRMs. We employed RNA sequencing analysis to interrogate the MoA of UPA on the endometrium and analyse a long-term effect if any.

**Methods:** Endometrial biopsies (n=27) were available through an embedded MoA component of the UCON Trial (EudraCT 2014-003408-65; REC14/LO/1602). Biopsies were obtained at baseline, before UPA treatment (n=9), at 6 months of UPA treatment (n=9), and at the end of UPA treatment following a withdrawal bleed (12 months; n=9). Biopsies were histologically staged: proliferative (n=5) and secretory (n=4) and stage-matched before and after UPA treatment. Each participant (n=9) was serially sampled. Total RNA was extracted, quality assessed and samples (RIN>8) underwent RNA Sequencing using Lexogen Quantseq platform. Results were analysed by bcBioRNASeq pipeline in Unix and differential expression analysis was performed using RStudio. Pathway analysis was conducted (Ingenuity Pathways Analysis software) and top differentially expressed pathways were identified based on log<sub>2</sub> fold change (<-1 and 1<) and p-value (<0.05). Results were compared for each group at baseline to six months on UPA treatment and at baseline to end of study. Validation of these results is in progress.

**Results:** Comparison of proliferative-phase pre-treatment versus UPA-treated endometrium revealed upregulation of 1421 genes and downregulation of 698 genes. Differentially expressed genes identified are involved in suppression of canonical pathways of the cell cycle, including centrosome separation and maturation, estrogen-mediated S-phase entry and cell-cycle progression. Genes encoding for cell cycle regulators such as cyclins A, B and E were significantly downregulated, including CDK1 and CDC25A. Comparison of secretory-phase pre-treatment versus UPA-treated endometrium identified significant upregulation of

metalloproteinases (MMP) signalling by downregulating genes encoding for MMP inhibitors (TIMP3 and TSP2). Notably, there were no enriched pathways between pre-treatment and post-UPA treated endometrium (twelve months), both in proliferative and secretory phase samples.

**Conclusion:** RNA Sequencing and pathway analysis demonstrates that SPRM (UPA) treatment affects the cell cycle and cell proliferation pathways in the human endometrium, and this effect appears reversible following discontinuation of UPA.

## O-104

### Next Generation of Human Endometrial Organoids: Bioengineering-Based Strategies for Preserving Tissue-Specific Extracellular Environment.

Emilio Francés-Herrero†,<sup>1,2</sup> Elena Juárez-Barber†,<sup>2</sup> Hannes Campo†,<sup>2,3</sup> Sara López-Martínez†,<sup>2</sup> Lucía de Miguel-Gómez†,<sup>1,2</sup> Amparo Faus,<sup>2</sup> Antonio Pellicer\*,<sup>4</sup> Hortensia Ferrero\*,<sup>2</sup> Irene Cervelló\*.<sup>2</sup>  
<sup>1</sup>Universitat de València, Valencia, Spain; <sup>2</sup>IVI Foundation-IIS La Fe, Valencia, Spain; <sup>3</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States; <sup>4</sup>IVIRMA-Rome, Rome, Italy.

**Introduction:** Organoids are defined as 3D *in vitro* cellular structures that preserve the functional and morphological characteristics of the tissues from which they come. To ensure the correct development of organoids it is necessary to use supportive matrix, such as Matrigel. However, these commercial preparations are unable to recapitulate tissue-specific composition signalling and dynamic changes. Here, we investigated the effect of an extracellular matrix hydrogel derived from decellularized endometrium (eECM) on the human endometrial organoids development when added to culture media.

**Methods:** Three human endometrial organoid lines were established (until passages 8-12) and cultured with standard (ExM), withdrawal of nicotinamide (ExM-Na) and hydrogel eECM supplemented (ExM+eECM; ExM-Na+eECM) media. Long-term chromosomal stability from early and late passages was evaluated using a genomic hybridization array. Glandular epithelial marker expression (PanCytokeratin), stromal components (Vimentin), mucopolysaccharide secretions (Muc-1 and PAS staining) and proliferation index (Ki67) were histologically and immunohistochemically assessed. Proliferation rates based on number of spheroids on days 2, 5 and 7 were compared between the different conditions.

**Results:** Organoids showed chromosomal stability from early culture stages until week 12. eECM supplementation did not modify the *in vivo* glandular epithelial phenotype, characterized by presence of PanCytokeratin and absence of stromal components, and endometrial secretions, showing Muc-1 and PAS positive staining, typical of human endometrial organoids. Day 2-7 proliferation rates showed three groups of statistical significance between the studied conditions: ExM+eECM (2.11±0.27), ExM and ExM-Na+eECM (1.84±0.33 and 1.94±0.28, respectively) and ExM-Na (1.55±0.22) (P<0.05). Ki67 proliferation assay confirmed the results based on counting the number of spheroids, significantly differentiating the groups supplemented with endometrial eECM (P<0.05).

**Conclusion:** In this study we have demonstrated the importance of mimicking a favourable native microenvironment for the development of human endometrial organoids. Thus, tissue-specific ECM hydrogels could be an optimal platform for *in vitro* organoid development, improving on common standard approaches.

EF and EJ contributed equally. SUPPORT: PI17/01039; PROMETEO/2018/137; FPU 18/06327; FI19/00110; ACIF/2017/118; CP19/00149; CP20/00120

## O-105

### Fibronectin-Integrin Interactions Regulate Placental Endothelial Cell Migration in Severe Fetal Growth Restriction.

Diane L Gumina†, Shuhan Ji, Kathryn McPeak, Emily J Su\*. University of Colorado Anschutz Medical Campus, Aurora, CO, United States.

**Introduction:** Placentas from pregnancies complicated by severe, early-onset fetal growth restriction with abnormal umbilical artery end-diastolic velocities (FGRadv) exhibit diminished vasculature mediated by impaired angiogenesis, but underlying mechanisms remain unknown.

We previously reported that FGRadv fetoplacental endothelial cells (ECs) exhibit reduced migratory capacity. However, this model does not account for EC-extracellular matrix (ECM) interactions, which are critical for regulating angiogenesis. Our lab has found that FGRadv placentas exhibit reduced fibronectin (FN), an ECM protein. FN interactions with integrins are responsible for dynamic and localized signaling required for angiogenesis. Of the FN-binding integrins,  $\alpha\beta3$  and  $\alpha5\beta1$  are known to regulate key angiogenic processes including EC migration. Thus, we hypothesized that FN- $\alpha\beta3$  and - $\alpha5\beta1$  signaling are dysregulated in FGRadv ECs, resulting in deficient migration.

**Methods:** Human fetoplacental ECs were isolated from uncomplicated term control (n=6) and FGRadv pregnancies (n=6) and were plated on FN in all experiments. Migration was assessed with scratch-wound assays. JBS5, AIB2 and LM609 (integrin  $\alpha5$ ,  $\beta1$ , and  $\alpha\beta3$  blocking antibodies, respectively) were used to inhibit integrin activation in both groups. Whole cell expression was assessed with qPCR and immunoblot. Focal adhesion complexes were isolated and compared via immunoblot. Non-linear regression and t-tests were utilized to assess statistical significance.

**Results:** Compared to control, FGRadv EC migration was significantly reduced when plated on FN (p<0.0001), suggesting possible  $\alpha\beta3$  and  $\alpha5\beta1$  deficiencies. Inhibition of  $\alpha5\beta1$  activation led to significantly compromised migration of both control and FGRadv ECs (p<0.0001). In contrast, inhibition of active  $\alpha\beta3$  diminished control EC migration (p<0.0001) but had no effect on FGRadv ECs (p=0.92), indicating integrin  $\alpha\beta3$  as a mechanistic candidate contributing to reduced migration in FGRadv. To understand cellular processes causing aberrant  $\alpha\beta3$  function, we first assessed expression and found no significant differences in  $\alpha\beta3$  transcript or protein between control and FGRadv ECs. However, as integrin signaling also requires dynamic activation and recruitment to focal adhesion complexes (FACs), we investigated  $\alpha\beta3$  in FACs and unexpectedly found that  $\alpha\beta3$  was significantly increased in FGRadv EC complexes (p=0.022).

**Conclusion:** FN- $\alpha\beta3$  and  $\alpha5\beta1$  interactions regulate fetoplacental EC migration overall, but integrin  $\alpha\beta3$  is dysregulated in FGRadv. This dysregulation is not caused by differences in expression but rather, recruitment to focal adhesion complexes. These findings suggest impaired focal adhesion dynamics and represent a previously unidentified mechanism contributing to deficient angiogenesis in FGRadv.

## O-106

### Characterization of Regulation of NLRP3 Inflammasome Activity in Placental Hofbauer Cells.

Magnolia G Wang†,<sup>1</sup> Seth Guller\*.<sup>2</sup>  
<sup>1</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>2</sup>Yale School of Medicine, New Haven, CT, United States.

**Introduction:** Hofbauer cells (HBCs) are fetal macrophages residing in the human placenta throughout pregnancy. HBCs play an important role in the regulation of placental innate immunity, and disruptions of their homeostasis are associated with complications of pregnancy including villitis of unknown etiology (VUE) and histological chorioamnionitis (HCA). Inflammasome NLRP3 is a key pathway in innate immunity, however, regulation of its activity in HBCs during normal pregnancy and pregnancy-related complications remain largely unelucidated due to limited tissue access and complex isolation procedure.

The objectives of the study are to investigate the regulation of NLRP3 inflammasome activation in placental HBCs, and to characterize key components in Signal 1 and Signal 2 pathways as compared to the established roles in other tissue-resident macrophages.

**Methods:** HBCs were freshly isolated from human placenta, and LPS and ATP were utilized to activate Signal 1 and Signal 2 of NLRP3 inflammasome, respectively. HBCs were pre-treated with caspase-1 inhibitors WEHD and VX765, P2RX7 receptor antagonist KN-62, or NLRP3-specific siRNA separately before treatment with LPS and ATP. ELISA was used to measure secreted IL-1 $\beta$  levels in culture media. For Western blotting, RIPA buffer was used to extract protein from HBCs, and proteins were separated via gel electrophoresis. Total cellular protein levels were quantified using DC protein assay. Membrane was incubated with multiple primary followed by secondary antibodies.

**Results:** In Signal 1 pathway, IL-1 $\beta$  was found to be a weak agonist that can stimulate its own production in an autocrine fashion; ROS was found to be indispensable to mediate downstream signaling events. In contrast to its established role in other tissue-resident macrophages, MEK was identified to mediate the Signal 2 pathway without any impact on Signal 1, underscoring unique aspects of NLRP3 regulation in HBCs. P2X7R, NLRP3 and caspase-1 demonstrated positive regulatory roles in the Signal 2 pathway, consistent with their roles in other tissue-resident macrophages. **Conclusion:** Unique aspects in Signal 1 and Signal 2 regulation of NLRP3 inflammasome activity were revealed in this comprehensive study of placental HBCs in comparison to their established roles in other tissue-resident macrophages, presenting HBC as a potential therapeutic target for the treatment of pregnancy-associated complications.

## O-107

### AKT Signaling Controls Trophoblast Development and Placentation.

Keisuke Kozai, Mae-Lan Winchester†, Mikaela E Simon†, Khursheed Iqbal, Masanaga Muto, Regan L Scott†, Chad Slawson\*, Michael J Soares\*. *University of Kansas Medical Center, Kansas City, KS, United States.*

**Introduction:** AKT serine/threonine kinase 1 (AKT1; also called protein kinase B alpha) is an intrinsic regulator of trophoblast cells. AKT1 is an integral component of signal transduction pathways regulating cell proliferation, differentiation, migration, survival, and metabolism and is implicated in placental development. In this project we utilize the rat, which possesses deep intrauterine trophoblast cell invasion similar to human placentation, to examine the role of AKT1 as an intrinsic regulator of placental development.

**Methods:** Crispr/Cas9 genome-editing was used to establish an AKT1 null rat model. Morphologic, biochemical, and molecular strategies were utilized to characterize the impact of an AKT1 deficiency on rat placentation. Combined immunoreactive AKT substrate pull-down and mass spectrometry was used to identify AKT substrates. Validation was performed in rat trophoblast stem cells and placental tissues.

**Results:** AKT1 null rats showed placental, fetal, and postnatal growth restriction. Each compartment of the AKT1 null placentation site showed abnormalities. AKT1 deficiency adversely affected labyrinth zone (barrier for placental-fetal transport) and junctional zone (interface with the uterus and the source of invasive trophoblast) development. The uterine-placental interface (metrial gland) also showed anomalies associated with trophoblast cell invasion. Junctional zone growth deficits were linked to dysregulated gene expression, including downregulation of transcripts critical to cell cycle progression. Mass spectrometry of AKT substrates from the junctional zone resulted in the identification of candidate downstream mediators of AKT action with known roles in regulating placental development. Forkhead box protein O4 (FOXO4), LLGL scribble cell polarity complex component 2 (LLGL2), and dual specificity protein phosphatase 9 (DUSP9) were identified as downstream AKT targets in trophoblast cells.

**Conclusion:** AKT1 is a key regulator of placental development. AKT1 acts through the orchestration of key regulatory proteins affecting trophoblast cell expansion and differentiation. Dissection of the actions of AKT1 and its substrates represents a roadmap for elucidating regulatory hubs critical for placentation and the identification of vulnerabilities associated with placental disease. (Supported by AHA fellowships to KK and MM, NIH grants HD020676; HD079363, HD099638, and the Sosland Foundation)

## O-108

### Changes in Lipid Profiles of Placental Exosomes in Maternal Plasma Characterize Pregnancies with a Small-for-Gestational Age Fetus.

Miira M Klemetti†, Ante BV Pettersson†, Porter R Tyler, Aafaque Khan, Premy Shan, Hannes Röst\*, Martin Post\*, Isabella Caniggia\*. <sup>1</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>2</sup>Peter Gilgan Centre for Research and Learning, Hospital for Sick Children, Toronto, ON, Canada; <sup>3</sup>Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada.

**Introduction:** Approximately 10% of all fetuses are born small-for-gestational age (SGA), which is associated with perinatal morbidity and mortality as well as adverse long-term health outcomes. Due to lack of predictive biomarkers, most SGA pregnancies are not recognized before birth. Placental extracellular vesicles, released into the maternal circulation from the first weeks of pregnancy, are known to carry bioactive molecules, including lipids, which are important mediators of foeto-maternal metabolism. The objective of the current study was to explore lipid profiles in circulating placental exosomes from pregnancies with an SGA vs. an appropriate- or large-for-gestational age fetus (AGA/LGA). **Methods:** Blood samples from women with normal pregnancies (AGA/LGA; birth weight  $\geq 10$ th percentile; n=220) and from normotensive women with an SGA fetus (birth weight <10th percentile; n=43) were collected by the Ontario Birth Study at 10-14 (G1), 16-22 (G2), and 26-32 (G3) weeks' gestation and at delivery (G4). Placental exosomes were harvested by ultracentrifugation, filtration and immunoprecipitation using placental alkaline phosphatase (PLAP) antibodies. Their size and concentration were quantified by nanoparticle tracking analysis, and exosomal and placental origin ascertained by immunoblotting for CD63, ALIX, TSG101 and PLAP. Placental exosomes were confirmed to be devoid of plasma lipoproteins by assessing ApoB concentrations with immunoblotting and flow cytometry. TripleTOF MS was used for unbiased lipidomic analyses.

**Results:** Placental exosome concentration was lower in SGA vs. AGA/LGA at G1, G3 and G4 (p<0.01 for all timepoints). MS/MS<sup>ALL</sup> analysis revealed significant differences in the lipid profile of placental exosomes across different gestational windows in both AGA/LGA and SGA pregnancies. The top 25 lipids that were found to be consistently increased across gestation (from G1 to G4) in SGA vs. CTR pregnancies included phosphatidyl choline (PC), phosphatidylethanolamine, phosphatidic acid, phosphatidyl serine (PS), phosphatidyl inositol (PI) and sphingomyelin species. In contrast, specific PCs and lyso-PCs were consistently decreased from G1 to G4 in SGA vs. CTR pregnancies. In volcano plot analysis, elevated PS, PI and ceramide species characterized placental exosomes of SGA pregnancies already at G1.

**Conclusion:** Discovery lipidomics demonstrated differences in the bioactive lipid cargo of circulating placental exosomes isolated from women with SGA vs. AGA/LGA fetuses already at 10-14 weeks, with potential predictive utility. (Supported by NIH)

## O-109

### Velocity-Selective Arterial Spin Labeling Perfusion Measurements

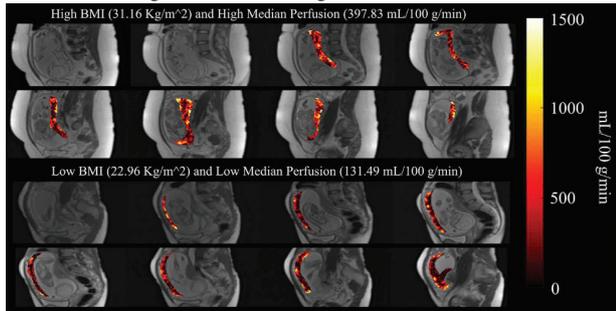
in 2<sup>nd</sup> Trimester Human Placenta. Daniel Seiter, Ruiming Chen, Kai Ludwig, Ante Zhu, Dinesh Shah, Sean Fain, Oliver Wieben, Kevin M Johnson\*. <sup>1</sup>UW-Madison, Madison, WI, United States; <sup>2</sup>GE Healthcare, Niskayuna, NY, United States.

**Introduction:** Perfusion magnetic resonance imaging (MRI) using arterial spin labeling (ASL) has shown value for noninvasive placental assessment. This study uses multislice velocity selective (VS)-ASL, allowing complete coverage and tagging of all vessels supplying blood to the placenta, to compare placental perfusion in early human pregnancy with clinical data.

**Methods:** A total of 74 patients (obese n=16, age=30.0 $\pm$ 2.8 yrs) were recruited and received MRI scans at gestational age (GA) 14- and 20-weeks. Clinical data such as BMI, blood pressure, and pregnancy outcomes were tracked. MRI was performed on a clinical 1.5T scanner in supine position. Two interleaved acquisitions of four 8 mm sagittal slices each with 16 tag/control pairs using a 2D single-shot fast spin echo readout were used for full placenta coverage. The order of slice acquisition was cycled to avoid post label delay bias. Placental perfusion (f, mL/100g/

min) was quantified by manually segmenting perfusion maps generated using Buxton's general kinetic model side by side with anatomical images. Correlation of median perfusion with clinical data listed was analyzed via linear regression. A Wilcoxon rank-sum test was used to compare the median perfusion between adverse and normal outcomes at both GAs.

**Results:** Of the 74 subjects, 16 patients (obese n=5) had adverse events including fetal growth restriction, gestational hypertension, preterm labor, gestational diabetes, or preeclampsia. Example perfusion maps overlaid on reference images are shown in Figure 1.



Regression analysis revealed a statistically significant positive relationship between perfusion and BMI at 20 weeks ( $R^2=0.099$ ,  $p=0.014$ ). Other relationships were not statistically significant. Comparisons between median perfusion of adverse and normal pregnancies were not statistically significant.

**Conclusion:** To our knowledge, this is the earliest measurement of perfusion in the human placenta using VS-ASL. Our analysis showed positive correlation with BMI and perfusion at 20 weeks, but no significant change in median perfusion pregnancies with adverse outcomes in early pregnancy. Possible explanations include perfusion compensation later in gestation in response to other placental restrictions (e.g. oxygen exchange). Further analysis is needed in larger and more uniform subjects with adverse outcomes. Analysis is ongoing to compare perfusion data with placental histopathology from delivery.

## O-110

**Placental NRF2 May Serve a Key Role in Maternal-Fetal Tolerance during Pregnancy.** *Kyunghee Hong*†, *Youn-Tae Kwak*, *Sribalashubashini Muralimanoharan*, *Carole R Mendelson*\*. *UT Southwestern Medical Center, Dallas, TX, United States.*

**Introduction:** A fundamental unanswered question in reproductive biology is: What protects the hemi-allogeneic fetus from rejection by the maternal immune system? We are testing the hypothesis that the multinucleated syncytiotrophoblast (SynT), formed by fusion of proliferative cytotrophoblasts (CytT) and covering the chorionic villi, may serve a critical role through production of immune modulators that act on the maternal decidua to protect the hemi-allogeneic fetus from rejection by the maternal immune system.

**Methods:** Human primary CytT and human trophoblast stem cells (hTSCs; provided by Drs. Okae and Arima, Sendai, Japan) cultured under conditions to promote CytT to SynT differentiation, or in a hypoxic environment, were analyzed at different times using RT-qPCR and Western blot. We also analyzed placentae of global *Nrf2* knockout (KO) vs. wild-type (WT) mice at 12.5 days post-coitum (dpc) for effects on immune modulator expression.

**Results:** We observed that genes involved in the induction and maintenance of immune tolerance were markedly upregulated upon CytT to SynT differentiation. These immune modulators include HMOX1, PD-L1, GDF15, and kynurenine pathway components, IDO1 and Ahr. Intriguingly, we discovered that the redox-regulated transcription factor NRF2 and co-regulated C/EBP $\beta$  and PPAR $\gamma$ , which serve critical roles in mouse labyrinthine trophoblast development, were also markedly induced during SynT differentiation. Notably, NRF2 knockdown prevented induction of C/EBP $\beta$ , PPAR $\gamma$  and the immune modulators, as well as induction of aromatase (CYP19A1), a key marker of SynT differentiation. ChIP-qPCR revealed that temporal induction of aromatase and the immune

modulators was associated with increased binding of endogenous NRF2 to putative response elements within their promoters, indicating that these transcription factors and immune modulators are direct downstream targets of NRF2. Consistent with our cell culture studies, placentas of global *Nrf2* KO mice at 12.5 dpc manifested a significant decrease in C/EBP $\beta$ , PPAR $\gamma$ , HMOX1 and Ahr mRNA, compared to those of WT mice. Importantly, NRF2 deficiency has been implicated in preeclampsia, a hypertensive disorder of pregnancy, associated with shallow implantation, inflammation and placental hypoxia. Notably, when hTSCs were cultured in a hypoxic (2% O $_2$ ) environment, the differentiation-associated induction of NRF2, C/EBP $\beta$ , aromatase and immune modulators was prevented.

**Conclusion:** Our compelling findings suggest that the O $_2$ -regulated transcription factors, NRF2, C/EBP $\beta$  and/or PPAR $\gamma$ , serve as key regulators of immune modulator expression during SynT differentiation. We propose that the immune modulators are secreted into maternal blood, or directly into the decidua, where they act on immune cells to maintain maternal tolerance to the hemi-allogeneic fetus during pregnancy. NIH-P01-HD087150

## O-111

**Premenstrual Dysphoric Disorder (PMDD) Is Associated with Estradiol-Dependent Aberrations in Cellular Ca $^{2+}$  Homeostasis and the Endoplasmic Reticulum Stress Response.** *Howard Li*†, <sup>1,2</sup> *Neelima Dubey*†, <sup>2</sup> *Jessica F Hoffman*†, <sup>2</sup> *David R Rubinow*, <sup>3</sup> *Peter J Schmidt*\*, <sup>2</sup> *David Goldman*\*, <sup>4</sup> *Yale School of Medicine, New Haven, CT, United States*; <sup>2</sup> *National Institute of Mental Health (NIMH), Bethesda, MD, United States*; <sup>3</sup> *UNC Chapel Hill, Chapel Hill, NC, United States*; <sup>4</sup> *National Institute of Alcohol Abuse and Alcoholism (NIAAA), Bethesda, MD, United States.*

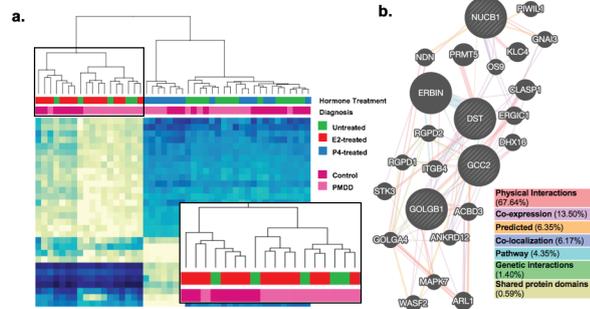
**Introduction:** Premenstrual Dysphoric Disorder (PMDD) is characterized by debilitating mood symptoms in the luteal phase of the menstrual cycle. Evidence suggests an etiology involving an abnormal response to ovarian steroids, but the molecular basis of a differential response to hormone remains poorly understood.

**Methods:** RNAseq analysis of lymphoblastoid cell lines (LCLs) from women with PMDD (n=10) and healthy controls (n=9) under untreated, estradiol-treated (E2) and progesterone-treated (P4) conditions.

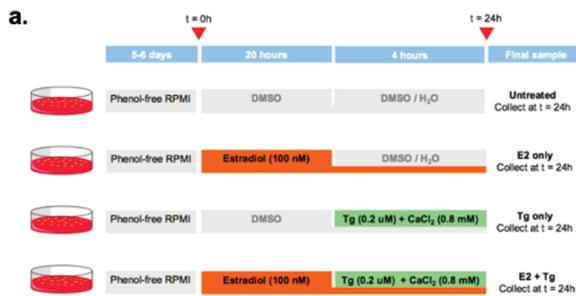
**Results:** Weighted gene correlation network analysis (WGCNA) identified 4 gene modules with significant diagnosis x hormone interactions, including 1 enriched for neuronal functions. Ontologic analysis of hub genes underlying neuronal enrichment signals revealed multiple pathways related to cellular Ca $^{2+}$  dynamics. Using differential expression analysis to compare transcriptional response to hormone, 1522 genes were differentially responsive to E2 (E2-DRGs) and 480 differentially responsive to P4 (P4-DRGs). Among top 10 E2-DRGs was a gene network (NUCB1, DST, GCC2, GOLGB1) involved in endoplasmic reticulum (ER)-Golgi function. qPCR validated a diagnosis x E2 interaction ( $F(1, 24)=7.01$ ,  $p=0.014$ ) in NUCB1, which regulates cellular Ca $^{2+}$  and the ER stress response (ERSR). We then used thapsigargin (Tg), which induces ER stress by disrupting cytoplasmic Ca $^{2+}$ , to test whether E2 induces differences in Ca $^{2+}$  homeostasis and ERSR. PMDD LCLs had a non-significant 27% decrease in Tg-induced XBP1 splicing (a measure of ERSR) compared to Controls. Addition of E2 resulted in a significant 38% decreased response ( $p=0.005$ ), with a significant diagnosis x treatment interaction ( $F(3,33)=3.51$ ,  $p=0.026$ ).

**Conclusion:** E2-dependent aberrations in cellular Ca $^{2+}$  handling and ER stress may contribute to the pathophysiology of PMDD.

**RNAseq Analysis of Control vs. PMDD transcriptomes**



**Thapsigargin challenge assays**



**O-112**

**Examination of Androgen Effects on Female Reproductive Axis: Hypothalamus-Pituitary-Ovary.** Sheng Wu\*, Olubusayo Awe†, Mingxiao Feng†, James Segars. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Hyperandrogenemia is a salient feature in many women who suffer irregular menses, oligo/anovulation and infertility, including women with polycystic ovary syndrome (PCOS), classic and non-classic (late-onset) congenital adrenal hyperplasia (CAH), exogenous testosterone treatment in female to male transsexuals, exogenous androgen use (body builders), or environmental toxicity. Women with elevated androgen have restored ovulation after long-term treatment with the competitive AR antagonist, flutamide, it implicates that androgen and its receptor (AR) plays a critical role in reproductive function, under both physiological and pathophysiological conditions. Animals with intact AR treated with dihydrotestosterone (DHT) have impaired estrous cyclicity, anovulation and infertility. However, what are the targets of androgen action and the mechanism underpinning the reproductive dysfunction is not known.

**Methods:** Since reproduction is tightly controlled by hypothalamus-pituitary-gonadal axis, our laboratory conditionally knock out the AR gene in AR<sup>fl</sup> mice (separately knockout by synapsin, aGSU and Cyp17 specific promoter driven Cre) in central nervous system (SynARKO), pituitary gonadotropes (PitARKO) and ovarian theca cells (ThARKO) specifically to explore the direct role of elevated androgen on the development of

reproductive dysfunction in female mice. Female mice were treated with 4mm DHT pellet for 4 weeks and mated with proven fertile males. Litters and pups were recorded. Circulated hormone levels were measured by Elisa or Millipore Luminex 200.

**Results:** Conditional knockout AR in ovarian theca cells, or pituitary gonadotropic cells partially prevented hyperandrogenemia induced acyclicity and infertility. We observed that conditional knockout AR in central nervous system in female mice (SynARKO) had no effect on normal puberty and reproductive function at physiological androgen levels. Puberty was examined by vaginal opening (27.6±2.7 vs 25.3±1.5 day post birth (DPB)) and by first estrus (38.1±1.9 vs 39.8±2.7 DPB) in SynARKO and wild type (WT) mice accordingly. Furthermore, LH, testosterone and estrogen levels were not altered. However, FSH levels were significantly reduced at estrous stage in SynARKO mice compared to WT. SynARKO mice showed consistent diestrus, and there was barely detectable corpora lutea in the ovaries after 4 weeks treatment with dihydrotestosterone (DHT). During 90 days mating, we did not observe any litters or pups in SynARKO-DHT female mice.

**Conclusion:** These findings highlight that ovarian theca and extra ovarian regulator gonadotropic AR (not central nervous AR) plays important roles in reproductive pathophysiology of hyperandrogenemia.

**O-113**

**Disparities in Seeking Infertility Care: Data from the 2017-2019 CDC National Survey of Family Growth.** Lauren E Barrison†, Allison R Kummnick†, Veronica Gomez-Lobo, Jaqueline Y Maher\*, MedStar Washington Hospital Center/Georgetown University Hospital, Washington, DC, United States; National Institutes of Health, Bethesda, MD, United States.

**Introduction:** Infertility in the US is a public health concern affecting millions of women across diverse backgrounds. Despite a National Public Health Action Plan that includes improving access to and eliminating disparities in infertility care, inequities persist. This study examined how race, education, and insurance contribute to differences in infertility care. **Methods:** We used data from the 2017-2019 National Survey of Family Growth Female Questionnaire (N=6141). We further analyzed those who reported speaking to a medical provider about help getting pregnant (N=478) and those who underwent infertility testing/treatment (N=139). Frequencies, Mann-Whitney tests, and multiple logistic regression models were utilized, with a p<0.05 significance cutoff.

**Results:** Of the 6141 respondents, within each race 10% White, 6.8% Hispanic, 6.7% Black, and 8.5% other race (N=478) reported speaking to a medical provider about help getting pregnant. Black and Hispanic women were less likely to speak to a provider about help getting pregnant compared to White women (OR 0.64, p=.001 and OR 0.65, p=.0004, respectively). Logistic regression models showed race to be a significant predictor of speaking to a provider, even when controlling for age (p=.016) and insurance status (p<.0001). Those with non-private insurance were less likely to speak to a provider than those with private insurance (p<.0001), even when controlling for age and race. In women over 30 years, those with 5+ years of higher education were more likely to talk to a provider than those with a bachelor degree (p<.05). Of the 478 who spoke to a provider, 57.3% were White, 20.9% Hispanic, 17.2% Black, and 4.6% other race. Among these participants there were no differences in infertility rate by race, insurance status, or insurance type (private vs non-private). There were no differences in how long participants tried to conceive prior to seeking infertility care by race, insurance type, or education level. From the 139 who sought infertility care and underwent testing/treatment, 50.4% were White, 15.1% Black, 30.2% Hispanic, and 4.3% other race. Treatment included ovulation induction, tubal surgery, intrauterine insemination, and IVF. There were no differences by race, insurance status, or insurance type in undergoing infertility testing/treatment.

**Conclusion:** We found significant differences in speaking to a provider about infertility by race and insurance type. However, it was encouraging to find that once this initial barrier was confronted no significant differences in receiving infertility testing/treatment persisted. Our findings suggest that future efforts to decrease infertility care disparities should target the initial discussion of infertility.

## O-114

**Normal Weight Women with Polycystic Ovary Syndrome (PCOS) Exhibit Oxidative Stress in Response to Saturated Fat Ingestion Even in the Absence of Abdominal Adiposity (AA).** Frank González\*,<sup>1</sup>Robert V. Considine,<sup>2</sup> Ola A. Abdelhadi,<sup>2</sup> Jiaping Xue,<sup>1</sup> Anthony J. Acton.<sup>2</sup> <sup>1</sup>University of Illinois at Chicago College of Medicine, Chicago, IL, United States; <sup>2</sup>Indiana University School of Medicine, Indianapolis, IN, United States.**Introduction:** We evaluated the effect of saturated fat ingestion on ROS generation and p47<sup>phox</sup> gene expression from mononuclear cells (MNC) of normal weight (NW) women with PCOS with and without AA, compared with NW body composition-matched ovulatory controls; and their relationship with insulin sensitivity and HCG-stimulated ovarian androgen secretion.**Methods:** We studied 16 NW women with PCOS (8 with & 8 without AA) diagnosed on the basis of oligo-amenorrhea and hyperandrogenemia and 14 NW ovulatory controls (7 with & 7 without AA) ages 18-40. AA was defined as the % ratio of truncal fat to total body fat measured by DEXA that was 2SD above the mean of controls without AA. ROS generation was measured by chemiluminescence, and p47<sup>phox</sup> mRNA and protein content was quantified by RT-PCR and Western blotting in MNC isolated from blood samples drawn fasting and 2, 3 and 5 hours after dairy cream ingestion (100 ml). Androgens were measured by RIA from blood samples drawn fasting and 24, 48 and 96 hours after HCG administration (5000 IU). Insulin sensitivity was derived by IS<sub>OGTT</sub>.**Results:** Compared with controls, the change from baseline (%) in prooxidant markers was greater ( $p < 0.0004$ ) in both PCOS groups at 2 hours (ROS generation - with AA:  $76 \pm 13$  vs.  $-2 \pm 8$ , without AA:  $74 \pm 9$  vs.  $-6 \pm 9$ ; p47<sup>phox</sup> mRNA - with AA:  $26 \pm 5$  vs.  $-4 \pm 3$ , without AA:  $26 \pm 6$  vs.  $-5 \pm 3$ ; p47<sup>phox</sup> protein - with AA:  $32 \pm 4$  vs.  $2 \pm 3$ , without AA:  $26 \pm 3$  vs.  $-5 \pm 3$ ) and 3 hours (ROS generation - with AA:  $55 \pm 7$  vs.  $-1 \pm 8$ , without AA:  $76 \pm 8$  vs.  $-17 \pm 7$ ; p47<sup>phox</sup> mRNA - with AA:  $31 \pm 6$  vs.  $-5 \pm 4$ , without AA:  $29 \pm 6$  vs.  $-9 \pm 5$ ; p47<sup>phox</sup> protein - with AA:  $34 \pm 4$  vs.  $2 \pm 3$ , without AA:  $28 \pm 4$  vs.  $-5 \pm 3$ ), and returned to baseline at 5 hours (ROS generation - with AA:  $2 \pm 2$  vs.  $-1 \pm 3$ , without AA:  $1 \pm 1$  vs.  $-2 \pm 2$ ; p47<sup>phox</sup> mRNA - with AA:  $1 \pm 1$  vs.  $-1 \pm 1$ , without AA:  $1 \pm 1$  vs.  $-1 \pm 1$ ; p47<sup>phox</sup> protein - with AA:  $1 \pm 1$  vs.  $-1 \pm 1$ , without AA:  $1 \pm 1$  vs.  $-1 \pm 1$ ). Compared with controls, both PCOS groups exhibited greater ( $p < 0.05$ ) HCG-stimulated area under the curve (AUC) for testosterone (T) (with AA:  $6466 \pm 774$  vs.  $3858 \pm 531$ , without AA:  $6157 \pm 1026$  vs.  $3064 \pm 587$ ) and androstenedione (A) (with AA:  $501 \pm 35$  vs.  $307 \pm 24$ , without AA:  $516 \pm 38$  vs.  $300 \pm 36$ ). For the combined groups, lipid-stimulated incremental AUC (iAUC) for ROS generation was positively correlated with HCG-stimulated androgen AUC (T:  $r = 0.54$ ,  $p < 0.005$ ; A:  $r = 0.72$ ,  $p < 0.0001$ ). IS<sub>OGTT</sub> was negatively correlated with lipid-stimulated iAUC for ROS generation ( $r = -0.42$ ,  $p < 0.03$ ), and p47<sup>phox</sup> gene expression (mRNA:  $r = -0.50$ ,  $p < 0.009$ ; protein:  $r = -0.59$ ,  $p < 0.002$ ). **Conclusion:** Lipid-stimulated ROS generation and p47<sup>phox</sup> gene expression are increased in PCOS independent of AA. We speculate that this prooxidant phenomenon in PCOS promotes hyperandrogenism and insulin resistance, and is further perpetuated by AA.

## O-115

**Preferred Timing of Efficacy of a New Therapeutic Candidate, Rytvela, for Prevention of Inflammation-Induced Preterm Birth and Fetal Growth Restriction.** Sarah-Eve Loisel<sup>†,1,2</sup> Renay Poupert,<sup>2</sup> Xin Hou\*,<sup>1</sup> Mathieu Nadeau-Vallée,<sup>2</sup> France Côté<sup>†,1,2</sup> Tiffany Habelrih<sup>†,1,2</sup> Christiane Quiniou\*,<sup>1</sup> Sylvain Chemtob\*,<sup>1,2</sup> <sup>1</sup>CHU Sainte-Justine, Montreal, QC, Canada; <sup>2</sup>Université de Montréal, Montreal, QC, Canada.**Introduction:** Over 2.5 million newborns die yearly and more than 80% of them are of low birthweight (LBW). LBW is a complex clinical entity composed of fetal growth restriction (FGR) and preterm birth (PTB). Surviving newborns face a higher risk of perinatal morbidities (such as bronchopulmonary dysplasia, necrotizing enterocolitis, cerebral palsy) due to the devastating effects of utero-fetal inflammation on vulnerable fetal organs. There is currently no efficient treatment for antenatal fetal protection. Among many proinflammatory mediators, IL-1 $\beta$  stands significantly in causing detrimental effects. The host lab has recently designed an IL-1 receptor antagonist, Rytvela, found to be effective

against PTB when given as a prophylaxis. The study objective is to further characterize Rytvela by evaluating its efficiency in preventing PTB and FGR when administered after inflammatory insult, thereby evaluating the optimal treatment timing and duration.

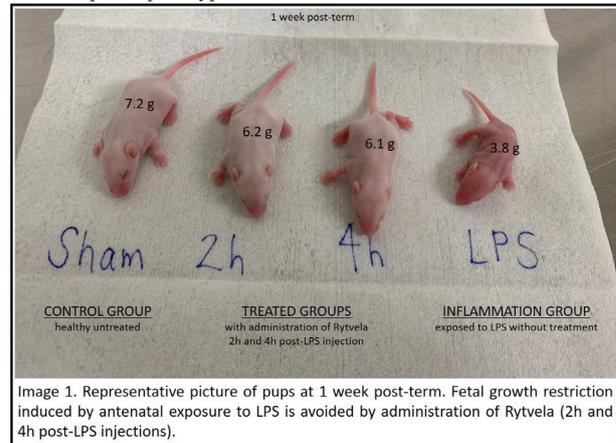
**Methods:** Pregnant CD-1 mice were injected with LPS (10  $\mu$ g i.p.) on gestational day 16.5 (G16.5). Rytvela (2 mg/kg/day s.c.) was administered at different time points (0.5, 1, 2, 4, 6 h) post-LPS or for different treatment durations (24, 36, 48 h). Prematurity rate (<G18.5), gestational length, newborn survival and weight were assessed. Histological analyses of the fetal lungs, intestines, and brain were performed.**Results:** Rytvela decreased LPS-induced PTB rate from 90% to 30% when administered 0.5 h post-LPS ( $p < 0.01$ ). Newborn survival, growth and weight were considerably improved with Rytvela administered up to 4 h post-LPS (see Image 1). Histological analysis revealed that Rytvela preserved fetal lung, intestine, and brain tissue integrity and growth when administered up to 6 h post-LPS. A 24 h treatment with Rytvela revealed a 2-fold increase in fetal survival whereas a 48 h treatment increased survival by 4-fold ( $p < 0.05$ ).**Conclusion:** Rytvela is efficient in preventing PTB and FGR when administered post-inflammatory insult. It revealed in murine model a maximum effect when administered 0.5 h post-LPS, for a period of 48 h; but Rytvela had significant benefits even when administered 6 h post-LPS, for a period of 24 h. Rytvela improved birth outcome by preserving fetal tissue integrity and growth. Hence, Rytvela could be a promising new and safe therapeutic prototype for treatment of PTB and FGR.

Image 1. Representative picture of pups at 1 week post-term. Fetal growth restriction induced by antenatal exposure to LPS is avoided by administration of Rytvela (2h and 4h post-LPS injections).

## O-116

**Comparative Adverse Effects of Antenatal Glucocorticoid Formulations: Studies in the Chicken Embryo.** T A Garrud<sup>†,1</sup>N Teulings<sup>†,1</sup> F G Conlon<sup>†,1</sup> W Tong<sup>†,1</sup> S G Ford<sup>†,1</sup> Y Niu<sup>†,1</sup> L M Nicholas<sup>†,1</sup> J B Derks\*,<sup>2</sup> S E Ozanne\*,<sup>2</sup> D A Giussani\*.<sup>1</sup> <sup>1</sup>University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>University Medical Centre, Utrecht, Netherlands.**Introduction:** Potential adverse side-effects of antenatal therapy with synthetic glucocorticoids (sGC) in threatened preterm birth have raised concern. Often different sGC with varying formulations are used. Here, we investigated whether different sGC formulations have differential effects on the developing cardiovascular system. We stripped as many confounding factors as possible to isolate direct mechanisms by using the chicken embryo. This model system permits investigation of the direct effects of therapy on the developing heart and vasculature, independent of effects on the mother and the placenta.**Methods:** Fertilised eggs were dosed at day 14 (term is 21 days; ~0.67 of gestation) with 0.1 mg/kg of sGC or water vehicle. There were 4 groups: Dexamethasone-phosphate (Dex), Betamethasone (Beta) acetate/phosphate (Celestone), Beta-phosphate (B-Phos) or Beta-acetate (B-Ace) (See Table 1). At day 19, cardiac and peripheral vascular function were determined (Langendorff and myography). In different cohorts, hearts were perfusion fixed for stereological analysis or frozen for molecular

analysis. Cardiomyocytes were also isolated and studied. Data were compared via Two-WAY ANOVA+Tukey.  $P < 0.05$  was considered significant.

**Results:** Treatment with any sGC promoted growth restriction, which was more severe following Beta treatment due to the acetate formulation (Table 1). Cardiac function was impaired by all sGC, again with Beta being more severe. Dex treatment resulted in enhanced peripheral constrictor reactivity, cardiomyocyte hypertrophy and induced oxidative stress, caspase 3-mediated apoptosis, with p38-mediated reduced proliferation. In contrast, Beta treatments impaired peripheral vasodilator reactivity, reduced total cardiomyocyte number, promoted excessive GR activation due to loss of negative feedback, and led to p53-mediated apoptosis with reduced cardiomyocyte proliferation. Beta acetate shared the loss of GR negative feedback and enhanced p53 expression, whereas Beta phosphate did not.

**Conclusion:** The data support direct and divergent adverse effects of sGC used in human clinical practise on the developing cardiovascular system. The work offers insight into mechanisms underlying detrimental effects, providing a platform to modify current clinical antenatal glucocorticoid therapy and make it safer for the treatment of the preterm baby.

*Support: The Wellcome Trust*

Outcome	Control	Dex	Celestone	B-Phos	B-Ace
Embryo weight (% egg weight)	39.2 ± 0.4 a	31.4 ± 0.8 b	18.3 ± 0.6 c	27.5 ± 1.3 d	21.5 ± 1.6 c
LV Developed Pressure (mmHg)	21.9 ± 2.2 a	16.3 ± 1.7 ab	9.5 ± 1.2 b	10.4 ± 1.2 b	12.8 ± 1.6 b
LV End Diastolic Pressure (mmHg)	6.8 ± 0.9 a	13.4 ± 1.5 b	18.9 ± 1.5 b	15.2 ± 3.9 b	18.5 ± 3.1 b
Cardiomyocyte Volume (µm)	471.1 ± 78.6 a	1519.0 ± 92.4 b	414.2 ± 54.7 a	516.0 ± 98.6 a	488.7 ± 107.6 a
Protein Carbonylation (% control)	100 ± 10.4 ab	130.4 ± 5.8 a	106.2 ± 8.0 ab	71.37 ± 5.1 bc	57.16 ± 8.8 c
P53 mRNA expression (% control)	100 ± 36 a	42.25 ± 18 a	457 ± 18 ab	608 ± 180 ab	852 ± 240 b

**Table 1. Cardiovascular Outcomes at embryonic day 19** All figures are mean ± SEM. Different letters are significantly different ( $P < 0.05$ ), one-way ANOVA with Tukey test. N = 8-12

## O-117

**Maternal Leukocyte DNA Methylation during Asymptomatic Pregnancy and Future Spontaneous Preterm Birth among Black American Women.** Shannon Gillespie, Chenggong Han, Zilu Liu†, Cindy Anderson, Shili Lin. *The Ohio State University, Columbus, OH, United States.*

**Introduction:** Preterm birth (PTB) is linked to more than one million deaths each year. In the U.S., 10% of births are preterm and Black women are at 1.5 times the risk compared to White women. Prevention is key. Yet, clinical tools guiding risk identification and intervention allocation are lacking. We aimed to identify markers of maternal leukocyte DNA methylation (i.e., epigenetic modifications that can affect gene expression) predictive of future spontaneous birth timing among Black American women.

**Methods:** Among a prospective cohort ( $n=96$ ), K2EDTA-treated whole blood was collected at 30±2 weeks of asymptomatic pregnancy and women were followed to birth. Leukocytes were isolated and DNA extracted. Using a nested case-control design, epigenome-wide DNA methylation was quantified using the Infinium MethylationEPIC BeadChip kit among five women with spontaneous PTB and 11 women with spontaneous full term birth matched for age, education, and nulliparity. Cases and controls were compared to identify differentially methylated loci (DMLs) and regions (DMRs). Pathway analyses were completed. Methylation at target priority amplicons was quantified by MassARRAY among all cohort members with spontaneous labor onset ( $n=50$ ). Kernel distance-covariance analyses estimated associations among average amplicon methylation and birth timing in unadjusted and adjusted (for maternal age, education, home ownership, insurance status, pre-pregnancy body mass index, smoking status, sleep quality, gravidity, parity, fetal sex, gestational hypertension, preeclampsia, and history of preterm birth) models.

**Results:** In epigenome-wide case-control comparison ( $\alpha=0.01$ ), 598, 3120, and 9672 DMLs were identified using the DEseq, Minfi, and BCurve methods, respectively. The three methods identified 38 overlapping DMLs (Relation to CpG island: 4 shelf, 7 shore, 11 island, 16 open sea). Minfi and

BCurve identified 3904 and 4192 DMRs, respectively. Pathway analysis of Minfi-identified DMLs predicted significant dysregulation of PI3K events in ERBB4 and ERBB2 signaling, G alpha signaling events, and NRAGE signals of death through JNK ( $p < 0.05$ ). Together, average beta values of the 18 target amplicons chosen for their relevance to immune-related parameters significantly predicted spontaneous birth timing in unadjusted ( $p=0.004$ ) and adjusted ( $p=0.044$ ) models.

**Conclusion:** To our knowledge, this study is the first to link maternal leukocyte DNA methylation during asymptomatic pregnancy to future spontaneous birth timing using epigenome-wide methods paired with targeted second method validation. This study will support the design of screening and clinical decision-making tools for the targeted prevention of inflammatory spontaneous PTB among Black American women.

## O-118

**Impact of Progesterone on Mechanism of Preterm Premature Rupture of Membrane.** Heejoong Lee,<sup>1</sup> Banghyun Lee\*<sup>2</sup> *The Catholic University of Korea, College of Medicine, Uijerngbu, Korea, Republic of;* <sup>2</sup>*Inha University, College of Medicine, Incheon, Korea, Republic of.*

**Introduction:** The role of the TLR/NLR family of proteins in maintaining placental homeostasis and contribution of altered TLR/NLR signaling to pathological states such as chorioamnionitis and PPRM remain understudied. Only a few researchers have been reported about the role of progesterone and its mechanism related to fetal membrane weakening and PPRM. Therefore, this study aim to investigate roles and molecular mechanisms of progesterone in fetal membranes under basal condition and stimuli with TLR/NLR agonists.

**Methods:** Fetal amniotic membranes were collected from uncomplicated pregnant women who had an elective cesarean at term prior to the onset of labor ( $n = 30$ ). Human primary amnion epithelial cells (hAECs) were pretreated with/without medroxyprogesterone acetate (MPA, 10 or 50µM) for 24 hours. After, TLRs/NLRs agonist alone or combination with MPA and agonist were treated under each of the following bacterial agonists: PDG for TLR2; MDP for Nod2. Expressions of TLRs/NLRs and their effects on pro-inflammatory cytokines in hAECs and effects of progesterone were evaluated with Quantitative real-time RT-PCR.

**Results:** Expressions of TLR2 and Nod2 genes were increased with treatment of each specific agonist. Co-stimulation of progesterone decreased expressions of TLR2 and Nod2 genes. Stimulation of specific agonists for TLR2 and Nod2 increased expressions of IL-1β and IL-8 genes. Co-stimulation of each specific agonist and progesterone decreased expressions of IL-1β and IL-8 genes. Expressions of TLR2 genes were decreased by progesterone stimulation alone compared with no treatment, whereas expressions of Nod2 genes were not changed. Expressions of IL-1β and IL-8 genes in treatment of all agonists were not changed by progesterone stimulation alone compared with no treatment

**Conclusion:** This study demonstrate progesterone is protective against PPRM through anti-inflammatory action.

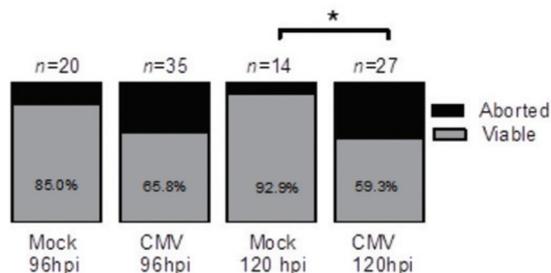
## O-119

**A Novel Murine Model of Cytomegalovirus Infection in Pregnancy.** Angela Shaddeau†,<sup>1</sup> Gregory Kirschen†,<sup>1</sup> Anna Chudnovets,<sup>1</sup> Quan Na,<sup>1</sup> Ayan Ghosh,<sup>2</sup> Halli Miller,<sup>2</sup> Jun Lei,<sup>1</sup> Ravit Boger,<sup>2</sup> Andrew Thagard,<sup>3</sup> Karen Racicot,<sup>4</sup> Irina Burd\*<sup>1</sup> *<sup>1</sup>Johns Hopkins School of Medicine, Baltimore, MD, United States; <sup>2</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>3</sup>Naval Medical Center Portsmouth, Portsmouth, VA, United States; <sup>4</sup>Michigan State University, East Lansing, MI, United States.*

**Introduction:** Cytomegalovirus (CMV) is a ubiquitous bloodborne virus that can cross the placenta and cause devastating effects in pregnancy, such as miscarriage or permanent neurological/neurocognitive effects. Yet, modeling of CMV disease in pregnancy, including vertical transmission and fetal effects, has been challenging in immunocompetent mice due to lack of transplacental transmission. We hypothesized that by utilizing intrauterine (IU) inoculation of murine CMV (mCMV) into pregnant, outbred, immunocompetent mice, bypassing systemic effects, we could develop a more representative model for CMV disease in pregnancy than what exists.

**Methods:** Timed-pregnant, CD1 mice were inoculated with either mCMV in doses ranging from  $5 \times 10^5$  to  $1 \times 10^7$  or vehicle (mock injection of phosphate buffered saline) by IU inoculation at embryonic day (E)10. Dams were euthanized at 96 or 120 hours post-infection (hpi) for quantification of fetal viability and tissue harvest. Immunocards for 96 immunomarkers were run on placentas. mCMV viral titers were conducted on uterine, placental, and fetal brain tissue. Placental and fetal brain tissue was evaluated for local mCMV antigens with immunohistochemistry (IHC) staining for pp65.

**Results:** A significant decrease in fetal viability was noted at 120 hpi after IU inoculation of mCMV compared with mock inoculation ( $p < 0.05$ ; Fig). These fetal miscarriages occurred without notable proinflammatory changes in placenta at 96 hpi. Viral specific antigens were observed in placental and fetal periventricular brain areas by IHC analysis.



**Figure:** mCMV intrauterine injection at E10 at a dosage of  $5 \times 10^5$ . Tissue harvest at 96 (E14) and 120 (E15) hpi. A significant decrease in fetal viability was observed at 120 hpi in mCMV compared to vehicle-infected controls. ( $p < 0.05$ )

**Conclusion:** Placental and fetal CMV infections occur following IU inoculation of mCMV in immunocompetent, outbred mice. We created a novel model which demonstrates CMV passage through placenta leading to fetal death, consistent with effects in humans. This model provides a platform for studying CMV vertical transmission as well as possible interventions to prevent fetal sequelae.

## O-120

### Psychological Distress during Pregnancy and Adverse Maternal and Perinatal Health Outcomes; the Role of Socioeconomic Status.

Leonie A. Daalderop<sup>†</sup>,<sup>1</sup> Jacqueline Legendijk<sup>†</sup>,<sup>1</sup> Eric A.P. Steegers\*,<sup>1</sup> Hanan El Marroun\*,<sup>1,2</sup> Anke G. Posthumus<sup>†</sup>.<sup>1</sup> <sup>1</sup>Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Erasmus School of Social and Behavioural Sciences, Erasmus University Rotterdam, Rotterdam, Netherlands.

**Introduction:** Having a low socioeconomic status (SES) may amplify the negative impact of psychological distress on maternal and perinatal health. We have therefore investigated the association between SES (conceptualised by educational level) and psychological distress during pregnancy. And, we examined the association between psychological distress and maternal and perinatal health among different SES groups.

**Methods:** This study was embedded in the Generation R study. The primary outcome was the prevalence of psychopathology and stress, measured with self-reported questionnaires. Differences between SES groups were tested with the chi-square test. Secondary outcomes were preterm delivery, small for gestational age, birth weight, gestational age, foetal growth restriction, pregnancy-induced hypertension, preeclampsia, and mode of delivery. Linear and logistic regression analyses were used to examine the associations between psychopathology/stress and the secondary outcomes. A principal component analysis was performed to reduce the number of predicting variables tested. A statistically significant association ( $p$ -value  $< 0.05$ ) between the principal component (PC) and one of the secondary outcomes was used as a benchmark to perform additional analyses for individual psychopathology and stress measures.

**Results:** 7,228 women were included in this study. Compared to women with a high SES, we found that women with a low SES experience

symptoms of psychopathology or stress twice as often. We found no associations between psychopathology or stress and any of the secondary outcomes among women with a low SES. Among women with a high SES, the psychopathology PC was positively associated with preterm birth (OR 1.07, 95%-CI 1.03;1.12,  $p$ -value  $< 0.01$ ) and negatively with birth weight ( $\beta$  -18, 95%-CI -24;-12,  $p$ -value  $< 0.01$ ). The stress PC showed a positive association with preterm birth (OR 1.14, 95%- CI 1.04;1.25,  $p$ -value 0.01) and negative associations with birth weight ( $\beta$  -48, 95%-CI -59;-37,  $p$ -value  $< 0.01$ ) and gestational age ( $\beta$  -0.07, 95%-CI -0.10;-0.04,  $p$ -value  $< 0.01$ ).

**Conclusion:** In this study, psychological distress during pregnancy was associated with perinatal health outcomes among women with a high SES, but not among those with a low SES. To clarify the complex associations between SES, psychological distress, and maternal and perinatal health, information regarding mental health should be included in standard national data.

## O-121

### MAP4K4: Identification of a Novel Candidate Gene Using Prenatal Exome Sequencing Data and Functional Modeling in Zebrafish.

Neeta L. Vora,<sup>1</sup> John Griffin<sup>†</sup>,<sup>2</sup> Kelly Gilmore,<sup>1</sup> Julie K. Holsclaw<sup>†</sup>,<sup>2</sup> Elizabeth Bhoj,<sup>3</sup> Erica E. Davis\*,<sup>4</sup> <sup>1</sup>UNC-Chapel Hill, Chapel Hill, NC, United States; <sup>2</sup>Duke University, Durham, NC, United States; <sup>3</sup>Children's Hospital of Pennsylvania, Philadelphia, PA, United States; <sup>4</sup>Northwestern University, Chicago, NC, United States.

**Introduction:** Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4 (MAP4K4) is an activator of the JNK pathway, which controls vital cellular processes, including embryonic development, proliferation, and apoptosis. In 2017, our group reported a de novo nonsense variant in MAP4K4 in a fetus with hypoplastic left heart and fused kidneys that was identified as part of our ongoing trio-based exome sequencing study. Query of MAP4K4 in the data sharing platform, GeneMatcher<sup>TM</sup>, identified another seven individuals from four families who harbor variants in MAP4K4. These individuals display dysmorphic facial features, neurologic dysfunction, cardiac anomalies and all survived past the neonatal period.

**Methods:** To test the functional relevance of MAP4K4 to patient phenotypes, we suppressed the zebrafish ortholog of MAP4K4 using morpholino antisense oligonucleotides. We examined renal, cardiac, and craniofacial abnormalities in zebrafish larvae, all of which have anatomical surrogates. We performed phenotyping during the first five days of development.

**Results:** We established knockdown efficiency of MAP4K4 morpholino and tested whether we could detect a dose-dependent phenotypic response. Depletion of MAP4K4 and assessment of renal tubule integrity using NaK-ATPase immunostaining did not produce a significant phenotype. However, evaluation of zebrafish larvae expressing a cartilage-specific transgene (*-1.4coll1a1:egfp*) displayed a dose-dependent increase in the angle of the ceratohyal cartilage, suggestive of altered craniofacial patterning. Cardiac structure was also significantly altered with atrial but not ventricular size significantly enlarged in larvae with depleted MAP4K4. Heart beat per minute was also significantly lower in the morpholino-injected batches compared to controls.

**Conclusion:** Our functional data, combined with a small cohort of individuals harboring rare MAP4K4 variants, support the candidacy of this signaling effector as a contributor to early developmental processes impacting the heart and craniofacial cartilage morphogenesis. Our ongoing work to test variant effect will elucidate the pathomechanism of this rare syndrome.

## O-122

**An Aberrant Endothelial Cell Response to Flow: Mechanistic Implications for Congenital Heart Defects in the Feto-Placental Unit.** Yalda Afshar,<sup>1</sup> Anhyo Jeong,<sup>1</sup> Christine Jang,<sup>1</sup> Gary Satou,<sup>1</sup> Mark Sklansky,<sup>1</sup> M. Luisa Iruela-Arispe,<sup>2</sup> <sup>1</sup>University of California, Los Angeles; David Geffen School of Medicine, Los Angeles, CA, United States; <sup>2</sup>Northwestern University, Chicago, IL, United States.

**Introduction:** Congenital heart defects (CHDs) are the most common cause of congenital anomalies. Among CHD, single ventricle (SV) phenotypes have the worst clinical prognosis with lifetime morbidity related to their vascular sequelae. We hypothesize that SV CHD emerges from maternal-fetal genetic predispositions coupled with abnormal environmental (flow) conditions that impact the fetal endothelial transcriptome and phenotype.

**Methods:** To understand and target the mechanisms underlying the prenatal vascular phenotype in SV CHD, we integrate data through the life course of these patients -- prenatal and postnatal. At delivery, primary endothelial cells were isolated from umbilical cords (n=20) of SV CHD fetuses. Cells were plated in an Ibidi monolayer under static versus laminar shear stress (15 dynes/cm<sup>2</sup> x 72-hours) to recapitulate *in vivo* conditions, and compared to controls (n=20). Cells were fixed so that deep cellular phenotyping and immunofluorescence for VE-cadherin, cytoskeleton, and Notch1 was done. Cell shape, angle relative to flow, and Notch1 polarization was quantitated. Global gene expression profiling by RNA-sequencing (RNAseq), assay for transposase-accessible chromatin-sequencing (ATACseq), to define open regions of chromatin, was conducted.

**Results:** Endothelial cells derived from CHD (versus controls) have perturbed responses to flow. SV endothelial cells demonstrate longer elongation factor (length cell/width cell), an abnormal angle relative to flow, abnormal adherens junctions, and lack of Notch1 polarization. The umbilical cord Doppler indices in these fetuses demonstrate that the cerebroplacental ratio (CRP, middle cerebral artery pulsatility index (MCA-PI) to umbilical artery-PI (UAPI) ratio) is lower in SV CHD versus controls (p<0.05), which correlates to the cellular findings (r=0.7, p=0.01). We define signaling pathways and gene networks that are altered in SV CHD. Global transcriptomics demonstrate significant changes in FABP4/5, APLN, DLL4, and FLT4 that are upregulated in SV CHD; whereas NOS3, BMP2, and ADAMTS1 are attenuated. ATACseq uncovers distinct regulatory elements of Notch1 open reading frame in SV CHD and motif enrichment at distal accessible chromatin regions as defined by SV CHD: Zic3, Tcf21, and Nkx2-5.

**Conclusion:** We define an aberrant endothelial cell population in the prenatal vascular phenotype of SV CHD. These endothelial cells possess distinctive ontogeny, gene expression patterns, and functional characteristics altered in SV CHD and point to sequelae of vascular morbidity.

## O-123

**Measuring *In Vivo* Arterial Stiffness in Fetal Life.** Nima Moghaddas<sup>†</sup>,<sup>1</sup> Beth J Allison,<sup>2</sup> Youguo Niu,<sup>1</sup> Kimberley J Botting,<sup>1</sup> Carmel M McEniery,<sup>1</sup> Dino A Giussani.<sup>1</sup> <sup>1</sup>University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>Monash University, Melbourne, Australia.

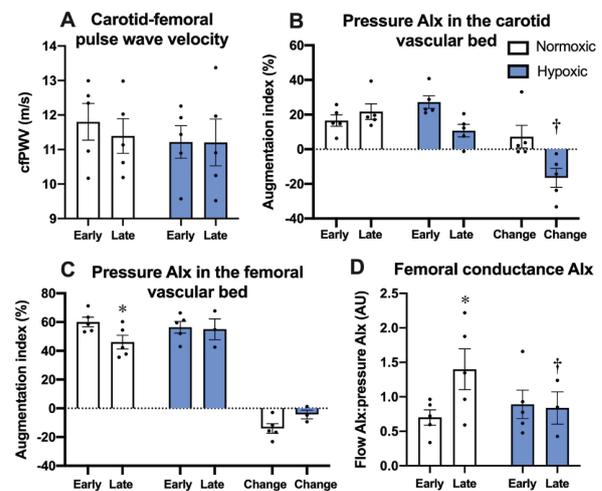
**Introduction:** Arterial stiffness is an independent predictor of cardiovascular disease and it is assessed clinically in patients by measuring the carotid-femoral pulse wave velocity (cfPWV; Laurent et al. *Hypert* 37:1236, 2001). To date, arterial stiffness has been impossible to measure in fetal life. By surgically implanting Transonic flow probes in the carotid and femoral vascular beds in fetal sheep, here we have developed novel technology to determine how fetal arterial stiffness is affected *in vivo* by advancing gestation and hypoxic pregnancy for the first time.

**Methods:** Under general anaesthesia, 10 fetal sheep were instrumented with flow probes and catheters in the carotid and femoral arteries at 116±2 days of gestation (dGA; term ~145 days). Following recovery, at 123±2, pregnancies were randomly exposed to normoxia (n=5) or hypoxia (10%O<sub>2</sub>, n=5) using isobaric chambers for 10 days. Carotid to femoral foot-to-foot transit times were obtained from 3 consecutive days of flow recordings before and at the end of treatment using the maximum point of

the second derivative (Chiu et al. *Am Heart J* 121:1460, 1991). Waveforms were analysed from 10 cardiac cycles every 5 min for 1 morning hour. Crown rump length (CRL) data from the same sheep breed (Fowden et al. *Q J Exp Physiol* 74: 703, 1989) were divided by transit times to yield fetal cfPWV. Simultaneous pressure and flow traces were analysed to calculate the pressure and flow augmentation index (AIx) independent of CRL, as described (Kelly et al. *Circ* 80:1652, 1989, Hirata et al. *Stroke* 37:2552, 2006). Flow AIx was divided by pressure AIx to index vascular conductance.

**Results:** Chronic hypoxia decreased the fetal PaO<sub>2</sub> from 20.8±0.7 to 12.3±1.0 mmHg (mean±SEM, P<0.05). Neither gestation or hypoxia affected fetal cfPWV (Fig.1A). However, a fall in the carotid pressure AIx occurred in hypoxic relative to normoxic fetuses (Fig.1B). Further, the ontogenic fall in femoral pressure AIx and the ontogenic increase in femoral conductance in normoxic fetuses did not occur in hypoxic fetuses (Fig.1C,D).

**Conclusion:** We show that it is possible to measure *in vivo* arterial stiffness in fetal life. Chronic hypoxia has significant effects on regional arterial stiffness in fetal life. These changes favour an increase in peripheral relative to central stiffness, supporting fetal brain sparing during chronic hypoxia. Analysis of cfPWV and of AIx in pressure and flow waveforms in fetal life provides a useful tool to diagnose fetal origins of vascular disease in adverse pregnancy.



**Figure 1. Chronic hypoxia alters stiffness in carotid and femoral vascular beds.** Values are mean±SEM for the cfPWV (A), the Pressure Augmentation Index (AIx) in the carotid vascular bed (B), the Pressure AIx in the femoral vascular bed (C) and the Flow AIx divided by the Pressure AIx in the femoral vascular bed to give an index of femoral vascular conductance (D) in sheep fetuses undergoing normoxic (white) or hypoxic (blue) pregnancy. Measurements and calculations were determined at 123±2 dGA prior to treatment (Early) or after 10 days of chronic hypoxia or chronic normoxia at 133±2 dGA (Late). Comparisons were performed using two-way ANOVA, a mixed-effects model and the Student's t test for unpaired data, as appropriate. Significant differences (P<0.05) are: \*Early vs. Late; †Normoxia vs. Hypoxia.

## O-124

**In Utero e-Cigarettes Exposure Enhances Neonatal Brain Ischemic Damage: Role of the miRNA-30c.** Andrew Walayat<sup>†</sup>, Yanyan Zhang, Yong Li, Asanjan Hosseini Maryam, Daliao Xiao\*. *Loma Linda University, Loma Linda, CA, United States.*

**Introduction:** Electronic-cigarettes (E-cigs) have rapidly become one of the most common tobacco products, a great concern for public health. Studies on the prevalence of E-cig use during pregnancy report higher rates of exclusive prenatal e-cigarette use than conventional cigarette use. To date, the safety of E-cigs to maternal and fetal development remains largely unknown. This study seeks to investigate how E-cig exposure in utero causes gender-dependent alterations of transcriptomic patterns, consequent development of brain hypoxic-ischemic (HI) sensitive phenotype in neonates, and the attenuation of the hypoxic-ischemic phenotype via miRNA inhibition.

**Methods:** Time-dated pregnant rats were exposed to chronic intermittent E-cig vaping in a chamber from gestational day 4 to 20 and control pregnant rats were kept in similar chambers. In the 1st set of experiments, brain tissues from exposed and control pups at postnatal day 9 (P9) were isolated to perform miRNA expression profile analysis and mRNA transcriptomic patterns by next-generation seq analysis and integrated with bulk RNA-seq to identify prospective miRNA targets. In the 2nd set of experiments, neonatal offspring (P7) underwent HI procedures. In the 3rd set of experiments, neonatal offspring (P5) underwent ICV injections of LNA-antagomir-30c and then underwent HI protocols at P7.

**Results:** There were 26 miRs/piRs in male and 11 miRs/piRs in female offspring that were significantly altered by e-cig exposure as compared with the controls. MicroRNA-30c was one of the differentially expressed miRNAs that was significantly up-regulated by e-cig exposure in male offspring. Similar to the miRNA-seq analysis, our RNA-seq analysis indicated gender-dependent alterations of gene expression patterns following E-cig exposure: 91 genes (55 up, 36 down) in males and 244 (120 up, 124 down) in females, with 8 common (Lsm6, Parp2, Megf8, Gm23134 down; Gnb1, Stmn1, Tubb5, Psmc1 up). Prenatal E-cig exposure enhanced HI-induced brain infarct size in male but not in female offspring as compared with the controls. However, the administration of LNA-antagomir-30c in male offspring demonstrated no significant differences of HI-induced infarct sizes in both e-cig exposed and control groups.

**Conclusion:** These findings provide novel evidence that E-cig exposure in utero is associated with gender-dependent alterations of epigenetic and transcriptomic patterns resulting in development of neonatal brain HI-sensitive phenotype in offspring. E-cig exposure-mediated changes of miR-30c may play a key role in the phenotype development.

## O-125

**Early and Mid-Gestation Zika Virus (Zikv) Infection in the Olive Baboon (*Papio anubis*) Leads to Neurological Birth Defects Due to Congenital Zika Syndrome (czs).** Sunam G Dockins†, Darlene N Reuter, Marta E Maxted, Ashley A Martin, Molly E Dubois, James F Papin, Dean A Myers\*. *OUHSC, Oklahoma City, OK, United States.*

**Introduction:** Early to mid-gestation (G) ZIKV infection in women is associated with the highest rate of birth defects due to congenital zika syndrome (CZS) including a wide range of neurological and neurodevelopmental anomalies. We reported vertical transfer and notable disruption in fetal frontal cortex development including loss of radial glial fibers, astrogliosis and oligodendrocyte damage at 21 days post ZIKV infection (dpi) at mid-G in the olive baboon (PMID: 30657788) predicting significant neuropathology by term gestation. In the present study we examined the fetal brain outcome at term following ZIKV infection during early or mid-G in baboons. We hypothesized that ZIKV infection would result in significant fetal brain defects by term gestation.

**Methods:** Timed-pregnant baboons were inoculated subcutaneously with ZIKV (PRVABC59;  $10^6$  ffu) during early (n=4; 55-58 dG) or mid-G (n=3; 92-97 dG). Dams were euthanized and tissues collected at 167-172 dG; term ~183 dG). Control fetal brains (n=3) were obtained at 167-172 dG. Immunostaining for inflammation (Iba-1), oligodendrocyte precursor cells (Olig-2), glia (GFAP) and neural progenitor cells (NPCs) (Nestin) were performed on the fetal cortex, cerebellar and hippocampal sections.

**Results:** Viremia was observed in 6/7 dams between 4-7 dpi, resolving by 14 dpi. ZIKV RNA was not detected in any fetal tissues sampled. Neuroinflammation (Iba-1) was significant in the frontal cortices of early-G but not mid-G fetuses compared to the control while both gestation ages had significant neuroinflammation in the cerebellum. Oligodendrocyte precursor cells (olig-2) were significantly reduced in cerebellum of early-G but not mid-G but not in the frontal cortex compared to the control. Significant increase in glial cells (GFAP) was observed in the cortex of both gestation groups compared to the control, a hallmark of astrogliosis. NPCs (Nestin) in the dentate gyrus (DG) of the hippocampus were significantly reduced with disorganized NPCs and loss or stunted neurites in the ZIKV fetuses compared to the control (p value 0.05).

**Conclusion:** We observed significant gestational age dependent neuropathology in fetal brains consistent with early to mid-G being the

most vulnerable to ZIKV. Although, ZIKV RNA was not observed at term in any fetal tissues, neuropathology observed in the affected fetal brains suggests vertical transfer occurred at some point during gestation, consistent with our earlier report demonstrating vertical transfer occurred within 14-21 dpi in baboons infected mid-gestation. The fetal brain injury resulting from early and mid-gestation ZIKV infection is comparable to neuropathology observed in human fetuses leading to the development of microcephaly and/or several neurodevelopmental abnormalities post-birth. (NIH: NS103772, OD010988).

## O-126

**Thyroid Hormone Dominates IGF1 Expression and Actions in Fetal Cardiomyocytes.** Natasha N Chattergoon<sup>1</sup>, Samantha Louey<sup>1</sup>, Sonnet Jonker<sup>1</sup>, George Giraud<sup>2,1</sup>, Kent L Thornburg\*. <sup>1</sup> *Oregon Health and Science University, Portland, OR, United States;* <sup>2</sup> *Portland VA Medical Center, Portland, OR, United States.*

**Introduction:** Both insulin-like growth factor 1 (IGF1), a stimulant of ovine fetal cardiomyocyte proliferation and triiodothyronine (T3), a proliferation suppressant increase in fetal plasma as term approaches. Because of their opposite but simultaneous influence on cardiomyocyte behavior, we sought to understand the independent effects of T3 and IGF1 [as analog long R<sup>3</sup> IGF1 (LR<sup>3</sup> IGF1)] on ovine fetal cardiomyocytes because the cardiomyocyte endowment at birth persists over the lifespan of offspring. Too few cardiomyocytes elevates risk for heart failure. Cardiac proliferation slows as term approaches so we speculated that T3 must override the actions of IGF1. We tested the hypothesis that T3 suppresses IGF1 signaling at the level of the IGF1-receptor 1 (IGF-R1) in fetal cardiomyocytes.

**Methods:** Four groups of fetal sheep (n=8/group) were studied between 125-130 days gestational age (dGA, term ~145 days): 1) Vehicle control, 2) T3 (54µg/d), 3) Long R<sup>3</sup> IGF1 (715µg/d), 4) T3+IGF1 (T3:54µg/d + IGF1:715µg/d). Fetal hemodynamics and blood chemistry were continuously monitored. At 130dGA, the fetal hearts were weighed and a section the left ventricle (LV) removed for tissue analysis using qPCR and Western blot analysis. Data are mean ± SEM and analyzed using multiple sample corrected ANOVA.

**Results:** Fetal aortic pressure did not change in the experimental period but fetal heart rate increased in IGF1 groups. Circulating glucose levels were reduced 50% in fetuses that received IGF1 alone but maintained at normal control levels in the T3+IGF1 group.

- Gene expression of **IGF-R1** was reduced by 30% in IGF1 fetuses and by 50% in T3+IGF1 fetuses compared to control.
- Thyroid receptor alpha1 (**TRα1**) was reduced by 50% in the T3+IGF1 group.
- Cardiac **IGF1** expression in T3 fetuses was twice that of hearts in the IGF1 and T3+IGF1 groups.
- Cell cycle inhibitor, **p21**, more than doubled by T3 treatment and significantly increased in T3+IGF1 compared to IGF1 alone.
- Cell cycle promoter, **cyclin D1**, was reduced by 20% in T3 and T3+IGF1 compared to Vehicle and IGF1 groups.

**Conclusion:** Our data suggest that while both T3 and IGF1 increase with gestation, T3 dominates IGF1's effects and regulates the expression of IGF1 related genes and their actions at the myocyte level. Reductions in IGF1 expression likely impede IGF1-stimulated cardiomyocyte proliferation. As term approaches, elevations in T3 concentration ensure that IGF-1 stimulated proliferation is mollified in favor of maturation of the myocyte in preparation for dramatic increases in workload after birth.

## O-127

**Black and White Women in the United States Military Have Comparable Rates of Preeclampsia and Post-Preeclamptic Cardiovascular Disease.** Andrea I Loewendorf\*,<sup>1</sup> Emily A Stone,<sup>2</sup> Lee Ann Zarzabal,<sup>2</sup> Thornton Mu,<sup>3</sup> Amelia Duran-Stanton.<sup>4</sup> <sup>1</sup>ImmunoVation, Pasadena, CA, United States; <sup>2</sup>Defense Health Agency, San Antonio, TX, United States; <sup>3</sup>Brooke Army Medical Center, San Antonio, TX, United States; <sup>4</sup>Chief, Ready and Resilient Integration Branch/Deputy Surgeon, Houston, TX, United States.

**Introduction:** Preeclampsia (PE), a hypertensive-inflammatory pregnancy condition, poses acute risks of seizures, stroke, and heart attack during the pregnancy; residual increased risks for heart attack and stroke linger for much longer, possibly decades. Racial differences may influence outcomes, but the underlying biology is not yet fully understood. We have designed a retrospective case-control military chart review project to begin to address this question.

**Methods:** Pregnancies and PE cases in the Military Health System (MHS) electronic health records (EHR) of Active Duty service women in the years 2006-2017 were identified.

**Results:** Between 2006-2017, 684,905 pregnancies including miscarriages and abortions were identified. The racial breakdown of PE prevalence in the United States Armed Forces between 1/1/2006-9/30/2017 is as follows: White 9.8%, Asian/Islander 8.7%, Black 10.2%, Other 8.4%, Unknown 6%. The prevalence of CVD as diagnosed by ICD code in preeclamptic (numerator) versus all pregnant/miscarriages (denominator) pregnancies is 15.8% for Whites, 15.7% for Asian/Islander, 17% for Blacks, 13.6% for Other and 9.2% for Unknown race. Stroke prevalence calculations were complicated by a change in the amount of reporting codes between the ICD9 and the ICD10 reporting system; the numbers and outcomes changed dramatically concomitantly and thus we decided to keep the datasets separate. The prevalence for stroke in the years 2006-2014 are between 145.8 and 266.7 with an average of 192.3 per 100 control pregnancies. Similarly, the incidence for stroke in preeclamptic pregnancies per 100 control pregnancies in the years 2006-2014 is between 110.4 and 218.6 with an average of 161.

**Conclusion:** The overall preeclampsia prevalence discovered in our datasets is in the higher ranges from what is reported (often between ~3-12%). Surprisingly, we find no difference of preeclampsia prevalence or CVD prevalence after preeclampsia between Whites, Asians, and Blacks despite the fact that these are large numbers of patients in our study; numerous previous studies have reported higher risks for preeclampsia in African Americans and also higher risks for CVD overall and after preeclampsia specifically. This raises the interesting question whether the lack of difference we observed is due to equal access to care and more similar lifestyles and employment situations within the Armed Forces versus in civilian populations.

## O-128

**Racial and Ethnic Disparities in Complications among Patients Undergoing a Trial of Labor after Cesarean Section at Mount Sinai Hospital.** Avisha Brielle Buckley†, Stephanie Sestito†, Tonia Ogundipe†, Natalie Cohen†, Mitchell Rosenberg†, Kelly Wang, Jill Berkin\*, Stoffels Guillaume, Chelsea DeBolt†, Jessica Peterson†, Joanne Stone\*, Angela Bianco\*, Luciana Vieira\*. *Icahn School of Medicine at Mount Sinai Hospital, Manhattan, NY, United States.*

**Introduction:** A patient's decision about whether or not to undergo a trial of labor after cesarean section (TOLAC) should be based on a discussion of the risks, benefits and alternatives for the individual. While numerous studies have worked to predict TOLAC success rates, few have examined factors associated with complications. We hypothesized that there would be a racial and ethnic differences in postpartum complications rates.

**Methods:** A retrospective chart review including all patients undergoing TOLAC from 2016 to 2019 was conducted. Records were excluded if they were incomplete or duplicate, and a total of 1275 were included. For this analysis, the following delivery complications were assessed: postpartum hemorrhage (PPH), blood transfusion, abruption, hysterectomy, uterine

rupture, D&C, intraamniotic infection (IAI), endometritis, wound infection, wound separation, or other postpartum complication. A Chi square or Fisher's exact test was used as appropriate.

**Results:** Among 1275 patients, 199 had at least one complication while 1076 experienced no complications. There was a significant association between the presence of complications and race and ethnicity status ( $p < 0.0001$ ). The distribution of each postpartum complication by race and ethnicity is shown in Table 1. Rates of PPH ( $p = 0.001$ ), abruption ( $p = 0.04$ ), intra-amniotic infection ( $p < 0.0001$ ) and other postpartum complications ( $p = 0.005$ ) differed significantly by race and ethnicity category. In the final multivariable model, after adjusting for maternal age, previous VBAC, pre-pregnancy BMI, term births, preterm births, living births, and insurance type, there was sufficient evidence to conclude that there was an association between experiencing complications and race and ethnicity ( $p = 0.02$ ). Specifically, the adjusted odds of at least one complication were significantly higher in individuals identified as Hispanic/LatinX compared to individuals identified as White/Caucasian (OR: 2.00, 95% CI: 1.28, 3.13,  $p = 0.002$ ).

**Conclusion:** In our patient population, there were significant racial and ethnic differences in overall postpartum complication rates and specifically in PPH, abruption, and IAI. These findings should be considered when counseling patients considering a TOLAC.

Table 1: Frequency of Complications by Race/Ethnicity

Variable	All (n=1275)	Asian or Other (n=74)	Hispanic/LatinX (n=230)	Non-Hispanic Black (n=180)	White/Caucasian (n=791)	p-value*
No Complications	1076 (84.4)	57 (77.0)	172 (74.8)	144 (80.0)	703 (88.9)	<0.001
At least one Complication	199 (15.6)	17 (23.0)	58 (25.2)	36 (20.0)	88 (11.1)	
PPH	126 (9.9)	13 (17.6)	35 (15.2)	14 (7.8)	64 (8.1)	0.001
Blood Transfusion	24 (1.9)	2 (2.7)	7 (3.0)	4 (2.2)	11 (1.4)	0.256
Abruption	10 (0.8)	2 (2.7)	0(0)	3 (1.7)	5 (0.6)	0.043
Hysterectomy	3 (0.2)	0(0)	1 (0.4)	0(0)	2 (0.3)	0.767
Uterine Rupture	14 (1.1)	1 (1.4)	4 (1.7)	0(0)	9 (1.1)	0.309
D&C	2 (0.2)	0(0)	1 (0.4)	0(0)	1 (0.1)	0.615
Intraamniotic Infection	40 (3.1)	2 (2.7)	16 (7.0)	14 (7.8)	9 (1.0)	<0.001
Endometritis	6 (0.5)	1 (1.4)	3 (1.3)	0(0)	2 (0.3)	0.080
Other postpartum complication	36 (2.8)	1 (1.4)	10 (4.3)	11 (6.1)	14 (1.8)	0.005

\*Chi square or Fisher's Exact Test were used

## O-129

**Potential Risk of Infection of First Trimester Placentas by SARS-CoV2.** Sampada Kallol,<sup>1</sup> Laura Martin-Sancho,<sup>2</sup> Donald Pizzo,<sup>1</sup> Sumit K Chanda,<sup>2</sup> Mana Parast,<sup>1</sup> Francesca Soncin.<sup>1</sup> <sup>1</sup>UCSD, La Jolla, CA, United States; <sup>2</sup>SBP, La Jolla, CA, United States.

**Introduction:** The outbreak of the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) in 2019 has led to an ongoing global pandemic of severe pneumonia-like disease, coronavirus disease 2019 (COVID-19). It is documented that SARS-CoV2 infection occurs by binding of viral spike (S) proteins to cellular receptor ACE2 and S protein priming by host cell protease TMPRSS2, localized on the cell surface of multiple organs, including heart, lungs, kidneys, testes, and intestine. However, data on ACE2 and TMPRSS2 expression in placenta, particularly in early gestation, are not well documented and the effect of viral exposure and potential vertical transmission during first trimester are unknown. Therefore, we aimed to study the potential for SARS-CoV2 infection of human placenta, specifically in early trophoblast cells, using the novel trophoblast stem cells (TSC) model.

**Methods:** We first performed ISH staining for ACE2 and TMPRSS2 on formalin-fixed paraffin sections of first trimester (n=6), second trimester (n=5) and term (n=5) human placentas. Then, we derived TSC from first trimester human placenta and differentiated into extravillous trophoblast cells (EVT) and syncytiotrophoblast cells (STB), as reported by Okae et al. Gene (qRT-PCR) and protein expression (western blotting) of ACE2 and TMPRSS2 were analyzed in TSC, EVT and STB. Furthermore, all three trophoblast cell types were infected with SARS-CoV2, with/without Remdesivir, which inhibits viral replication, and samples collected up to 48h post-infection. SARS-CoV2 titer was measured by plaque assay on cell culture supernatant and SARS-CoV2 replication was measured by qRT-PCR on cell lysates.

**Results:** ISH staining revealed that ACE2 and TMPRSS2 were absent from cytotrophoblast at all gestational ages. ACE2 was moderately expressed in first trimester STB, decreasing in this compartment at term; it was also highly expressed in term EVT. TMPRSS2 showed moderate

expression in EVT, but less expression in STB, across gestation. *In vitro*, ACE2 and TMPRSS2 were predominantly expressed in the differentiated trophoblast cells, with higher expression in EVT than STB, while no/low expression was detected in undifferentiated TSC, both at mRNA and protein levels. Moreover, infected EVT and STB showed higher viral titer and mRNA levels compared to undifferentiated TSC. In this context, treatment with the inhibitor of viral replication, Remdesivir, reduced viral load in both assays.

**Conclusion:** The higher levels of ACE2 and TMPRSS2 in differentiated trophoblast, compared to cytotrophoblast/trophoblast progenitor cells, suggest that SARS-CoV2 can infect placental tissues through either STB or EVT, both of which are adjacent to maternal blood. The higher expression of these receptors in first trimester STB suggests that SARS-CoV2 infection during early gestation might present a higher risk to an ongoing pregnancy.

### O-130

**Impact of SARS-CoV-2 Infection during Pregnancy by Maternal Race-Ethnicity.** Darios Getahun,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiaxiao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Michael J Fassett,<sup>7</sup> <sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States.

**Introduction:** To compare the consequences of prenatal COVID-19 on pregnancy outcomes between racial/ethnic groups.

**Methods:** The KPSC electronic health records between 04/06/2020-12/31/2020 were used to build a retrospective cohort (n=29,323). SARS-CoV-2 infections were identified by PCR-based testing that was universally offered to all women. Outcomes were compared between non-Hispanic white (White), non-Hispanic black (Black), Latina, and Asian/Pacific Islander (Asian/PI) women. Multivariable logistic regression was used to estimate the odds ratios (OR).

**Results:** Among Whites, prenatal SARS-CoV-2 infection was associated with placenta previa (adj.OR:1.85, CI:1.03-3.34), preterm birth (PTB) (overall PTB [adj.OR:1.81, CI:1.00-3.26]); indicated PTB [adj.OR:2.42, CI:1.19-4.92]), neonatal SARS-CoV-2 infection (adj.OR:18.0, CI:3.18-101), postpartum maternal hospital stays >2 days (adj.OR:1.5, CI:1.07-2.11), and maternal ICU admission (adj.OR:29.27, CI:4.69-182), and lower risk of LGA (adj.OR:0.46, CI:0.22-0.95). Among Blacks, SARS-CoV-2 infection was associated with Apgar score <7 (adj.OR:3.03, CI:1.03-8.93) and neonatal SARS-CoV-2 infection (adj.OR:36.73, CI:1.91-707). Black-White disparities in preeclampsia, PTB, cesarean delivery, postpartum hemorrhage, NICU admission, neonatal sepsis, and RDS were observed. Among Latinas, SARS-CoV-2 infection was associated with GDM (adj.OR:1.28, CI:1.06-1.56), neonatal SARS-CoV-2 infection (adj.OR:6.26, CI:3.19-12.28), and maternal ICU admission (adj. OR:4.82, CI:1.74-13.34). Latina-White disparities in GDM, preeclampsia, preterm PROM, PTB, chorioamnionitis, and LGA were observed. Among Asian/PI, SARS-CoV-2 infection was associated with neonatal SARS-CoV-2 infection (adj.OR:43.6, CI:1.2-153). Asian/PI -White disparities in GDM, preeclampsia, PTB, chorioamnionitis, and postpartum LOS ≥2 days were observed.

**Conclusion:** These findings suggest that the clinical consequences of prenatal SARS-CoV-2 infection differ markedly between race/ethnicity groups. Further studies are needed to ascertain the biological or sociological factors that are responsible for this race-disparity.

### O-131

**Stressors in Fertility Treatment: The Impact of the COVID-19 Pandemic.** Sarah Dynia†, Caroline Peschansky†, Safina Usmani, Sonia Patel†, Kayla Vitale†, Jawaria Amir, Royi Lynn†, Lauren Grimm, Erica Loudon, Roohi Jeelani, Angie Beltsos. *Vios Fertility Institute, Chicago, IL, United States.*

**Introduction:** Prior research has demonstrated that the psychological impact of an infertility diagnosis is comparable to other serious medical conditions, including cancer. Over the past year, the ongoing COVID-19 pandemic has been associated with increased stress, anxiety, and feelings of depression across almost all populations. For those already experiencing heightened levels of stress and anxiety prior to COVID-19, the effect of increased stress and worry may have detrimental effects on health outcomes. While the cause-and-effect relationship between stress and infertility is still unclear, it is important to consider how COVID-19 leads to increased distress among infertility patients and how this may impact perceived treatment outcomes. Thus, we sought to better understand how various stressors related to COVID-19 impacted the patient experience during fertility treatment.

**Methods:** An anonymous survey was distributed to patients at a private fertility clinic via the patient portal. Survey questions investigated patient demographics and feelings of anxiety regarding specific COVID-19 related stressors. Baseline levels of anxiety were measured by GAD-7, a standardized 7-question generalized anxiety disorder scale. Patient confidence in their fertility treatment and potential changes to their treatment plan due to COVID-19 were also assessed. Responses ranged from “not at all” to “all of the time”. Survey responses were analyzed to determine average response and response frequencies.

**Results:** Of the 290 participants, 89% were women with an average age of 33.8, and of whom 58.9% have been pursuing fertility treatment for at least a year. The average GAD-7 Anxiety Severity Score was 5.79, indicating an overall feeling of mild anxiety among respondents. 31% reported financial concerns related to the COVID-19 pandemic as at least one of the causes of their anxiety and worry. Of those that reported financial concerns, 64% came from patients who made less than \$100,000 per year. 59% of respondents reported COVID-19 health concerns as one cause of stress, but only 29% reported delaying treatment or considering delaying treatment due to the pandemic. Fortunately, 54% of respondents indicated that seeking treatment has improved the way they feel about their fertility.

**Conclusion:** According to our results, patients seeking fertility treatment report a mild level of anxiety and are impacted by many stressors such as financial concerns and the ongoing uncertainty of COVID-19. While these stressors may impact patients differently, providers should provide increased consideration for the psychological impact of infertility during COVID-19 via regular check-ins and resources to improve mental health. Further studies should focus on the impact of infertility and COVID-19-related stressors among minority communities.

### O-132

**Expression of SARS-CoV-2 Entry Molecules at Maternal-Fetal Interface and Regulation of Proinflammatory Cytokines and Coagulation Factor III in Human Placental Microvascular Endothelial Cell by Spike Protein.** Xiaofang Guo, Umit A. Kayisli, Asli Ozmen, Nihan Semerci, Kellie Larsen, Zhi Tian, Frederick Schatz, Diane Allen-Gipson, Ozlem Guzeloglu-Kayisli, Charles J. Lockwood. *University of South Florida, Tampa, FL, United States.*

**Introduction:** Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) is responsible for the pandemic of COVID-19, it infects cells via binding to angiotensin converting enzyme 2 (ACE2), subsequent entry cells through transmembrane protease serine type 2 (TMPRSS2). Evidence showed that SARS CoV-2 was detected in placenta in infected pregnant women, which indicating it might be responsible for the pregnancy complications such as preterm birth, abruption, fetal growth restriction and/or preeclampsia. Therefore, we hypothesize that SARS CoV-2 infection of placental microvascular endothelial cells (PMVECs) induces proinflammatory cytokines release to contribute to placental dysfunction, which impairs pregnancy outcomes.

**Methods:** ACE2 and *TMPRSS2* expression were measured by qPCR in human primary cultured term cytotrophoblasts (CTBs), syncytiotrophoblast (STBs), term and first trimester decidual cells (TDCs and FTDCs, respectively) as well as PMVECs (n=4/each cell type). Cultured PMVECs were treated with 10, 100, 1000 ng/ml of recombinant SARS CoV-2 S-protein  $\pm$  10 ng/ml IFN $\gamma$ . Expression levels of proinflammatory cytokines IL1 $\beta$ , IL6 and IL8, chemokines CCL2, CCL5, CXCL9 and CXCL10, and coagulation factor III (*F3*) that is the prime initiator of the extrinsic coagulation cascade were measured by qPCR.

**Results:** Weak ACE2 and *TMPRSS2* levels were detected in CTBs and STBs as well as PMVECs. ACE2 levels were significantly higher in CTBs and STBs vs. TDCs or FTDCs, while *TMPRSS2* levels were not detected in TDCs, FTDCs or PMVECs. qPCR analysis revealed that S-protein in all concentrations that used significantly increased IL1 $\beta$ , IL6, IL8, CCL2, and CCL5 levels, whereas only higher concentration S-protein significantly induced *F3* levels in PMVECs compared to mock treated control. However, any concentration of S-protein did not alter CXCL9, and CXCL10 levels in PMVECs. Previous studies showed increased IFN $\gamma$  levels in serum samples of COVID-19 patients as well as at the maternal-fetal interface during early pregnancy and in preeclampsia. Therefore, we treated PMVECs with IFN $\gamma$  alone or in combination with 10 ng/ml S-protein. IFN $\gamma$  alone significantly increases proinflammatory cytokines and chemokines as well as *F3* levels, however, in combination of S-protein further increase all inflammatory markers and *F3* levels in PMVECs.

**Conclusion:** These findings indicated that SARS CoV-2 infection in placental microvasculature: 1) induces proinflammatory cytokine and chemokine release, which contributes to cytokine storms in pregnant women and causes placental dysfunction; and 2) elevates *F3* expression that may trigger vascular thrombosis in placenta.

### O-133

**Chorionic Somatomammotropin RNA Interference Reduces Global Nutrient Uptake and Umbilical Blood Flow Resulting in Intrauterine Growth Restriction.** Amelia R Tanner $\dagger$ ,<sup>1</sup> Cameron S Lynch,<sup>1</sup> Victoria C Kennedy,<sup>1</sup> Asghar Ali,<sup>1</sup> Quinton A Winger,<sup>1</sup> Paul J Rozance,<sup>2</sup> Russell V Anthony\*.<sup>1</sup> <sup>1</sup>Colorado State University, Fort Collins, CO, United States; <sup>2</sup>University of Colorado School of Medicine, Aurora, CO, United States.

**Introduction:** Previously we have described two distinct phenotypes in response to chorionic somatomammotropin (CSH) RNA interference (RNAi) near term: 1) pregnancies with intrauterine growth restriction (IUGR), and 2) pregnancies in which fetal and placental size were not impacted. To better understand the biological functions of CSH in both phenotypes, we employed steady-state techniques to measure changes in blood flow and nutrient uptake near term (130 days of gestational age; dGA). In the absence of IUGR, the *in vivo* physiological ramifications of CSH RNAi included perturbed uterine blood flow and placental glucose metabolism. In the current study, we describe the *in vivo* consequences of CSH RNAi in pregnancies with IUGR.

**Methods:** The trophoblast of hatched blastocysts (9 dGA) were infected with a lentivirus expressing either a scrambled control (NTS) or CSH-specific shRNA (tg6), prior to transfer into synchronized recipient sheep. At 90 dGA, umbilical hemodynamics and fetal measurements were assessed by Doppler ultrasonography. At 120 dGA, pregnancies were fitted with maternal and fetal vascular catheters. At 130 dGA, pregnancies underwent steady-state metabolic studies with the <sup>3</sup>H<sub>2</sub>O transplacental diffusion technique. Uterine (maternal), umbilical (fetal), and uteroplacental (placental) uptake rates of oxygen, glucose, lactate, and amino acids were calculated using the Fick principle. Tissues were subsequently harvested. Data resulting from scrambled control (NTS; n=4) and CSH RNAi (tg6; n=4) pregnancies were compared by Student's T-test.

**Results:** CSH RNAi reduced (P $\leq$ 0.05) fetal weight and uterine weight by 30% and 43% respectively. Umbilical blood flow (mL/min) was reduced (P $\leq$ 0.05) by 36% at 90 dGA and by 40% at 130 dGA in CSH RNAi pregnancies. Furthermore, CSH RNAi resulted in reduced (P $\leq$ 0.01) umbilical IGF1 concentrations, as well as reduced (P $\leq$ 0.05) umbilical uptakes of oxygen, glucose, lactate, and several individual amino acids

(mmol/min). Additionally, CSH RNAi reduced (P $\leq$ 0.05) uterine uptakes of oxygen, glucose, and many individual amino acids. Uteroplacental glucose uptakes were also reduced (P $\leq$ 0.05) by CSH RNAi.

**Conclusion:** In the present study, CSH RNAi reduced umbilical blood flow and global nutrient uptake which ultimately led to reduced fetal and uterine weights. These data suggest that CSH is not only important for uterine blood flow and uteroplacental glucose uptake, but it also facilitates adequate umbilical blood flow necessary for the uptakes of oxygen, oxidative substrates, and hormones necessary to support fetal growth. Additionally, these data suggest that CSH may have uterotrophic actions. Supported by NIH HD093701.

### O-134

**Trophoblast-Specific Knockdown of the System A Amino Acid Transporter, *Slc38a2*/SNAT2, Causes Fetal Growth Restriction in Mice.** Owen R Vaughan,<sup>1,2</sup> Elena Silva,<sup>1</sup> Kenneth Barentsen,<sup>1</sup> Russell V Anthony,<sup>3</sup> Thomas L Brown,<sup>4</sup> Theresa L Powell,<sup>1</sup> Thomas Jansson.<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University College London, London, United Kingdom; <sup>3</sup>Colorado State University, Fort Collins, CO, United States; <sup>4</sup>Wright State University, Dayton, OH, United States.

**Introduction:** System A transporters/SNATs mediate sodium-dependent accumulation of small neutral amino acids in the trophoblast. Their activity in the human placenta is associated with birth weight. Placental System A activity is reduced in pregnancies complicated by fetal growth restriction but the mechanistic importance of individual placental SNAT isoforms for intrauterine growth is unknown. We hypothesized that trophoblast-specific SNAT2/*Slc38a2* knockdown, using lentiviral small-hairpin (sh) RNA delivery to the blastocyst trophectoderm, reduces placental growth, transplacental amino acid transfer and fetal weight in late gestation mice.

**Methods:** Embryonic day (E) 3.5 mouse blastocysts were transduced with 5 x 10<sup>5</sup> transforming units of a lentiviral vector containing either a U6 promoter-driven, *Slc38a2* targeting shRNA (*Slc38a2*KD), or a non-targeting control shRNA (SCR), both with green fluorescent protein (GFP). After 4hr, SCR and *Slc38a2*KD blastocysts were transferred to contralateral uterine horns of pseudopregnant CD-1 female recipients (n=29). On E18.5, recipients were euthanized and fetuses and placentae collected and weighed. *Slc38a2* expression was determined by qPCR. Trophoblast plasma membrane SNAT2 abundance and *in vivo* System A transport capacity were determined by western blot and maternal-placental <sup>14</sup>C-methylaminoisobutyric acid clearance per gram placenta, respectively. Litter mean outcome measures were calculated for SCR and *Slc38a2*KD conceptuses and differences determined by paired Student's t-test.

**Results:** Both SCR and *Slc38a2*KD transduced conceptuses exhibited trophoblast-specific GFP fluorescence. Placental, but not fetal, *Slc38a2* expression was 59% lower in *Slc38a2*KD compared to SCR conceptuses (P<0.001, n=6 litters). Placenta-specific *Slc38a2* knockdown reduced both fetal weight (-11%) and placental weight (-18%) compared to SCR controls (P<0.01, n=29 litters). Trophoblast plasma membrane SNAT2 abundance and *in vivo* System A amino acid transport capacity were also 20-30% lower in *Slc38a2*KD than SCR placentas (P<0.05, n=9-13 litters). *Slc38a2* knockdown did not alter embryo implantation rate but diminished fetal viability, as measured by the proportion of transferred blastocysts surviving to term.

**Conclusion:** This study demonstrates, for the first time, a cause-and-effect relationship between reduced placental expression of the System A amino acid transporter *Slc38a2*/SNAT2 and fetal growth restriction in mice. We speculate that *Slc38a2*/SNAT2 deficiency mechanistically contributes to placental insufficiency in human pregnancies complicated by fetal growth restriction.

### O-135

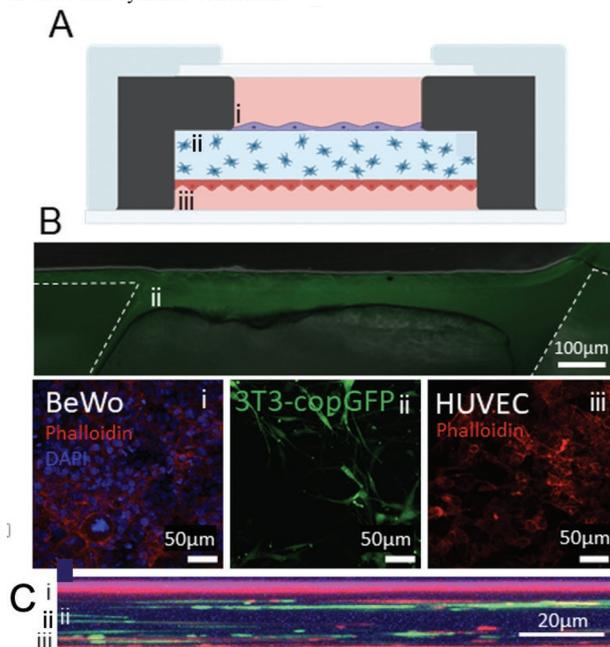
**An *In Vitro* Placenta Model with a Native Stroma for Mechanistic Transport Studies.** Katherine M. Nelson $\dagger$ , Sarah A. Geissler, Jason P. Gleghorn\*. University of Delaware, Newark, DE, United States.

**Introduction:** Transplacental transport of many medications is poorly understood, leaving pregnant women vulnerable to the unknown risks of many therapeutics that may be necessary during pregnancy. The

ability to study placental drug transport is severely limited due to ethical concerns and technical challenges. Our objective was to develop an *in vitro* microfluidic placental model incorporating multiple cell types and placenta derived extracellular matrix (pECM). This model can be used to delineate mechanisms of transplacental drug transport and perform drug screening.

**Methods:** BeWo cells and HUVECs were purchased from ATCC or Lonza. Human primary trophoblast and placental endothelial cells were isolated from healthy term placenta. Placenta were decellularized using alternating hypo- and hypertonic washes with water and 10% NaCl, digested with pepsin, and gelled via neutralization with 1M NaOH. pECM was used to create thin membranes by drying gelation solution on silicon channel inserts at 37°C. The pECM was also used to create a stromal layer capable of housing stromal cells, such as fibroblasts. These pECM structures were incorporated into a microfluidic device by clamping the silicon insert between two acrylic pieces containing microfluidic channels (Fig. 1A).

**Results:** Confluent trophoblast and endothelial cell layers were formed on the obverse and reverse of the pECM membrane forming maternal and fetal fluidic channels. Fibroblasts were cultured within a hydrated stromal layer in addition to the epithelial and endothelial monolayers (Fig. 1 B & C). Barrier formation was confirmed with transepithelial electrical resistance (TEER). Culture was maintained over 7 days. Antipyrine, sodium-fluorescein, and glucose were used to validate transport dynamics in static and dynamic conditions.



**Fig. 1.** (A) A schematic cross-section of the model and (B) fluorescent images of the insert after 3 day culture with BeWos on the maternal side (i), 3T3 cells in the stromal layer supported by the insert (dotted line) (ii), and HUVECs on the fetal side (iii). (C) Cross-section of the three cell layers (i,ii,iii).

**Conclusion:** We have successfully created a placenta-on-a-chip device using multiple cell types and pECM that enable the investigation of passive and active mechanisms of transplacental transport and the impacts of fluid flow on these processes. Additionally, the incorporation of pECM allows for mechanistic studies of cell-ECM interactions and the use of potentially pathological ECM to study cellular interactions in the context of preeclampsia or preterm birth.

## O-136

**Obesity Downregulates Lipid Metabolism Genes in First Trimester Placenta.** Aisha Rasool<sup>†</sup>,<sup>1</sup> Begum Aydogan Mathyk,<sup>2</sup> Danielle Roncari,<sup>1</sup> Perrie O'Tierney-Ginn\*,<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>Brandon Regional Hospital, Brandon, FL, United States.

**Introduction:** Maternal obesogenic environment affects placental fatty acid (FA) metabolism and shapes fetal growth trajectory. Placentas of obese women have low mitochondrial beta-oxidation of FA and accumulate lipids in late pregnancy. This reduces ATP production and creates a lipotoxic environment, impairing placental efficiency. The chronology of these changes during gestation are poorly understood. We hypothesize that placental fatty acid metabolism is impaired in women with obesity from early pregnancy.

**Methods:** RNA was extracted from placentas of 27 healthy women (13 obese [body mass index (BMI)>35]; 14 lean [BMI<25]) collected in early pregnancy (gestational age (GA): 7.2-13.4 weeks). Maternal fasting triglycerides were measured in plasma collected at the time of procedure. Placental expression of 35 fatty acid metabolism genes was analyzed using multiplex nCounter assay (Nanostring). Expression was normalized to stable housekeeping genes (L19, YWHAZ and beta-actin), log transformed, and analyzed using nSolver Software (4.0.70). Analysis covariates included gestational age, race, smoking and fetal sex. False discovery rate (Q) was calculated by Benjamini Krieger Yekutieli test. Q values <0.05 were significant. Spearman rank testing was used for correlations.

**Results:** Expression of genes associated with FA oxidation (ACOX1; Q=0.00008, CPT2; Q=0.0009, AMPK $\alpha$ ; Q=0.004), FA uptake (LPL; Q=0.001, LIPG; Q=0.008, MFSD2A; Q=0.002), FA synthesis (ACC; Q=0.004) and storage (PLIN2; Q=0.002) were significantly reduced in placentas of obese (BMI: 35.8  $\pm$  6.5 kg/m<sup>2</sup>) compared to lean (BMI: 21.2  $\pm$  1.9) women. Maternal age, race, GA and smoking were not different between BMI groups. Maternal circulating triglyceride levels were positively correlated with BMI (r=0.55, p=0.02), and negatively correlated with lipid synthesis genes ACS2 (r=-0.64, p=0.005), ACSL5 (r=-0.50, p=0.03) and SREBP1 (r=-0.61, p=0.007).

**Conclusion:** We demonstrate that obesity and hyperlipidemia impact placental FA metabolism from the first trimester of pregnancy. As hypothesized, markers of FA oxidation were decreased in placentas of obese women suggesting impairments in mitochondrial (CPT2) and peroxisomal (ACOX1) function. Markers associated with FA synthesis and uptake were downregulated in placentas of obese women, contrary to what is seen at term. FA synthesis genes were also negatively correlated with high maternal triglycerides. In early pregnancy, lipid uptake and synthesis pathways are critical for phospholipid production and growth, and disruption of these pathways may affect placental efficiency later in pregnancy. As these changes begin as early as 7 weeks, interventions designed to improve outcomes in pregnancies complicated by obesity must start prior to conception or from first trimester for maximal effectiveness.

## O-137

**Thymic Progesterone Receptor Expression Regulates Maternal Thymic Involution in Murine Pregnancy.** Soo Hyun Ahn<sup>†</sup>,<sup>1</sup> Sean L Nguyen,<sup>1</sup> Tae-Hoon Kim,<sup>2</sup> Jae-Wook Jeong,<sup>2</sup> Ripa Arora,<sup>1</sup> Margaret G Petroff\*,<sup>1</sup> <sup>1</sup>Michigan State University, East Lansing, MI, United States; <sup>2</sup>Michigan State University, Grand Rapids, MI, United States.

**Introduction:** The thymus, a lymphoid organ that is instrumental for the maturation of self-tolerant T cells, undergoes temporary involution in both weight and cellularity during pregnancy. This phenomenon can be recapitulated by injecting non-pregnant (NP) female mice with ovarian hormones, estrogen and progesterone. However, the mechanism of how these hormones affect thymic involution is unknown. Here, we examine the role of the thymic nuclear progesterone receptor (PGR) receptor in pregnancy-induced thymic involution and thymocyte development.

**Methods:** Using RT-qPCR, we measured transcript expression of *Pgr* and other steroid hormone receptors in the thymus throughout pregnancy in WT mice. We determined the cellular localization of PGR using immunofluorescence microscopy. To study the role of PGR in thymocyte maturation and thymic involution, we generated a thymic epithelial cell

(TEC)-specific *Pgr* knockout mouse model (TEC<sup>PRKO</sup>) by crossing mice harboring Cre driven by the TEC-specific *Foxn1* promoter with *Pgr*<sup>fl/fl</sup> mice. Thymic weights, cellularity, and T cell development were evaluated in NP and pregnant mice using multi-parameter flow cytometry in both WT and TEC<sup>PRKO</sup> mice.

**Results:** Pregnancy induced a 4-7 fold upregulation of *Pgr* mRNA in WT females, while transcript levels for *Esr1*, *Esr2*, *Pgrmc1*, and *Pgrmc2* remained similar between NP and pregnant mice. PGR protein expression increased by 2-4 fold across gestation, and localized almost exclusively to cortical TECs in the thymus. In WT, both the thymic weight and cellularity decreased with advancing gestation; they remained unchanged in TEC<sup>PRKO</sup> females. Flow cytometric analysis showed significant increase in the proportions of the most immature thymocyte population (CD44<sup>+</sup>CD25<sup>-</sup>; DN1) in comparison with thymocytes of later maturation stages across gestation; this remained similar in TEC<sup>PRKO</sup> females. Finally, we examined the role of thymocyte death in thymic involution using Cleaved Caspase 3 (C-casp3) expression in thymocytes to signify cells either undergoing death by neglect (C-casp3<sup>+</sup> CD5<sup>+</sup>TCRβ<sup>lo</sup>) or clonal deletion (C-casp3<sup>+</sup> CD5<sup>+</sup>TCRβ<sup>+</sup>). In the WT thymus, pregnancy induced increased proportion of thymocytes undergoing death by neglect and decreased proportion of thymocytes undergoing clonal deletion. On the other hand, both selection processes were reduced in NP thymus of TEC<sup>PRKO</sup> mice.

**Conclusion:** Using a combination of molecular, flow cytometric, imaging, and novel mouse model approaches, we show that PGR expression by TECs is responsible for thymic involution during pregnancy. Further, our results indicate that PGR plays a fundamental role in both positive and negative selection during pregnancy and in normal T development in the thymus.

### O-138

**A Role for the IL-33-ILC2 Axis in Driving Uterus-Intrinsic Parturition Pathways in Mice.** *Johan Siewiera*†, Madelene Dahlgren, Kelly Cautivo, Damon Rideaux, Ari Molofsky, Adrian Erlebacher. *UCSF, San Francisco, CA, United States.*

**Introduction:** The pathways that initiate parturition remain largely undefined. One critical step is thought to be “decidual activation,” the process whereby the decidua increases its expression of COX-2 and produces prostaglandins that stimulate myometrial contraction. Functionally dissecting the upstream triggers for decidual activation has been difficult, however, since model organisms such as mice normally initiate parturition via luteolysis, the process through which the ovary stops producing progesterone.

**Methods:** Wild-type and mutant mice were treated with a single subcutaneous injection of depot medroxyprogesterone acetate (DMPA; also known as Depo-Provera) on E12.5 to sideline luteolysis-induced parturition and instead assay uterus-intrinsic parturition pathways. We measured parturition timing and analyzed late gestation uteri by flow cytometry, immunofluorescence, RNA-Seq and prostaglandin ELISA.

**Results:** We find that mice deficient in the alarmin IL-33 show a severe parturition delay when we experimentally force circulating progesterone levels to remain high, thus ensuring that parturition will occur through uterus-intrinsic pathways. Strikingly, IL-33 expression in late gestation and consequent activation of the key IL-33 target cell type, the Group 2 Innate Lymphoid Cells (ILC2), are confined to the myometrium and more specifically to the mesometrial triangle, the histologically-distinct segment of myometrial tissue that remains in contact with the decidua throughout the entirety of gestation. We also find that IL-33 is required for optimal uterine contraction in a model of inflammation-induced preterm labor. Surprisingly, disruption of the IL-33-ILC2 axis decreases COX-2 expression by the decidua and its production of PGE<sub>2</sub>.

**Conclusion:** These results reveal a contribution from IL-33, acting through ILC2s, in driving the uterus-intrinsic parturition pathway in mice, as well as a previously unappreciated upstream role for the myometrium in controlling decidual activation and the timing of labor induction. Supported by NIH grant R01AI150191 to A.E.

### O-139

**Single-Cell RNA Sequencing Identifies Novel Uterine Macrophage Population Increased by Regulatory T Cells and Associated with Decreased Fetal Loss.** *Emma L Lewis*†, *Paige M Porrett*, *Michal A Elovitz*\*. <sup>1</sup>*University of Pennsylvania, Philadelphia, PA, United States;* <sup>2</sup>*University of Alabama, Birmingham, AL, United States.*

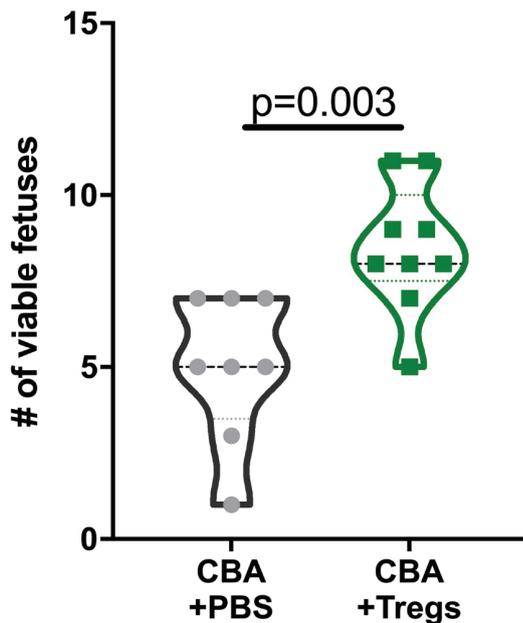
**Introduction:** Intrauterine fetal demise (IUFD) - fetal loss after 20 weeks - affects 6 pregnancies per 1,000 live births in the United States. At least one-third of IUFDs are of unknown etiology and mechanistic studies of IUFD are rare. Therefore, we investigated immunologic mechanisms of IUFD using the CBA mouse model of mid-late gestation fetal loss.

**Methods:** CBA and C3H/HeN (C3H, control) female mice were mated to DBA/2 males and sacrificed at multiple gestational ages (E8-E18) for immune phenotyping of the maternal-fetal interface by flow cytometry (N=4/strain/age). We also performed an adoptive transfer of 2x10<sup>5</sup> regulatory T cells (Tregs) or PBS to CBA dams at E2. Treg and PBS treated CBA mice were sacrificed at E14 and E18 to determine rates of fetal loss and immune compositional changes by flow cytometry (N=7-9 dams/group). To further elucidate uterine immunologic shifts, we performed single cell RNA-sequencing (scRNA-Seq) on CD11b<sup>+</sup> cells sorted from the uterus of Treg and PBS treated CBA mice (N=3/group). Repeat experiments comparing CBA and C3H mice were used to confirm cell types identified by scRNA-Seq by flow cytometry (N=6-8/group).

**Results:** Compared to C3H dams, CBA dams have 3.5-fold higher rates of fetal loss (p=0.0001). Mid-gestation CBA dams also have 4.2-fold fewer uterine Tregs (p=0.001) and 3-fold fewer decidual macrophages (p=0.0004) than C3H dams. Treg transfer restores CBA pregnancy to be more similar to a C3H pregnancy phenotype by decreasing fetal loss 2.7-fold (p=0.04), increasing pup viability (Fig A), and increasing uterine Tregs 2.3-fold (p=0.05) as assessed by flow cytometry. scRNA-Seq identified 15 uterine CD11b<sup>+</sup> populations, with Trem2<sup>+</sup> Apoe<sup>+</sup> macrophages increased by Treg transfer (p=2.2e-12). These uterine Trem2<sup>+</sup> Apoe<sup>+</sup> macrophages are enriched for gene sets from macrophages subsets found in other tissues, including those in atherosclerotic plaques (p<0.0001). We then identified this Trem2 macrophage by flow cytometry and found that pregnant CBA mice had 2-fold fewer of this macrophage subtype than C3H mice (p=0.004).

**Conclusion:** These data identify a novel uterine macrophage population that may be critical for healthy pregnancy and demonstrate that systemic Treg transfer alters tissue immune populations, which has broad implications for immunotherapy. scRNA-Seq also reveals specific uterine cells and gene pathways that suggest potential mechanisms of and therapeutic targets for IUFD.

**Figure A:  
Viability, E18**



#### O-140

**Pluripotent Stem Cell Derived Endometrial Stromal Fibroblasts Co-Culture with Endometrial Epithelial Organoids to Form a 3-Dimensional Model of the Human Endometrium.** [Virginia Chu Cheung†](#),<sup>1</sup> Chian-yu Peng,<sup>1</sup> Mirna Marinic,<sup>2</sup> Noboru J Sakabe,<sup>2</sup> Ivy Aneas,<sup>2</sup> Vincent J Lynch,<sup>3</sup> Carole Ober,<sup>2</sup> Marcelo A Nobrega,<sup>2</sup> John A Kessler\*.<sup>1</sup> <sup>1</sup>Northwestern University, Chicago, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States; <sup>3</sup>Univeristy of Chicago, Chicago, IL, United States.

**Introduction:** The human endometrium is a highly dynamic organ - cyclically proliferating, differentiating, and shedding in response to ovarian hormones. Defects in this organ could result in endometrial, pregnancy, and birth related diseases. Mouse models have fundamental differences that make it a poor model for the human endometrium. Current *in vitro* models using human biopsied cells only represent the adult endometrium and have not been used to study interactions between the different cell-types of the endometrium. We developed a model of the human endometrium combining human pluripotent stem cell-derived endometrial stromal fibroblasts (PSC-ESF) and endometrial epithelial organoids (EEO) in a 3-dimensional (3D) organoid system.

**Methods:** PSCs were differentiated into ESF were able to decidualize *in vitro*. The protocol guided the cells through developmental stages: differentiating to intermediate mesoderm (IM), endometrial stromal progenitors (ESP), and finally ESF. Differentiation was characterized by qRT-PCR and ICC. EEO were isolated from term placenta and expanded in culture as organoids following a published protocol. PSC-ESF and EEO were independently dissociated and combined to allow self-assembly in 3D. The two-cell organoids were then exposed to two 16-day periods of decidualization cues, separated by a period of hormone withdrawal and were maintained up to 52 days.

**Results:** Differentiating cells at the IM stage upregulate T, LHX1, and PAX2 as measured by qRT-PCR. The PSC-ESP have increased expression and cell surface localization of AMHR2 as well as region specificity characterized by expression of HOXA10 and HOXA11, but not HOXA9 or HOXA13. PSC-ESF lost expression of AMHR2 while upregulating expression of hormone receptors. PSC-ESF also expressed receptors for

ligands expressed by EEO, such as LIFR, SMO, and NOTCH1/2/3 and were able to co-culture with primary EEO. The PSC-ESF organized in a layer surrounding the EEO and cell type specific expression of stromal (HOXA11, PDGFR $\beta$ , VIM) and epithelial (KRT7, EpCAM, acetylated- $\alpha$ -Tubulin) markers.

**Conclusion:** We have generated a two-cell, 3D model of the endometrium that will enable the study of how these two human cell types interact *in vitro*. Using induced PSC from patients with known disease outcomes, our model can investigate these cell types and how their interaction is affected in diseases such as endometriosis and endometrial cancer. Furthermore, this model will be a valuable platform for the addition of other cell types, such as vascular endothelial cells or fetal trophoblasts, to enable more complex and more representative modeling of the human endometrium *in vitro*.

#### O-141

**The Vertical Transfer of Maternal Immune Cells during Pregnancy Promotes Neonatal Immunity against Viral Infections in Mice.** [Ina Stelzer](#),<sup>1,2</sup> Christopher Urbschat,<sup>1</sup> Kristin Thiele,<sup>1</sup> Ioanna Trivai,<sup>1</sup> Julian Kottlau,<sup>1</sup> Felix Stahl,<sup>1</sup> Maria Emilia Solano\*,<sup>1</sup> Petra Arck\*.<sup>1</sup> <sup>1</sup>University Medical Center Hamburg-Eppendorf, Hamburg, Germany; <sup>2</sup>Stanford University, Stanford, CA, United States.

**Introduction:** The function of vertically transferred immune cells from mother to fetus and their long-term retention in offspring's organs remains poorly defined. We previously detected maternal microchimeric (MMc) cells with an adaptive immune cell phenotype in fetal bone marrow at late murine gestation (E18.5). We here aimed to elucidate a potential functional contribution of MMc to fetal immune development and early life immunity.

**Methods:** F1 offspring were generated from an allogeneic mating model (female (F) C57Bl/6J CD45.2 H-2<sup>b</sup> x male (M) Balb/c CD45.1 H-2<sup>d</sup>), allowing to detect and sort maternal CD45.2 H-2<sup>b</sup> cells from fetal CD45.1/2 H-2<sup>db</sup> cells by flow cytometry. For functional tests, we established a reductionist approach by generating allogeneic pregnancies (F Rag2<sup>-/-</sup>yc<sup>-/-</sup> x M WT) that lack the vertical transfer of adaptive-immune MMc cells (MMc<sup>low</sup>). Offspring from reciprocal matings (F WT x M Rag2<sup>-/-</sup>yc<sup>-/-</sup>) served as controls (MMc<sup>pos</sup>). Neonates were inoculated with murine cytomegalovirus (MCMV) on their first day of life, and viral load determined on neonatal day 7.

**Results:** The presence of MMc promoted the preferential differentiation of sorted fetal hematopoietic stem and progenitor cells (HSPC, E18.5) towards myelopoiesis in comparison to HSPC cultured alone, as observed in an *in vitro* co-culture system. Consistently *in vivo*, MMc<sup>low</sup> fetuses with significantly reduced levels of MMc showed decreased monocyte frequencies among myeloid cells in the bone marrow (E18.5). Strikingly, MMc<sup>low</sup> neonates showed a higher viral load after neonatal MCMV infection in comparison to MMc<sup>pos</sup> controls, which could be rescued by replenishing MMc in dams during pregnancy via adoptive transfer of peripheral adaptive immune cells. The direct involvement of neonatal bone-marrow derived monocytes in reducing MCMV viral load was confirmed *in vitro*, as evident in a significant reduction of MCMV plaque sizes in presence of monocytes.

**Conclusion:** The functional capacity of MMc cells to support stem cell differentiation, monocyte development, and subsequently neonatal immunity establishes that 'cellular parenting' mediated by MMc cells during pregnancy ultimately reduces the severity of early life infections.

#### O-142

**Differentiation of Mouse iPSCs into Functional Oocytes.** [Raymond M. Anchan\\*](#), Nicholas W Ng, Kha U Dam, Ankrish Milne, Emily R Disler, Nicole Dunn, Kevin M Elias, Elizabeth Ginsburg. *Brigham & Women's Hospital, Harvard Medical School, Boston, MA, United States.*

**Introduction:** Premature ovarian failure (POF) can be genetic or treatment-related as seen with reproductive age women exposed to chemotherapy. POF results in the loss of endocrine function and fertility with limited treatment options. In this mouse study, we present a novel concept for fertility and endocrine function restoration using mouse

granulosa cell-derived induced pluripotent stem cells (mGriPSCs) to generate functional oocytes, augment endocrine function, and restore fertility.

**Methods:** mGriPSCs were labeled with the green fluorescent protein (GFP) reporter gene and differentiated in three-dimensional suspension culture into steroidogenic ovarian cells and presumptive oocytes. These differentiated cells were then isolated by fluorescence activated cell sorting (FACS) for the ovarian surface antigen, anti-Mullerian hormone receptor 2 (AMHR2), which by RT-PCR analysis is also expressed in mouse oocytes. Following FACS, 17,000 AMHR2 positive cells were then injected into each ovary of five subfertile, immunodeficient mice. Mice were hyperstimulated and underwent either oocyte retrieval or mating with wild type males to assess neogametogenesis and fecundity.

**Results:** FACS revealed 0.4% of differentiated stem cells were AMHR2 positive. These sorted cells continued to produce estrogen, progesterone, and AMH *in vitro*. One month after injection of sorted cells into mouse ovaries, 44% of oocytes obtained from these mice expressed the GFP reporter, confirming their stem cell origin. Further, these stem cell-derived oocytes were calcium activated or fertilized *in vitro*. When stem cell-injected mice were mated with wild type mice, we obtained three litters, the first of which was sacrificed at embryonic day 18, the second at embryonic day 20, and the third delivered live pups. Overall, 33% of the offspring showed GFP reporter expression by immunohistochemistry (IHC). Additionally, IHC analysis of host mouse ovaries and embryos displayed contribution of the GFP cells to various cell types, confirming the chimeric composition of these tissue.

**Conclusion:** We believe that these exciting results provide translational opportunities to further explore this experimental paradigm using human iPSCs. Patients could potentially be treated with autologous stem cells to restore endocrine function and fertility, therefore providing additional novel options for women with POF.

#### O-143

**HO-1 Genetic Variants Display Racial Diversity and May Impact Hypertensive Disorders in Pregnancy.** Tianyanxin Sun<sup>†</sup>,<sup>1</sup> Nima Mousavi<sup>†</sup>,<sup>2</sup> Ronald J Wong,<sup>1</sup> Nazish Sayed,<sup>1</sup> Joseph C Wu,<sup>1</sup> David K Stevenson,<sup>1</sup> Melissa Gymrek,<sup>2</sup> Virginia D Winn\*.<sup>1</sup> <sup>1</sup>Stanford University School of Medicine, Palo Alto, CA, United States; <sup>2</sup>University of California San Diego, San Diego, CA, United States.

**Introduction:** Hypertensive disorders in pregnancy (HDP) are a leading cause of maternal and fetal morbidity and mortality worldwide. Racial diversity exists for HDP with African women having higher risk while Asian women having overall decreased risk than White women. Genetic polymorphisms in the heme oxygenase-1 (HO-1) regulatory region have been linked to cardiovascular and metabolic diseases. HO-1 is the rate-limiting enzyme that catalyzes the degradation of heme to produce carbon monoxide (CO) and bilirubin, which have both anti-inflammatory and cytoprotective properties. HO-1 polymorphisms at rs3074372 (GTn repeat, S/M/L) and rs2071746 (A/T SNP) can affect HO levels, with shorter GTn repeats and A allele associated with higher HO-1 expression. To explore whether HO-1 genetic variants can predispose women to HDP, we first determined the frequencies of HO-1 variants (GTn repeat length, A/T SNP) among 5 different racial populations [African, American (Hispanic), European (White), East Asian, and South Asian] from the 1000 genome project. We then compared the White HO-1 variant frequencies to an available White HDP cohort.

**Methods:** Sequences of GTn repeats and data on A/T SNP of female individuals were extracted from the 1000 genome phase 3 database using HipSTR v0.6.2 and from ensembl.org, respectively. Genotypes of GTn repeats and A/T SNP of HDP patients were determined using DNA fragmentation analysis and Taqman SNP genotyping, respectively. GTn repeat lengths were defined as short (S,  $\leq 27$ ), medium (M,  $>27$  and  $\leq 35$ ), or long (L,  $>35$ ). Chi-square test was used for statistical analysis ( $p < 0.05$  was considered statistically significant).

**Results:** Both HO-1 genetic variants showed significant racial differences (GTn repeats,  $p < 0.00001$ ; A/T SNP,  $p < 0.00001$ ). The S allele frequency was highest in East and South Asian women; M and A alleles were highest in Hispanic and White women; and L and T alleles were highest in African

women. Of note, the highest risk genotype LL-TT was most common in African women compared to others. The HDP cohort had a higher ratio of the L allele ( $p = 0.02$ ) than White controls while no difference in A/T allele ratios was observed.

**Conclusion:** The two genetic variants of HO-1 show different distributions across racial populations. African women more often had alleles associated with low HO-1 expression in contrast to Asian women who had alleles with highest HO-1 expression. We speculate that these genetic variants may contribute to the racial diversity in HDP. Of the two HO-1 variants, GTn repeat length more likely plays a role in any genetic predisposition to HDP as there was no difference in the A/T SNP ratios between the HDP and control populations.

#### O-144

**Surgically Increasing Uteroplacental Impedance Results in Attenuated Uterine Vascular Remodeling and Preeclampsia-Related Placental Secretomics during Pregnancy.** Nga Ling Ko\*,<sup>1</sup> Narmin Mukhtarova,<sup>1</sup> Catrina Hood,<sup>2</sup> Ying Wai Lam,<sup>2</sup> George Osol.<sup>1</sup> <sup>1</sup>The University of Vermont, Burlington, VT, United States; <sup>2</sup>Proteomics Facility, Vermont Genetics Network, The University of Vermont, Burlington, VT, United States.

**Introduction:** Maternal uterine vascular adaptations are important for maintaining normal uteroplacental (UP) perfusion and preventing pregnancy complications such as preeclampsia (PE) and intrauterine growth restriction. The venous compartment is the major blood reserve of the body. Inadequate venous reserve or compliance, and venous insufficiency during pregnancy are linked to poor reproductive outcome in women. Yet, little is known about the contribution of venous hemodynamics to gestational uterine vascular remodeling. We hypothesized that (1) increasing UP venous impedance leads to slower upstream arterial blood flow (hence less shear stress) and less arterial remodeling; and (2) the placenta alters its secretion of signaling molecules to compensate for, or as a consequence of its underperfusion.

**Methods:** (1) We developed a surgical model of increased uteroplacental impedance (IUPI) in pregnant Sprague-Dawley rats (IUPI;  $n = 10$ ) that involved ligating the main uterine artery and vein (MUA+MUV) at the cervical end to allow single blood inflow/outflow from the ovarian end and putting a silver clip on the MUV at the ovarian end to partially restrict venous outflow. The sham-operated contralateral horn served as control. (2) To analyze the secreted placental proteins using a proteomics approach (secretomics), two placentas from each ligated vs. control horn were collected ( $n = 4$  animals) for *ex vivo* culture for 20 hrs. Proteins in the conditioned media were concentrated by acetone precipitation, then analyzed by WB and liquid chromatography mass spectrometry (LCMS) using isobaric tandem mass tag (TMT).

**Results:** (1) Compared to the control horn, pup weight in the IUPI horn decreased  $5 \pm 1\%$  and placental efficiency (pup/placental weight ratio) was significantly lower ( $6.0 \pm 0.2$  vs  $6.7 \pm 0.2$ ;  $p = 0.02$ ). The anticipated expansive MUA and MUV remodeling was completely abolished with venous flow restriction. (2) Proteomics analysis detected 2305 proteins and 92 of which were differentially regulated ( $p < 0.05$ ). Some upregulated proteins (e.g. fibrinogen  $\beta$  and hemoglobin zeta chain) and down-regulated proteins (e.g. ribosomal proteins, asparagine synthetase and heat shock protein 90) are related to PE. WB analysis verified the upregulation of fibrinogen  $\beta$  subunit in the ligated samples.

**Conclusion:** Increased uteroplacental impedance due to reduced venous outflow decreases maternal uterine vascular remodeling and alters some placental secretory proteins in a way similar to PE, suggesting a significant role of venous hemodynamics in assuring healthy pregnancy outcome. Our novel rat surgical IUPI model may prove useful in identifying some of the underlying mechanisms.

## O-145

**The New Generation Antiplatelet Agent Prasugrel Represents an Exciting Novel Candidate Therapy for Preeclampsia.** Natalie Hannan\*,<sup>1</sup> Natasha De Alwis†,<sup>2</sup> Natalie Binder,<sup>1</sup> Sally Beard,<sup>1</sup> Vi Nguyen,<sup>1</sup> Kaitu'u-Lino Tu'uhevaha,<sup>1</sup> Stephen Tong.<sup>1</sup> <sup>1</sup>University of Melbourne, Parkville, Australia; <sup>2</sup>University of Melbourne, Heidelberg, Australia.

**Introduction:** Preeclampsia (PE) is a serious complication of pregnancy, claiming over 60,000 mothers worldwide and far more neonatal loss. Central to the pathophysiology is the systemic maternal endothelial dysfunction; culminating in hypertension and major end organ damage. Prasugrel, a new generation antiplatelet drug used clinically to prevent in cardiovascular events and stroke, demonstrates key anti-thrombotic, anti-inflammatory properties and thus may be effective in preventing the development of PE. **Objective:** To examine whether prasugrel can mitigate the pathophysiology in human and mouse models of preeclampsia, and compare the effects of prasugrel directly to the antiplatelet aspirin, the current most widely prescribed drug to prevent PE.

**Methods:** Functional studies were performed on human primary: 1) cytotrophoblast 2) endothelial cells 3) and PE placental explants. We tested the effects of prasugrel and aspirin on human tissues at increasing doses (0-100µM) to determine effects on oxidative stress, mitochondrial function, sFlt1 production, endothelial dysfunction (in multiple in vitro models) and vascular reactivity wire myograph studies using pregnant arteries from both women and mice. Importantly we tested the effects of prasugrel in two mouse models of PE.

**Results:** Prasugrel reduced reactive oxygen species (ROS) production and induced nuclear translocation of the transcription factor, Nrf2, as well as enhancing Nrf2 target gene expression. Prasugrel potentially reduced sFlt1 secretion from preeclamptic placental explants and reduced pro-inflammatory cytokine production from preeclamptic placental explants and endothelial dysfunction models. Prasugrel reduced markers of dysfunction (VCAM1 and ET-1) and models of dysfunction (monocyte-endothelial adhesion) and enhanced phosphorylation of endothelial nitric oxide synthase (eNOS). Strikingly, administration of prasugrel significantly reduced blood pressure (hallmark characteristic), as well as reducing circulating markers associated with PE in two mouse models.

**Conclusion:** Prasugrel potentially enhances placental cryoprotection, decreases sFlt1 and pro-inflammatory mediator release, reduced both endothelial dysfunction and blood pressure mouse models of disease. Given they are classified as category B/C drugs and used in the newborn, they represent exciting candidate therapies to prevent and/or treat PE.

## O-146

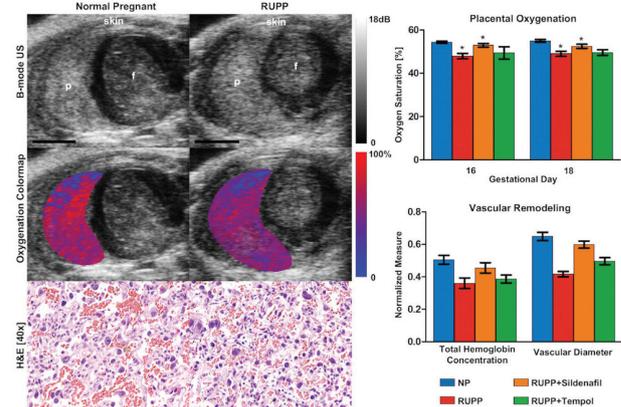
**Sildenafil Improves Placental Function and Remodeling in Preeclamptic Rats.** Dylan J Lawrence†, Carolyn L Bayer\*. Tulane University, New Orleans, LA, United States.

**Introduction:** Preeclampsia is a pregnancy-induced hypertensive disorder that results from abnormal vascular remodeling and placental ischemia during early development. Tempol, a superoxide dismutase mimetic, and the phosphodiesterase inhibitor sildenafil are two potential treatments for preeclampsia that target different mechanistic pathways. While both therapies been shown to improve the maternal symptoms of preeclampsia in preclinical models, the effects on *in vivo* placental function have not been demonstrated. Our aim was to use spectral photoacoustic (sPA) imaging to monitor the longitudinal placental response to tempol or sildenafil treatment in the reduced uterine perfusion pressure (RUPP) rat model of preeclampsia.

**Methods:** Sprague Dawley rats (n=8) were administered tempol or sildenafil via drinking water beginning on gestational day (GD) 12. High-resolution ultrasound (US) and sPA images of the placental environment were acquired on GD14, 16, and 18 with the RUPP surgical procedure implemented immediately after the first imaging session. All sPA images were exported for processing and a linear spectral unmixing algorithm was performed to estimate the concentration of oxyhemoglobin and deoxyhemoglobin. The placenta was then manually segmented using the co-registered B-mode US images of anatomy and the average placental oxygenation and total hemoglobin concentration, a measure of vascular remodeling, were calculated.

**Results:** Placental oxygenation in the RUPP was decreased after the surgical intervention, as compared to normal pregnant (p<0.05). Vascular remodeling was also found to be impaired in RUPP animals. Administration of sildenafil, but not tempol, significantly improved placental oxygenation (p<0.05) and increased vascular remodeling in the RUPP in comparison to untreated RUPP animals.

**Conclusion:** Using ultrasound-guided sPA imaging, we have shown that sildenafil treatment restores placental function in the RUPP rat model of preeclampsia. Our work demonstrates that sPA imaging could be used to monitor differential longitudinal effects of therapeutic intervention on placental growth, independent from maternal symptom management.



**Figure 1:** High-resolution B-mode US images of normal pregnant (left) and RUPP (right) placentas on GD16 acquired using a 20 MHz transducer. A custom oxygenation colormap overlaid on the placenta (p) shows RUPP placentas have increased blue coloring demonstrating the placental hypoxia induced by the RUPP surgery. sPA images of total hemoglobin concentration indicate that vascular remodeling is impaired in the RUPP on GD18, confirmed by maternal vascular space diameter measurements on H&E stained placental sections. Placental oxygenation and vascular remodeling were both improved in RUPP animals receiving sildenafil but unaffected by tempol treatment. Scale bars = 3mm on US images; 0.1mm on H&E images.

## O-147

**Differential Distribution of Tryptophan-Metabolites in Fetal and Maternal Circulations during Pregnancy: Preeclampsia-Elevated Aryl Hydrocarbon Receptor Ligands.** Ying-jie Zhao†,<sup>1</sup> Chi Zhou,<sup>1</sup> Ying-ying Wei,<sup>2</sup> Hui-hui Li,<sup>1</sup> Kai Wang\*,<sup>2</sup> Jing Zheng\*.<sup>1</sup> <sup>1</sup>University of Wisconsin-Madison, Madison, WI, United States; <sup>2</sup>Tongji Univ. School of Medicine, Shanghai, China.

**Introduction:** Preeclampsia (PE) is a leading cause of maternal and fetal morbidity and mortality during pregnancy. L-Tryptophan (Trp) can be metabolized into various biologically active metabolites, including aryl hydrocarbon receptor (AhR; a transcription factor) ligands. These Trp-metabolites regulate many critical biological processes (e.g., vascular and immune responses). However, the concentrations of most Trp-metabolites are unknown in maternal and fetal circulations, particularly in PE.

**Methods:** 25 Trp-metabolites were measured in paired maternal (Mat) and umbilical cord vein (UCV) sera from women with normotensive (NT) pregnancy and PE using LC-MS/MS. 20 women with singleton pregnancies were included in each group (10 with male and 10 with female fetuses).

**Results:** 20 Trp-metabolites were detected in Mat and UCV sera, among which 9 are AhR ligands. The concentrations of 9 Trp-metabolites including 4 AhR ligands (L-Kynurenine, Cinnavalinamine, Indole-3-lactic acid [ILA], and Indoleacetate [IAA]) were higher (P < 0.05) in UCV vs. Mat, whereas 2 (5-hydroxy-L-Tryptophan [L-5-HTP] and Serotonin, an AhR ligand) were lower (P < 0.05) in UCV vs. Mat. Only ILA was elevated (P < 0.05) in PE-Mat (45% above NT-Mat) and PE-UCV (27% above NT-UCV). The concentration of IAA in UCV was 30% higher (P < 0.05) in NT with male vs. female fetuses. Positive correlations between Trp-metabolites (Trp and 3-Indoxyl sulfate in Mat, and ILA in UCV) and maternal mean artery pressure were identified in NT-, but not PE-Mat and UCV. Trp-metabolites in Mat (Kynurenic acid, Picolinic acid, L-5-HTP, and Indoxyl-b-D-glucuronide) and UCV (ILA and Indole-3-propionic acid) were also positively correlated with maternal proteinuria in PE patients.

**Conclusion:** These data indicate that the placenta is a major source of Trp-metabolites and fetal sex does not significantly affect levels of most Trp-metabolites in maternal and fetal circulations during NT and PE. In addition, Trp-metabolites are associated with blood pressure regulation in normal pregnancy; however, PE uncouples these associations. Thus, Trp-metabolites may regulate maternal and fetal endothelial/vascular function during pregnancy. The PE-induced ILA elevation suggests that dysregulation of endogenous AhR ligands may contribute to PE-associated endothelial/vascular dysfunction.

#### O-148

**Can WS1442 Ameliorate Maternal Vascular Dysfunction in Preeclampsia?** Stephanie A Worton†, Yasmin Mills†, Susan L Greenwood\*, Jenny E Myers\*. *University of Manchester, Manchester, United Kingdom.*

**Introduction:** In pre-eclampsia (PE), dysfunction of maternal resistance arteries, which contributes to vasospasm, hypertension and organ hypoperfusion, is characterised by increased sensitivity to vasoconstrictors and impaired endothelium-dependent vasorelaxation. The *Crataegus* (hawthorn) extract WS1442, which is licensed for treatment of heart failure, relaxes resistance arteries via both nitric oxide (NO) and endothelium-derived hyperpolarising factor (EDHF) pathways, but its effects have not been assessed in pregnancy. **Hypothesis:** WS1442 will reduce vasoconstrictor sensitivity and cause endothelium-dependent relaxation in arteries from women with normal pregnancy and PE.

**Methods:** (i) Using wire myography, vasoconstrictor responses to U46619 (thromboxane mimetic) were assessed in omental arteries (<400µm) from normal pregnancy (NP; N=6) and PE (N=3) before and after treatment with WS1442 (100µg/ml; 1hr). (ii) In pre-constricted omental arteries (U46619 EC<sub>80</sub>), relaxation in response to increasing WS1442 concentrations (1-1000µg/ml) was assessed (NP N=8, PE N=5). (iii) To investigate the mechanism of WS1442 activity, concentration-response experiments were repeated in endothelium-denuded omental arteries (N=6), and mouse mesenteric arteries obtained from wild type (WT; N=4) and endothelium-derived NO synthase deficient (eNOS<sup>-/-</sup>; N=4) pregnant animals. Data were compared by 2-way ANOVA.

**Results:** (i) WS1442 treatment reduced U46619-induced constriction in omental arteries from women with NP (post-treatment constriction WS1442 36.8±11.3% vs. vehicle control 77.8±6.9%; p<0.0001), and women with PE (52.5±19.0% vs. 87.4±8.8%; p=0.006). (ii) WS1442 relaxed pre-constricted arteries from NP (remaining constriction WS1442 10.5±1.73% vs. control 36.1±8.8%, p=0.0001), but not PE (32.6±9.4% vs. 47.8±12.8%). (iii) Endothelium removal prevented WS1442-induced relaxation (39.5±11.1% vs. 60.1±10.1%). WS1442-induced relaxation occurred in mesenteric arteries from WT mice (41.0±4.1% vs. 67.5±5.0%; p<0.001), and persisted in eNOS<sup>-/-</sup> mice (34.2±12.3% vs. 70.2±3.5%; p<0.0001).

**Conclusion:** WS1442 substantially reduced agonist-induced constriction of omental arteries from NP and PE, and relaxed pre-constricted arteries from NP but not PE. WS1442-induced relaxation was endothelium-dependent, but independent of eNOS, implicating an EDHF contribution to vasorelaxation. WS1442 merits further investigation as a potential treatment for PE.

#### O-149

**Evaluation of ATR Inhibitors for Targeted Therapy of Ovarian Clear Cell Carcinoma.** Jing Ji,<sup>1</sup> Zhigui Li†,<sup>1</sup> Emily Sherman†,<sup>1</sup> Olorunfoba Osagie†,<sup>1</sup> Shijun Mi,<sup>3</sup> Whitney Soble,<sup>1</sup> Jessie Li†,<sup>1</sup> Gloria Huang\*. <sup>1</sup>Yale School of Medicine, New Haven, CT, United States; <sup>2</sup>Xi'an Jiaotong University, Xi'an, China; <sup>3</sup>Albert Einstein College of Medicine, Bronx, NY, United States.

**Introduction:** ARID1A mutations occur in approximately 50% of ovarian clear cell carcinomas (OCCC) and result in deficient ARID1A protein expression. ARID1A deficiency impairs the DNA damage response that maintains genome integrity. We therefore hypothesized that ARID1A-mutated OCCC may be vulnerable to further inhibition of the DNA damage response using ATR inhibitors. The objective of this study was to

evaluate the usefulness of ATR inhibitors in OCCC and to determine the relationship between ATR sensitivity and ARID1A status using isogenic cell line and xenograft models.

**Methods:** Using stable lentiviral transfection, we generated paired isogenic human OCCC cell lines (ES2 and OVISe) with or without ARID1A expression. ARID1A wildtype ES2 cells were transfected with short hairpin RNA targeting ARID1A, or non-targeting shRNA control. ARID1A mutant OVISe cells were transfected with doxycycline (Dox)-inducible full-length ARID1A cDNA or doxycycline-inducible control cDNA (LacZ). Cells were treated with two different ATR inhibitors (AZD6738 or VX-970), and their effects on cell growth quantified using the Sulforhodamine B (SRB) assay. Immunoblotting and immunofluorescence (IF) were done to evaluate ARID1A knockdown and restoration, and to detect γ-H2AX (a DNA damage marker). For the *in vivo* evaluation of ATR inhibitor therapy, ES2-shARID1A xenografts were grown in athymic nude mice. After randomization, xenograft-bearing mice were treated with an ATR inhibitor (AZD6738) or vehicle control. Comparisons were performed using two-tailed *t*-tests or One-way ANOVA, as appropriate, and a P-value <0.05 was deemed statistically significant.

**Results:** Immunoblotting and IF showed efficient ARID1A knockdown and restoration compared to isogenic controls. ES2-shARID1A cells (ARID1A knockdown) were more sensitive to ATR inhibitors compared to ES2-shCon cells (control); P<0.01 for AZD6738, P<0.01 for VX-970). OVISe-ARID1A+Dox cells (ARID1A restoration) were more resistant to ATR inhibitors than OVISe-ARID1A (no Dox) or OVISe-LacZ+Dox cells (control); P<0.001 for AZD6738, P<0.01 for VX-970. Following ATR inhibitor treatment, increased expression of γ-H2AX by immunoblotting and IF was observed in ARID1A-deficient cancer cells compared to ARID1A-expressing cells. *In vivo*, AZD6738 treatment effectively suppressed tumor growth of ARID1A deficient OCCC; mean tumor volume of treatment group vs vehicle control, P<0.001.

**Conclusion:** ARID1A deficient OCCC is sensitive to ATR inhibition *in vitro* and *in vivo*. These data support clinical evaluation of ATR inhibitors in OCCC and consideration of ARID1A mutational status as a potential predictive biomarker of response to ATR inhibitors.

#### O-150

**Endoplasmic Reticulum Stress Response to Hyperinsulinemia in Endometrial Epithelial Cells Depends on PIK3CA Mutation.** Mike R Wilson†, Ronald L Chandler\*. *Michigan State University, Grand Rapids, MI, United States.*

**Introduction:** Endometrial cancer (EC) was the first cancer identified with an increased risk among obese populations. Obesity is associated with over 60% of EC occurring in the United States and incidence of EC is rising steadily due to the obesity epidemic. Hyperinsulinemia is also known to contribute to EC independent of obesity. However, obesity or hyperinsulinemia alone do not result in EC. Genetic mutations driving EC are well characterized, but these mutations alone are not sufficient for EC pathogenesis as they exist in uteri without cancer. While the PI3K pathway gene PIK3CA is highly mutated EC, recent studies have identified that PIK3CA mutations also occur at a high rates in the endometrial epithelium of women without EC. In normal cells, the PI3K pathway maintains metabolic homeostasis, but in cancer the PI3K pathway is activated to promote cell survival. There is an urgent need to understand the mechanisms by which obesity and genetic mutation interact to promote EC. Metabolic signaling influences protein production and folding. The unfolded protein response pathway maintains protein quality control, and is activated upon endoplasmic reticulum stress. During endoplasmic reticulum stress, if proteostasis cannot be achieved, the unfolded protein response is activated, resulting in cell death. Obesity results in systemic endoplasmic reticulum stress, with hyperinsulinemia as a contributing factor. Our hypothesis is that endoplasmic reticulum stress induced by hyperinsulinemia contributes to the development of EC, and that the effect of hyperinsulinemia depends on endometrial mutation.

**Methods:** We performed *in vitro* studies using immortalized endometrial epithelial cells. Cells were transfected with siRNAs and plasmids and

treated with insulin over 3 days. RNA and metabolites were isolated from the cells for RNA-seq and mass spectrometry, respectively. Statistical tests were performed on all experiments.

**Results:** We identified a unique role for mutant PIK3CA in regulating endoplasmic reticulum stress in response to insulin treatment, an effect which was not observed following loss of EC tumor suppressors PTEN, PIK3R1 or ARID1A. Mutant PIK3CA antagonizes endoplasmic reticulum stress induced by insulin, promoting survival through resistance to unfolded protein response-mediated cell death mechanisms. Using RNA-seq and metabolomics approaches in tandem, we have identified a role for nucleotide metabolism in regulating this process.

**Conclusion:** PIK3CA-mutant endometrial epithelia may be better suited to survival in a hyperinsulinemic environment, and as such, hyperinsulinemia may promote the spread of PIK3CA-mutants throughout endometrial glands. Through understanding endoplasmic reticulum stress resistance in the endometrium, we hope to develop preventative interventions for obese women at risk for EC.

## O-151

**ACS NSQIP - Personalised Risk Prediction Tool for Postoperative Complications in Gynaecology Surgery?** Lusine Sevinyan<sup>†</sup>,<sup>1</sup> Sadie Jones<sup>†</sup>,<sup>2</sup> Jonathan Home<sup>†</sup>,<sup>3</sup> Rasiyah Bharathan,<sup>3</sup> Anil Tailor,<sup>1</sup> Simon Butler-Manuel,<sup>1</sup> Peter Williams,<sup>4</sup> Thumuluru Kavitha Madhuri.<sup>1,5</sup> <sup>1</sup>Royal Surrey NHS Foundation Trust, Guildford, United Kingdom; <sup>2</sup>Cardiff University, Cardiff, United Kingdom; <sup>3</sup>University Hospitals of Leicester NHS Trust, Leicester, United Kingdom; <sup>4</sup>University of Surrey, Guildford, United Kingdom; <sup>5</sup>University of Brighton, Brighton, United Kingdom.

**Introduction:** Despite the informed consent process, patients' understanding of potential post-operative complications is often limited, making it difficult to call the decision an informed one, so estimating the risk of postoperative complications is important for shared decision making and to help multidisciplinary teams plan postoperative care. Increased incidence of gynaecological cancers and operations, especially technically challenging minimally invasive surgery (MIS) in older, obese and patients with multiple comorbidities, requires accurate prediction of the likelihood of mortality and morbidity and patient involvement in joint decision making about the management. ACS-NSQIP (American College of Surgeons National Surgical Quality Improvement Program) risk calculator is a validated web-based tool based on 21 preoperative risk factors to predict 8 post-operative outcomes. The objective of our study was to explore the validity of ACS NSQIP in gynaecology for perioperative prediction of postoperative complications.

**Methods:** A retrospective multicentre cohort study evaluated 1552 patients who underwent surgery at a tertiary oncology centre. Data collection undertaken through dedicated database. Data collated on 764 patients undergoing robotic, 248 laparoscopic and 540 open surgery for suspected or confirmed gynaecological malignancy. All missing data collated from patient notes. Following data lock with the actual post-op event/complication that occurred in this retrospective cohort, ACS NSQIP used to count predictive scores for each patient. Data analysis evaluating ACS-NSQIP validity and relevance in gynaecological oncology patients and its ability to predict postoperative complications performed.

**Results:** ACS-NSQIP was found to best predict mortality (AUC - 0.900), it was most accurate for prediction of complications as follows: discharge to rehabilitation (AUC-0.866), cardiac complications (AUC-0.844), sepsis (AUC-0.795), pneumonia (AUC-0.779), VTE (AUC-0.715), return to theatre (AUC-0.715), surgical site infection (AUC-0.684), readmission (AUC-0.680), renal failure (AUC-0.665). Poor result in the prediction of UTI (AUC-0.561) was noted.

**Conclusion:** ACS-NSQIP risk calculator appears to predict major complications and post-operative mortality making it useful as an informed consent tool. Preliminary data suggests that further validation is required to fully evaluate if the risk scores may be used to inform patients pre-operatively of their risk of complications and is currently being undertaken.

## O-152

**High NLRP7 Expression Promotes Choriocarcinoma Development: Proof of Concept from Clinical and Preclinical Studies.** Deborah Reynaud<sup>†</sup>,<sup>1</sup> Roland Abi Nahed<sup>†</sup>,<sup>1</sup> Nicolas Lemaitre<sup>†</sup>,<sup>1</sup> Pierre-Adrien Bolze\*,<sup>1</sup> Touria Aboussaouira\*,<sup>2</sup> Padma Murthi\*,<sup>3</sup> Rima Slim\*,<sup>4</sup> Mohamed Benharouga\*,<sup>1</sup> Nadia Alfaidy\*.<sup>1</sup> <sup>1</sup>INSERM U1292, Grenoble, France; <sup>2</sup>Hassan II University, Casablanca, Morocco; <sup>3</sup>Monash Biomedicine Discovery Institute, Victoria, Australia; <sup>4</sup>McGill University, Montreal, QC, Canada.

**Introduction:** The inflammatory gene *NLRP7* is the major gene responsible for recurrent complete hydatidiform moles (CHM), a benign abnormal pregnancy that develops in 5-20% of cases into the most malignant form of trophoblastic disease, the gestational choriocarcinoma (CC). Yet, the role of *NLRP7* in the development of CC has not been investigated.

**Methods:** Three approaches were employed to define the role of *NLRP7* in CC development. *i)* a clinical study that analyzed a distinctive collection of human placenta and sera collected from uncomplicated control (n=29), HM (n=26), and CC (n=11) patients *ii)* an *in vitro* study investigating the impact of *NLRP7* knockdown on the proliferation, migration and invasion of the CC cell line, JEG3, using novel 2D and 3D cultures systems, and *iii)* an *in vivo* study using three CC mouse models, including the newly developed CC orthotopic model. Immunohistochemistry, Western blotting, Antibody-array and RT-qPCR analyses were used to characterize the impact of *NLRP7* invalidation on the maternal tumor-associated microenvironment.

**Results:** We demonstrated that placental *NLRP7* is highly expressed in CC patients and in JEG3 cells and functions in an inflammasome-independent manner. *NLRP7* knockdown decreased JEG3 proliferation and tumor organization. *In vivo* models substantiated the direct involvement of *NLRP7* in CC aggressiveness by creating an immunosuppressive microenvironment that fosters the growth and dissemination of tumor cells.

**Conclusion:** The study characterized the critical role of *NLRP7* in CC and proposes that it plays a significant role in CC growth and dissemination. This finding highlights *NLRP7* therapeutic promise as a molecular target.

## O-153

**Microbial-Driven Preterm Labour Involves Crosstalk between the Innate and Adaptive Immune Response.** Denise Chan<sup>†</sup>,<sup>1</sup> Phillip R Bennett,<sup>1,2</sup> Yun S Lee,<sup>1,2</sup> TG Teoh,<sup>1,2</sup> Malko Adan,<sup>1,2</sup> Saqa M Ahmed,<sup>1</sup> Richard G Brown,<sup>1</sup> Anna L David,<sup>3</sup> Holly Lewis,<sup>1</sup> Belen Gimeno-Molina,<sup>1,2</sup> Jane E Norman,<sup>4,5</sup> Sarah J Stock,<sup>4</sup> Vasso Terzidou,<sup>1,2</sup> Pascale Kropf,<sup>1,2</sup> Marina Botto,<sup>1,2</sup> David A MacIntyre,<sup>1,2</sup> Lynne Sykes\*.<sup>1,2</sup> <sup>1</sup>Imperial College, London, United Kingdom; <sup>2</sup>Imperial March of Dimes PRC, London, United Kingdom; <sup>3</sup>UCL, London, United Kingdom; <sup>4</sup>Edinburgh University, Edinburgh, United Kingdom; <sup>5</sup>University of Bristol, Bristol, United Kingdom.

**Introduction:** The vaginal microbiota has a key role in the pathogenesis of spontaneous preterm birth (sPTB). Whilst *Lactobacillus crispatus* has been demonstrated to be protective against cervical shortening and sPTB, depletion of *Lactobacillus* species and high-diversity vaginal microbiota are associated with increased risk of preterm prelabour rupture of membranes and sPTB. Interactions between the host innate and adaptive immune response and vaginal microbiota are not well understood. In this study, we investigate the vaginal microbiota and cervicovaginal fluid immunophenotype of women at high risk of sPTB.

**Methods:** Women at high risk of preterm birth were recruited from preterm birth surveillance clinics at five UK hospitals (n=133). Cervicovaginal fluid (CVF) was collected at three points during pregnancy, 12-16, 20-24 and 30-34 weeks. Bacterial DNA was extracted from CVF and the composition assessed using MiSeq-based 16S rRNA gene sequencing surveying the V1-V2 region and cytokine, complement and immunoglobulin immunoassays were performed on the matched CVF supernatant.

**Results:** *Lactobacillus* species depleted, high-diversity vaginal microbiota were associated with increased mannose binding lectin (MBL), IgM, IgG1-4, and C3b (all p<0.0001), C5 (p<0.001), IL-8 (p<0.01), IL-6 (p<0.001) and IL-1β (p<0.0001). Women with these microbial compositions who subsequently delivered preterm had significantly higher concentrations of MBL, IgM, C3b, C5 and IL-8, IL-6 and IL-1β compared to those who

delivered at term. Cervical shortening was associated with increased relative abundance of *Lactobacillus iners* ( $p < 0.05$ ) and elevated IgM ( $p < 0.01$ ), IgG2 ( $p < 0.05$ ), C3b ( $p < 0.01$ ), C5a, IL-6 and IL-1 $\beta$  (all  $p < 0.05$ ). Vaginal progesterone had no effect on the local immune milieu. In contrast, insertion of a braided cervical cerclage resulted in an augmented immune response and increased risk of sPTB compared to monofilament cervical cerclage (57% vs 20%,  $p = 0.02$ ).

**Conclusion:** We propose that a dysregulated innate and adaptive immune response, bridged by the complement system, can play a role in the mechanism of microbial driven PTB, and provide support for exploring live biotherapeutics and complement therapeutics for preventing sPTB.

### O-154

**Complement Blockade with Eculizumab for Treatment of Pregnant or Postpartum Women with Severe Coronavirus Disease 2019.** Richard M Burwick\*,<sup>1</sup> Gabriela Dellapiana†,<sup>1</sup> Rachel Newman†,<sup>1</sup> Sarah Smithson†,<sup>1</sup> Mariam Naqvi,<sup>1</sup> John Williams III,<sup>1</sup> Melissa Wong,<sup>1</sup> Martha Bautista,<sup>1</sup> Anna Gaden,<sup>1</sup> Shamsah Kazani,<sup>2</sup> Mark Ma,<sup>2</sup> Sanjay Mitter,<sup>2</sup> Jon Monteleone,<sup>2</sup> Stephan Ortiz,<sup>2</sup> Mark Zakowski,<sup>1</sup> Sara Ghandehari,<sup>1</sup> Noah Merin,<sup>1</sup> Ananth Karumanchi\*.<sup>1</sup> <sup>1</sup>Cedars-Sinai Medical Center, Los Angeles, CA, United States; <sup>2</sup>Alexion Pharmaceuticals, Boston, MA, United States.

**Introduction:** Complement activation is increased in patients with severe coronavirus disease 2019 (COVID-19). Eculizumab, an inhibitor of complement protein C5, may aid in the treatment of severe COVID-19, but published reports are limited to non-pregnant adults. We sought to determine if eculizumab is safe and effective for treatment of pregnant or postpartum women with severe COVID-19.

**Methods:** Open label, multicenter, Expanded Access Program (EAP), evaluating eculizumab for treatment of severe COVID-19, beginning August 2020 at Cedars-Sinai Medical Center. Hospitalized patients, including pregnant and postpartum women, were eligible if they had severe COVID-19 with bilateral pulmonary infiltrates and supplemental oxygen requirement. Those with mild to moderate disease were excluded. Eculizumab was given on day 1 (1200mg IV) with additional doses if still hospitalized (Day 4, 8, 15, 22; Day 12, 18 optional). The primary outcome was survival at Day 15. Secondary outcomes were number of days alive and free of mechanical ventilation at Day 15 and 29, improvement of oxygenation, and duration of ICU and hospital stay. We assessed safety and adverse maternal and neonatal outcomes, and pharmacokinetic, pharmacodynamic and complement biomarker studies.

**Results:** The trial is ongoing at time of submission. We present data from eight pregnant or postpartum women enrolled from August 2020 to February 2021; six enrolled during pregnancy (mean 30±4.0 weeks gestation; range 25-35 weeks) and two enrolled postpartum. Baseline oxygen requirement ranged from 2L/min nasal cannula to 12L by non-rebreather mask. The median number of doses of eculizumab was 2 (range 1-3), and median time to discharge was 5.5 days (range 3-12). All participants met the primary outcome of survival at Day 15, and all were alive and free of mechanical ventilation at Day 15 and 29. None required ICU admission, and all were free of supplemental oxygen by Day 29. Free C5 levels were  $< 0.5$   $\mu\text{g/ml}$  after eculizumab treatment (free C5:  $296 \pm 84$  vs.  $0.04 \pm 0.01$   $\mu\text{g/ml}$ ), consistent with complete terminal complement inhibition. Soluble C5b-9 levels also decreased after treatment (sC5b-9:  $1254 \pm 441$  vs.  $400 \pm 179$   $\text{ng/ml}$ ). None of the pregnant participants required preterm delivery due to COVID-19. One delivered at 36w3d for placenta previa; the rest had term deliveries. There were no serious maternal or neonatal adverse events at 3 months of follow up.

**Conclusion:** In this series of pregnant and postpartum women with severe COVID-19, treatment with eculizumab was safe and effective. A larger, controlled study in pregnant women is indicated.

### O-155

**Zika Virus Vertical Transmission Dynamics in the Pregnant Rhesus Macaque.** Michelle R Koenig†,<sup>1</sup> A Mitzey,<sup>1</sup> L T Keding,<sup>1</sup> T A Treadway,<sup>1</sup> H Simmons,<sup>1</sup> A Mejia,<sup>1</sup> M I Bliss,<sup>1</sup> A M Weiler,<sup>1</sup> T Friedrich,<sup>1</sup> X Zeng,<sup>2</sup> D H O'Connor,<sup>1</sup> E L Mohr,<sup>1</sup> T G Golos\*.<sup>1</sup> <sup>1</sup>University of Wisconsin Madison, Madison, WI, United States; <sup>2</sup>Fort Detrick, Frederick, MD, United States.

**Introduction:** In utero, Zika virus (ZIKV) exposure is associated with fetal brain and visual system defects, along with fetal demise and pregnancy loss. Human placental studies show evidence of ZIKV infection; however, little is known about the time course of vertical transmission or maternal-fetal interface (MFI) responses during acute stages of infection. We used pregnant rhesus macaques to determine the trajectory and cellular pathways of vertical ZIKV transmission, hypothesizing that earlier maternal inoculation would increase fetal infection risk.

**Methods:** To evaluate the timing of vertical transmission, we challenged pregnant rhesus macaques with ZIKV and terminated the pregnancy 7, 14, or 30 days post-infection (dpi). MFI and fetal tissues were examined for histopathology, and tissue viral load was measured by qRT-PCR. To test the effect of gestational timing, we infected pregnant macaques at two different times during early pregnancy. Macaques were infected with a Puerto Rican isolate ZIKV-PRVABC59 (ZIKV-PR), or a Senegal isolate ZIKV-DAK AR 41524 (ZIKV-Dak) to compare the vertical transmission of Asian or African lineage viruses. A total of 19 pregnant macaques were inoculated with ZIKV and compared to 10 control pregnancies.

**Results:** We challenged 11 pregnant macaques with ZIKV-PR at gestational day (gd) 45; with pregnancy terminated at 7 dpi (n=2), 14 dpi (n=4) and 30 dpi (n=5). At 7 dpi, 1/2 had virus only in the MFI, at 14 dpi 2/4 had virus detected in the MFI and 1/4 had virus detected in cord blood, and at 30 dpi 3/5 had virus detected in the MFI and 1/5 pregnancies had virus detected in the fetus. We challenged 4 additional pregnant macaques at gd 30 with ZIKV-PR with termination at 14 dpi; 1/4 resulted in fetal demise with no virus in the fetus. 1/4 had virus detected in cord blood and amniotic fluid, and 4/4 had virus detected in the MFI. We then sought to compare the results of ZIKV-PR to ZIKV-Dak. Four pregnant macaques were challenged with ZIKV-Dak at gd 45 and terminated 14 dpi, and virus was detected in 1/4 of the fetuses and 4/4 in MFI tissue.

**Conclusion:** Although vertical ZIKV transmission is well-documented, the window for vertical transmission timeline and mechanism of fetal infection are unknown. Our results suggest a permissive window for ZIKV vertical transmission 1-2 weeks after maternal infection. The fetal demise that occurred, ZIKV-PR at gd 30, suggests that infection during very early pregnancy may be more susceptible to adverse pregnancy outcomes. Additionally, our results suggest that ZIKV-Dak, an African lineage virus, compared to ZIKV-PR, an Asian lineage virus, may lead to more productive infection in the MFI. Future studies will continue to evaluate the effects of genetically diverse viral isolates and gestational timing of infection on ZIKV vertical transmission.

### O-156

**Tracking Postnatal Behavioral Outcomes in a Novel Mouse Model of Congenital CMV Infection.** Gregory Wohl Kirschen†,<sup>1</sup> Anguo Liu,<sup>1</sup> Yang Liu,<sup>1</sup> Ashley Coggins,<sup>1</sup> Jun Lei,<sup>1</sup> Karen Racicot,<sup>2</sup> Andrew Thagard,<sup>3</sup> Irina Burd\*.<sup>1</sup> <sup>1</sup>Johns Hopkins, Baltimore, MD, United States; <sup>2</sup>Michigan State University, East Lansing, MI, United States; <sup>3</sup>Uniformed Services University, Bethesda, MD, United States.

**Introduction:** Cytomegalovirus (CMV) is a ubiquitous bloodborne virus that can cross the placenta and cause devastating effects in pregnancy, such as miscarriage or permanent neurological/neurocognitive effects on the human fetus. Our group recently developed a novel mouse model of congenital CMV infection, allowing for an enriched understanding of the underlying pathophysiology of this disease. Whether this mouse model recapitulates neurodevelopmental behavioral features of human CMV infection remains unknown. Such knowledge would add to the validity of this model and would serve as a springboard for further work into the underlying neural disruptions that results from in utero infection with CMV.

**Methods:** Timed-pregnant, CD1 mice were inoculated with  $5 \times 10^5$  m murine (m)CMV or vehicle (mock) by intrauterine (IU) inoculation at embryonic day (E)10 (n=5 dams/group). We tested newborn pups on

postnatal day (PND) 5 and 9 on three assays that require integration of multiple sensory systems and gross motor coordination: cliff aversion, negative geotaxis, and surface righting. Standard statistics were employed.

**Results:** Plaque assays of placenta and fetal brain at 120 hours post-inoculation confirmed vertical transmission of the virus. mCMV-infected dams gave birth to significantly fewer viable pups (86.0% vs. 69.1%,  $p < 0.001$ ). At PND5, mCMV-infected pups performed significantly poorer than controls on cliff aversion and negative geotaxis tasks, with no difference on surface righting ( $p < 0.01$ ,  $p < 0.05$ ,  $p > 0.05$ , respectively). We found no differences between groups on any of the tasks on PND9.

**Conclusion:** These findings suggest that our model recapitulates elements of congenital CMV infection by demonstrating the impairment in early postnatal neurologic function. Our data raise interesting questions regarding the underlying neurodevelopmental programs and network connectivity that may be disturbed by CMV-induced neural injury/inflammation, and compensatory mechanisms that the plastic, neonatal brain is able to enact to counteract such perturbations..

### O-157

**Adopting 3D Methods to Culture Endometrial Stromal Cells Enhances Their Decidualization Response.** Kira Buttrey†, Juan S Gnecco, Alexander Brown, Clara Ives, Linda G Griffith\*. *Massachusetts Institute of Technology, Cambridge, MA, United States.*

**Introduction:** Endometrial stromal cells (ESCs) are hormone responsive, fibroblast-like cells which undergo decidualization upon exposure to progesterone. The mechanisms driving decidualization remain poorly characterized despite the importance of the process in both healthy and diseased endometrial states. Traditionally, ESCs are cultured and expanded on polystyrene, which gives cells non-physiological cues and leads to loss of hormone responsiveness with increasing passages. We hypothesized that adopting 3D synthetic hydrogels to ESC culture would provide an environment more representative of tissue, allowing for an enhanced hormone response over a greater number of passages.

**Methods:** Primary samples of healthy endometrial stromal cells were isolated from tissue biopsies collected from patients at Newton Wellesley Hospital under informed consent (n=3). 3D cultures involved encapsulating cells in 3uL, fully defined hydrogel droplets, while 2.5D samples were plated on top of flat layers of gel (30,000 cells / sample for all groups). Within each group, cells were treated with either estrogen (E2) or E2 with Medroxyprogesterone acetate (MPA). Cultures were maintained for 15 days, and prolactin (PRL) secretion (ELISA) was measured as a marker of decidualization and analyzed using a t-test. Cells were phenotypically characterized by immunostaining and confocal microscopy to assess morphological state and relevant protein expression.

**Results:** Compared to 2D cultures, ESC cultured in 3D hydrogels had an enhanced progestin-induced decidualization response (8.3 vs 65.7-fold change from E to EP, respectively;  $p < 0.05$ ). ESCs remained significantly hormone responsive in 3D cultures through at least passage 7 while 2D cultures were largely unresponsive by passage 5. Adjusting gel stiffness led to changes in ESC remodeling but not PRL secretion, emphasizing how different environmental contexts can induce difference ESC morphologies while suggesting that the decidualization response is not tied to a single visual morphology in 3D. Culturing ESCs in 2.5D did not induce the enhanced PRL secretion seen in 3D ( $p < 0.05$ ). This suggests that the dimensionality of 3D cultures more so than the softness allows for an enhanced decidualization response. The enhanced response in 3D was seen in both traditional and fully defined media formulations.

**Conclusion:** Culturing ESCs in 3D hydrogels allows for a more robust progestin-induced decidualization response than traditional 2D cultures. Furthermore, this responsiveness is maintained over a greater number of passages and does not depend on a particular media context. 3D culturing methods therefore extend the application of primary ESCs and provide a means to investigate the role of the extracellular matrix in decidualization.

### O-158

**Dysregulation of Primordial Follicle Growth Activation Regulatory Factor Expression in a Granulosa Cell Model of Galactosemic Primary Ovarian Insufficiency.** John Rushing†, Evelyn Llerena Cari, Synneva Hagen-Lillevik, Amanda Kallen, Alex J. Polotsky, Kent Lai, Joshua Johnson\*. <sup>1</sup>University of Colorado School of Medicine (AMC), Aurora, CO, United States; <sup>2</sup>University of Utah School of Medicine, Salt Lake City, UT, United States; <sup>3</sup>Yale University School of Medicine, New Haven, CT, United States.

**Introduction:** Classical Galactosemia (CG) arises from loss of function of galactose-1-phosphate uridylyltransferase (GALT). CG is often characterized by early primary ovarian insufficiency (POI) despite maintenance of a lifelong galactose-restricted diet. Some information is available about toxic metabolites produced in the absence of GALT, but mechanistic understanding of CG-POI is lacking. We generated mouse granulosa cells (GC) null for *Galt* to test how CG specifically impacts GCs. We compared *GaltKO:GC* versus parent OV3121 cell growth, and their expression of regulators of primordial follicle growth activation (PFGA) *Tumor necrosis factor alpha receptor 2 (Tnfr2)*, and *Antimüllerian hormone receptor 2 (Amhr2)*. Last, we assessed cisplatin (CIS) sensitivity to test if loss of *Galt* renders cells defective in their response to genotoxic insult.

**Methods:** CRISPR:Cas9 was used to generate *GaltKO:GC* cells. Comparison of cell growth was performed by direct cell counts and also CellTag fluorescence. qRT-PCR was performed on replicates of parent or *GaltKO:GC* cells treated as follows. Cells were cultured in DMEM:F12 media (5% FBS + Pen/Strep), treated with vehicle(s) PBS or EtOH, or, with 10 ng/ml Tnfa, 10 ng/ml Amh, combined Tnfa + Amh, or, 20 uM CIS. Statistical tests used were Welch's two sample t-test or ANOVA with Bonferroni post-hoc test. P-values <0.05 were considered statistically significant.

**Results:** *GaltKO:GC* cells grow significantly slower than parents, doubling every 27.6 hours versus 21.2 hours, respectively. qRT-PCR revealed altered *Amhr2* and *Tnfr2* expression. *GaltKO:GC* express significantly lower levels of *Amhr2* (47% of control) and *Tnfr2* (77% of control) transcripts. Treatment with Amh, Tnfa, or both agents resulted in a distinct response in *GaltKO:GC* where both *Amhr2* and *Tnfr2* were upregulated approximately two-fold versus vehicle controls. Parent cells treated identically did not upregulate *Amhr2* or *Tnfr2*. No difference was detected in the fraction of cells killed by CIS after 24 hours. Despite this, CIS treatment resulted in a 50% decrease in *Amhr2* expression in surviving parent cells, but no such decrease occurred in *GaltKO:GC*.

**Conclusion:** *GaltKO:GC* cells appear to be a robust tool suitable to probe the consequences of the loss of GALT function in CG-POI. Combined with prior published work, these preliminary studies suggest that key positive (*Tnfr2:Tnfa*) and negative (*Amhr2:Amh*) regulators of PFGA are aberrantly regulated in the absence of *Galt*. In addition, the lack of enhanced sensitivity to CIS-induced death does not favor compromised response to genotoxic insult as contributing to follicle loss in CG.

### O-159

**A Multiomics Approach to Placental Dysfunction in Common Obstetrical Syndromes.** Oren Barak†, Samantha Piekos†, Tianjiao Chu, Elena Sadovsky, Jean-Francois Moulliet, Yingshi Ouyang, Lee Hood, Nathan Price, Yoel Sadovsky\*. <sup>1,2</sup>Magee Womens Research Institute, Pittsburgh, PA, United States; <sup>2</sup>University of Pittsburgh, Pittsburgh, PA, United States; <sup>3</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>4</sup>Onegevity Health, New York, NY, United States.

**Introduction:** Despite its high prevalence, the pathogenesis of human placental dysfunction (PD) and its related clinical complications remains unknown. We used a high throughput multiomics approach to better define the molecular phenotypes of conditions related to PD, including preeclampsia (PE), fetal growth restriction (FGR), and spontaneous preterm delivery (sPTD).

**Methods:** Using our comprehensive Magee Obstetrical Maternal Infant (MOMI) Database and Biobank, we identified women with the following conditions: severe PE (n=75), FGR (birthweight<3rd centile; n=37), FGR with a hypertensive disorder (FGR+HDP; n=29), sPTD (n=72), and two control groups: 1) uncomplicated, term pregnancy with birthweight >10th centile (n=113) 2) Induced PTD or elective cesarean section without PE, FGR or preterm labor (n=16). Placental biopsies, snap-frozen in liquid

nitrogen, were used for targeted proteomics (453 proteins), untargeted metabolomics, and transcriptomics (processing underway). We deployed unsupervised dimensionality reduction algorithm, partial least squares-discriminant analysis (PLS-DA), and hierarchical clustering to evaluate differences between cohorts. We executed differential analysis among the cohorts to identify misregulated proteins and metabolites. Finally, we performed n-of-1 analysis in which outlier analytes (not in the normal baseline [1,99]) represent biomolecular signatures indicative of the disease state.

**Results:** While there were no clear clusters between cohorts and the controls using the unsupervised algorithms, applying PLS-DA supervised learning to the metabolomics data displayed clear separation of the cohorts from the controls. This was driven by a higher expression of lipid and vitamin metabolism pathways in the FGR/FGR+HDP groups. We also identified analytes that were differentially expressed between each disease group and the controls. We validated our approach using known differentially expressed analytes, such as sFLT-1 and PIGF. Finally, individual women had a significantly higher number of outlier protein values across the pathological cohorts, compared to the control. Transcriptomics analysis as well as hierarchical clustering to reveal placental disorder subgroups is currently underway.

**Conclusion:** Multiomics analyses of the placental tissue provide new insights into the biological pathways underlying PD and identify potential biomarkers and biosignatures indicative of a disease state. Future integration of this multiomics approach with clinical and histopathological data may improve our ability to define and treat clinical syndromes that emanate from PD.

## O-160

**Determining the Impact of Environmental Toxin Cadmium at the Feto-Maternal Interface Using an Organ-on-Chip (FMI-OOC) Device.** Sungjin Kim†,<sup>1</sup> Lauren Richardson†,<sup>2</sup> Enkhtuya Radnaa†,<sup>2</sup> Zunwei Chen†,<sup>1</sup> Ivan Rusyn,<sup>1</sup> Ramkumar Menon\*,<sup>2</sup> Arum Han\*.<sup>1</sup> <sup>1</sup>TAMU, College Station, TX, United States; <sup>2</sup>UTMB, Galveston, TX, United States.

**Introduction:** At term, labor is associated with multiple fetal- and maternal-derived signals that coordinate to initiate delivery of the fetus. Exposure to toxic environmental chemicals may prematurely trigger labor-associated inflammation at the feto-maternal interface (FMI: decidual-fetal membrane interface), leading to preterm birth (PTB). However, cell-specific response leading to FMI cell inflammation that contributes to PTB onset remains unclear. Using a recently developed and characterized microfluidic organ-on-a-chip system for FMI (FMI-OOC) that mimics the physiological functions and responses of *in vivo* FMI, this study tested the effect of maternal exposure to cadmium (Cd), a well-reported environmental toxin associated with PTB.

**Methods:** The FMI-OOC contains four concentric circular chambers designed to culture immortalized and well-characterized fetal membrane cells (decidua, chorion trophoblasts, and amnion [mesenchyme and epithelium]) and collagen matrix. Decidual cells were treated with CdCl<sub>2</sub> (1 and 10 μM) mimicking maternal exposure, and its effects across the FMI towards the fetal cell compartments. Effects on cell cycle (flow cytometry), cell death (apoptosis/necrosis staining), and inflammation (Luminex assay) were analyzed; along with concentrations of CdCl<sub>2</sub> across FMI.

**Results:** Within the FMI-OOC, CdCl<sub>2</sub> propagated to the chorion within 48h. CdCl<sub>2</sub> induced cell death ( $p=0.02$ ) and a pro-inflammatory environment in the decidua (low IL-10 and high TNF- $\alpha$  secretion) ( $p=0.02$ ), but had minimal effect on the fetal chorion cells with no effect on the amnion. The effect was dose dependent.

**Conclusion:** FMI-OOC maintained intercellular interactions as seen *in vivo* and allowed us to determine the response from a specific compartment. CdCl<sub>2</sub> produced a maternal, but not fetal, response within the FMI-OOC. This observation is in line with *in vivo* patient data which has shown that Cd-mediated adverse effects are mediated by maternal pathophysiological response rather than fetal-derived triggers of preterm labor. We postulate that intact chorion layer functions as a barrier for various substances and its compromise is essential for propagation of toxic substances.

## O-161

**Entinostat Increases Sensitivity to Olaparib in a Homologous Recombination Proficient Syngeneic Mouse Model of Ovarian Cancer.** Vijayalaxmi G Gupta\*,<sup>1</sup> Yosklay L Fernandez,<sup>2</sup> Katherine F Roby,<sup>2</sup> Fiona Yull,<sup>3</sup> Marta A Crispens,<sup>4</sup> Andrew J Wilson,<sup>4</sup> Harsh B Pathak,<sup>2</sup> Andrew K Godwin,<sup>2</sup> Andrea Jewell,<sup>2</sup> Dineo Khelele.<sup>1</sup> <sup>1</sup>Washington University St. Louis, St. Louis, MO, United States; <sup>2</sup>University of Kansas Medical Center, Kansas City, KS, United States; <sup>3</sup>Vanderbilt School of Medicine, Nashville, TN, United States; <sup>4</sup>Vanderbilt University Medical Center, Nashville, TN, United States.

**Introduction:** Ovarian cancer is the deadliest gynecologic malignancy. Approximately 50% of high grade serous ovarian cancers are homologous-recombination (HR) proficient and relatively resistant to poly (ADP-ribose) polymerase inhibitors (PARPi). We have shown that combining the histone deacetylase inhibitor entinostat with the PARPi olaparib has preclinical efficacy in human xenograft immunocompromised models of ovarian cancer. Here, we determine the effects of entinostat plus olaparib in an immunocompetent syngeneic mouse model.

**Methods:** We treated HR proficient ID8 mouse ovarian cancer cells with entinostat, olaparib, or both. We performed cell viability, clonogenicity, and DNA damage assays. Cell viability was replicated in genetically defined ID8-P53<sup>-/-</sup> (HR proficient) and ID8-P53<sup>-/-</sup>BRCA2<sup>-/-</sup> (HR deficient) cells. We injected ID8 cells intraperitoneally into syngeneic mice, which were treated with entinostat, olaparib, or both for 21 days. We performed immunohistochemistry staining for markers of: proliferation (Ki67), anti-tumorigenic M2 macrophages (mannose receptor), pro-tumorigenic M1 macrophages (CCL3), and an immune checkpoint (PD-L1).

**Results:** Compared to olaparib, entinostat plus olaparib significantly decreased ID8 cell viability ( $P<0.0009$ ), had no effect on clonogenicity ( $P<0.2$ ), and significantly increased DNA damage ( $P<0.0001$ ). Entinostat plus olaparib was no more effective than olaparib alone in inhibiting viability in ID8-P53<sup>-/-</sup> ( $P<0.67$ ) or ID8-P53<sup>-/-</sup>BRCA2<sup>-/-</sup> ( $P<0.06$ ) cells. Compared to olaparib, ID8 tumors from mice treated with entinostat plus olaparib showed significantly decreased Ki67 ( $P<0.0006$ ), and mannose receptor ( $P<0.0001$ ), whereas increased CCL3 ( $P<0.0001$ ) and PD-L1 ( $P<0.0001$ ).

**Conclusion:** Entinostat plus olaparib reduced HR proficient ID8 mouse ovarian cancer cell growth *in vitro* and *in vivo*. ID8 tumors from mice treated with the combination showed more anti-tumorigenic M1-like macrophages and fewer pro-tumorigenic M2-like macrophages, along with increased PD-L1 expression. This suggests that entinostat plus olaparib in HR proficient ovarian cancer not only has direct effects on tumor cells but also modulates the tumor immune microenvironment. Future studies will investigate the possibility that entinostat sensitizes tumors to a PD-L1 inhibitor.

## O-162

**Factors Associated with Referral-Based Receipt of Fertility Consultation Among Reproductive Age Women with Pre-invasive or Invasive Gynecologic Malignancies.** Ruoxi Yu†, Anna L Beavis\*, Mindy S Christianson\*, Kala Viswanathan\*, Akila N Viswanathan\*, Rebecca L Stone\*. Johns Hopkins University School of Medicine, Baltimore, MD, United States.

**Introduction:** For reproductive age women diagnosed with pre-invasive or invasive gynecologic malignancies (GM), access to reproductive endocrinologists and infertility (REI) specialists is key to understanding fertility preserving options prior to gonadotoxic cancer treatment. We examined patient factors associated with successful completion of fertility consultation after referral to REI in this population.

**Methods:** We performed a retrospective cohort study of women ages 18-45 seen and referred for newly diagnosed cervical cancer (CC), endometrial intraepithelial neoplasia (EIN) or endometrial cancer (EC), and borderline ovarian tumor (BOT) or ovarian cancer (OC) at a single academic center between 2015-2019. ANOVA and Kruskal-Wallis test were performed to compare differences between disease type. We then examined patient factors associated with our primary outcome, completed REI consultation, using univariate log binomial regression. Due to small sample size, Firth's logistic regression was used to analyze factors with  $p$ -value  $<0.1$  in a multivariable model.

**Results:** Of 92 total patients with documented fertility referral, 65 patients (71%) completed REI consultation. Of those referred, 87% of women with

CC (n=13) consulted REI, compared to 74% with BOT/OC (n=29) and 61% with EIN/EC (n=23). Age, BMI, race, income, relationship status, having  $\geq 1$  child, and known metastasis were not significantly associated with REI consultation after referral. Groups by disease type did not differ significantly by age or parity. In the multivariable model, women with government-sponsored insurance were 73% less likely to complete REI consultation compared to those with private insurance (OR=0.27, 95% CI[0.08,0.90];p=0.03). While not statistically significant, compared to women with BOT/OC, women with EIN/EC were 47% less likely to complete REI consultation (OR=0.53, 95% CI[0.19,1.4];p=0.21), whereas those with CC were 179% times more likely (OR=1.79, 95% CI[0.42,10.6];p=0.45).

**Conclusion:** The majority of women with pre-invasive or invasive GM completed REI consultation. However, having government-sponsored insurance was independently associated with lower likelihood of accessing fertility resources, and women with EIN/EC were least likely to complete REI consultation. This demonstrates the need for improvement in access to fertility counseling at the time of pre-invasive or invasive GM.

Characteristics of Patients Referred to REI			
	Total Referred (n=92)	Completed REI Consultation	
		Yes (n=65)	No (n=27)
Median Age (years) at Diagnosis [Min, Max]	33 [19, 45]	33 [19, 45]	33 [19, 43]
Median BMI (kg/m <sup>2</sup> ) at Diagnosis [Min, Max]	27.3 [18.7, 59.1]	26.4 [18.7, 55.7]	28.5 [18.9, 59.1]
Race			
White/Caucasian	46 (50%)	32 (49%)	14 (52%)
Black/African American	27 (29%)	17 (26%)	10 (37%)
Other	19 (21%)	16 (25%)	3 (11%)
Insurance Type			
Private	77 (84%)	59 (91%)	18 (67%)
Government Sponsored	13 (14%)	6 (9%)	7 (26%)
Other	2 (2%)	0 (0%)	2 (7%)
Median Household Income (\$) [Min, Max]	85,400 [38,700, 203,000]	86,400 [38,700, 203,000]	78,800 [40,200, 147,000]
Relationship Status at Diagnosis			
Partnered	43 (47%)	32 (49%)	11 (41%)
Unpartnered	49 (53%)	33 (51%)	16 (59%)
Had at Least 1 Child			
Yes	11 (12%)	7 (11%)	4 (15%)
No	81 (88%)	58 (89%)	23 (85%)

## W-001

**Association between Daily Melatonin and Cortisol Rhythms and Gestation Length.** Ronald T. McCarthy,<sup>1</sup> Peinan Zhao,<sup>1</sup> Anjana Delhi,<sup>1</sup> Nandini Raghuraman,<sup>1</sup> Emily S Jungheim,<sup>1</sup> Justin C Fay,<sup>2</sup> Erik D Herzog,<sup>3</sup> Sarah K England\*.<sup>1</sup> <sup>1</sup>Washington University School of Medicine, St. Louis, MO, United States; <sup>2</sup>University of Rochester, New York, NY, United States; <sup>3</sup>Washington University in St. Louis, St. Louis, MO, United States.

**Introduction:** The ability to identify women at high risk of preterm birth (PTB) remains limited. One risk factor for PTB is shift work (1), suggesting that altered circadian rhythms may play an important role. Two convenient circadian markers are the hormones melatonin and cortisol, which peak at night and in the morning, respectively. Here, we asked whether alterations in melatonin and cortisol rhythms are associated with length of gestation and risk of preterm birth.

**Methods:** Pregnant volunteers wore wrist actigraphy devices (MotionWatch8, CamNtech, Cambridge, UK), completed sleep surveys

(Munich Chronotype Questionnaire), and provided saliva every four hours for a 24-hour period each trimester. Melatonin and cortisol were measured by ELISA (Salimetrics, USA). Cosinor analysis was used to define hormone profiles as having strong rhythms (sinusoidal with high peak-to-trough amplitude) or low amplitude (not sinusoidal). We analyzed the time of peak hormone concentration and daily mean, maximum, and minimum concentrations. We compared length of gestation and the risk of PTB between patients with strong and low amplitude cortisol and melatonin rhythms.

**Results:** We enrolled 1459 participants and 1220 had live births. Of those with live births, 429 (35.2%) had melatonin and 505 (41.4%) had cortisol data that were included in the 1<sup>st</sup> trimester analysis. Study participants' melatonin and cortisol profiles were reproducible across trimesters. For melatonin, 340 (79.3%) participants had strong rhythms, whereas 89 (20.7%) had low amplitude rhythms. For cortisol, 410 (81.2%) had strong rhythms, and 95 (18.8%) had low amplitude rhythms. Women with low amplitude rhythms in both melatonin and cortisol also had greater daily variations in their sleep period start times. Although women with low amplitude melatonin rhythms had shorter gestations (low group =38.3wks vs strong group = 39.0wks,  $P < 0.001$  by log-rank test), there was no difference in rate of PTB <37 weeks between low amplitude and strong melatonin rhythms. (20.2% vs. 12.9%,  $P = 0.12$ ). Women with low amplitude cortisol rhythms had similar gestation lengths ( $P = 0.14$  by log-rank test, low group =39.0wks vs strong group =39.0wks) and no difference PTB <37 weeks (17.9% vs. 12.9%,  $P = 0.27$ ) compared to strong cortisol rhythms.

**Conclusion:** Women with low amplitude melatonin rhythms have shorter gestations than those with strong rhythm profiles whereas low amplitude cortisol rhythms are not associated with length of gestation.

## W-002

**Neurosteroids and Steroid Hormones in Preterm Birth.** Gabriella Mayne<sup>†</sup>, Peter E. DeWitt,<sup>2</sup> Brandy Ringham,<sup>2</sup> Anna Warener,<sup>1</sup> Uwe Christians,<sup>2</sup> Dana Dabelea,<sup>2</sup> K. Joseph Hurt\*.<sup>3</sup> <sup>1</sup>University of Colorado, Denver, CO, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>University of Colorado School of Medicine, Aurora, CO, United States.

**Introduction:** Neurosteroids such as allopregnanolone (ALLO) play important roles in stress pathophysiology. Low stress-responsive ALLO is associated with perinatal depression, but preterm birth is also associated with maternal stress. Animal models show associations between low ALLO and reduced gestational length, and neurosteroids may directly inhibit uterine contractility via myometrial  $\gamma$ -aminobutyric acid receptor activation. We hypothesized that women who deliver preterm have lower maternal ALLO compared with women who deliver at full term. We evaluated maternal serum ALLO and other steroid hormones at two points in gestation and investigated a preterm birth prediction model using clinical and maternal demographic factors.

**Methods:** We performed a nested case-control study using banked serum samples. We included healthy women with singleton pregnancy and excluded mothers with major medical illness, preeclampsia, or chronic hypertension. We matched preterm cases with term controls (1:1) by gestational age (GA) at first blood sample and the least difference in time between samples (N=27 per group). We used a novel, validated high-performance liquid chromatography tandem mass spectrometry assay for ALLO and five other steroids. We used ANOVA, T-test, linear regression and AUROC for statistical analyses and predictive modelling.

**Results:** We found no significant difference in maternal preterm or term ALLO at the examined timepoints (sample 1 at 16.9 weeks mean GA: 4.46 $\pm$ 1.71 ng/mL vs 4.38 $\pm$ 1.70 ng/mL, p=0.87; sample 2 at 26.5 weeks mean GA: 7.44 $\pm$ 3.01 vs 7.84 $\pm$ 3.77, p=0.67). However, we found that preterm cases had higher cortisol levels for sample 1 (325  $\pm$  120 ng/mL vs. 253  $\pm$  107 ng/mL, p=.024) and lower percent change between samples (53  $\pm$  63% vs. 103  $\pm$  96%, p=.029). We created a model for preterm birth with cortisol and demographics (AUROC 0.64). We improved the model slightly by adding ALLO and five other clinical and demographic characteristics (AUROC = 0.69).

**Conclusion:** We found no significant difference in ALLO levels during gestation between pregnancies ending with pre- or full-term delivery. Further evaluation in larger cohorts with additional gestational timepoints may be informative. Cortisol showed some utility as an early pregnancy biomarker for preterm birth prediction. Our models suggest future studies using maternal clinical and demographic factors and steroid hormone serum concentrations for preterm birth risk assessment.

#### W-003

**Adverse Events Due to Inflammation Are Successfully Prevented by an Allosteric Modulator of IL-6R in a LPS Mouse Model of Preterm Birth.** Elizabeth Prairie,<sup>1</sup> France Côté†,<sup>1</sup> Laurence Gobeil,<sup>2</sup> Sarah-Eve Loisel,<sup>1</sup> Xin Hou,<sup>1</sup> Christiane Quiniou,<sup>1</sup> David Olson,<sup>3</sup> Sylvain Chemtob\*.<sup>1</sup> <sup>1</sup>Université de Montréal, Montreal, QC, Canada; <sup>2</sup>Université de Sherbrooke, Sherbrooke, QC, Canada; <sup>3</sup>University of Alberta, Montreal, QC, Canada.

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

**Methods:** An established LPS-induced PTB model on timed pregnant mice was used to evaluate the degree of inflammation in GT, maternal plasma and amniotic fluid as well as fetal lungs and intestines. Pregnant mice were injected with LPS (10µg/kg i.p.) at gestational day 16 in presence or absence of **HSJ633** (1mg/kg/12h), Tocilizumab (TOC; 10mg/kg/12h), or vehicle. All experiments were compared with TOC, an anti-IL6R antibody commercially available.

**Results:** Concomitant treatment with **HSJ633**, and to a lesser extent TOC, reduced the gene expression of pro-inflammatory cytokines and UAP (*IL-1β*, *IL-6*, *TNFα*, *CCL2*, *Casp1*, *OxTR*, *IL-12* and *PGES*) and expression of pro-inflammatory proteins (IL-1β, IL-6 and TNFα) in GT, maternal plasma and amniotic fluid. Furthermore, **HSJ633** reduced abnormal morphologies in the offspring induced by inflammation (number and level of atrophy of the intestinal villi as well as the number and size of the pulmonary alveoli). Immuno-histological analysis of **HSJ633**-FITC revealed presence in the placenta but not in the fetus.

**Conclusion:** Collectively, our data show that **HSJ633** antagonized the activity of IL-6R in a LPS-induced PTB model, and improved birth outcome by increasing survival and preserving fetal organ integrity. These findings highlight the importance of IL-6 and uncover in vivo pharmacological efficacy of a novel IL-6R modulator. **HSJ633** is a promising new therapeutic prototype in prevention of PTB.

#### W-004

**Progesterone Withdrawal-Induced Inflammatory Drive of Cervix Ripening and Preterm Birth Is Linked to Morphology of Macrophage Phenotypes.** Olivia G Beck, Michael A Kirby, Steven M. Yellon\*. Loma Linda University, Loma Linda, CA, United States.

**Introduction:** In mammals before term, the cervix ripens with loss of response to progesterone (P4) as characterized by degradation of cross-linked extracellular collagen, reduced density of cell nuclei in the stroma, and increased density of resident macrophages (Mφs). In vitro, Mφ morphology distinguishes anti-inflammatory (elongated M2, eM) from inflammatory (round M1, rM) phenotypes. Thus, the objective of this in vivo study was to determine whether premature cervix ripening before preterm birth (PTB) reflects a shift to an inflammatory-like Mφ morphology.

**Methods:** Pregnant CD1 mice were given the P4 receptor (PR) antagonist RU486 or were ovariectomized (Ovx) on pregnancy day 16 to induce PTB within 24h. Other Ovx mice were given the PR agonist R5020 to block PTB. Cervix sections were obtained from prepartum mice 6h after

treatment (day 16.5). Cervix sections from vehicle-treated and sham-operated (days 15, 16.5 and 18) served as controls. The density and shape of F4/80-stained Mφs were evaluated in the cervix stroma.

**Results:** In the cervix stroma from controls, total Mφs, as well as the major rM and eM subtypes increased by the day before birth (day 18 vs 15 or 16.5). A novel, though minor polymorphic subset (pM) also increased by day 18 vs 16.5 of pregnancy. Within 6h of RU486 treatment, the density of all Mφ subtypes increased vs controls, ~15h before PTB (day 17), a >2 day advance before term birth. Moreover within 6h of Ovx and before PTB, resident rM increased, but neither eM nor pM density changed. Importantly, PR agonist treatment of Ovx mice not only prevented PTB, but blocked the rise in rM and increased the presence of eM without an affect on pM density. Thus, the density of the rM phenotype was temporally related to loss of P4 efficacy (PR antagonist or Ovx-reduced systemic concentrations) that advanced cervix ripening and induced PTB. By contrast, the purported anti-inflammatory eM phenotype correlated with PR agonist-forestalled cervix remodeling and blocked PTB in R5020-treated Ovx mice.

**Conclusion:** Findings support the hypothesis that the balance of Mφ phenotypes tips towards a pro-inflammatory subtype which may promote cervix stroma remodeling in these models for PTB. Efficacy of a PR agonist to block preterm parturition following loss of systemic P4 appears to promote an anti- over pro-inflammatory Mφ phenotype. These models for the mechanism of parturition before labor advance the possibility that biomarkers for Mφ polarization in the prepartum cervix may forecast PTB risk. In conjunction with previous findings that inflammatory changes related to reduced CN density and a decline in cross-linked collagen in the cervix stroma of mice days before birth, the results may lead to innovative local immunotherapeutic approaches that block or promote inflammatory-mediated ripening before or at term.

#### W-005

**The Anti-Inflammatory Properties of MicroRNA-125 Limit the Vasoobliteration in a Rat Model of Oxygen-Induced Retinopathy.** Maëlle Wirth†,<sup>1,2</sup> Michel Desjarlais,<sup>1</sup> Isabelle Lahaie,<sup>1</sup> Samy Omri,<sup>1</sup> Rabah Dabouz,<sup>1</sup> José-Carlos Rivera,<sup>1</sup> Sylvain Chemtob.<sup>1,3</sup> <sup>1</sup>Maisonneuve-Rosemont Hospital Research Center, Montréal, QC, Canada; <sup>2</sup>Université de Montréal, Montréal, QC, Canada; <sup>3</sup>Centre Hospitalier Universitaire Sainte-Justine Research Center, Montréal, QC, Canada.

**Introduction:** Excessive retinal inflammation is a hallmark in the progression of the retinopathy of prematurity (ROP), and is strongly associated to retinal vasoobliteration. Dysregulation of microRNAs (miRs), key negative regulators of genes expression, has been implicated in the ocular inflammation. However, the role of miRs in inflammatory process during ROP pathogenesis, and especially on activated-microglial cells, remains to explore. This study aimed to investigate the potential anti-inflammatory role of miR-125 in a rat model of Oxygen-Induced Retinopathy (OIR).

**Methods:** qRT-PCR and western blot were performed respectively to evaluate the expression of miR-125 and inflammatory cytokines in retinal tissues of OIR rats compared to normoxic healthy rats and also in activated microglial cells (SIM-A9) subjected or not to hyperoxia and LPS. In vitro: miR-125 function on inflammation on SIM-A9 by LPS or hyperoxia was performed using a miR-125 mimic. The angiogenic properties of Human Retinal Microvascular Endothelial Cells (HRMEC) treated with the culture medium of SIM-A9 pre-subjected to hyperoxia and transfected or not with miR-125 mimic, were analyzed by a matrigel assay. In vivo: OIR rat pups were intravitreally supplemented with miR-125 mimic (10 nmol/kg) or a control-miR at P5 during the vasoobliteration phase of the cycling OIR model (50/10% O<sub>2</sub> from P1 to P14). Retinal tissues were collected at P10 to analyze the inflammatory markers and the vascular density by retinal lectin-immunostaining.

**Results:** We found that miR-125 expression is significantly reduced in the retina and choroid of OIR rats compared to control rats, and in SIM-A9, correlated with an upregulation of key proinflammatory cytokines including TNF-α, IL-6 and IL-16. Interestingly, we found a significant decrease of TNF-α, IL-6 and IL-16 expression in SIM-A9 transfected with miR-125. HRMECs exposed with the culture medium of SIM-A9

pre-subjected to hyperoxia showed a decrease in their angiogenic capacity, which is recovered by the overexpression of miR-125. In vivo, OIR rat pups intravitreally supplemented with miR-125 mimic displayed a significantly decrease of TNF- $\alpha$ , IL-6 and IL-16 in the retina, associated with lower vasoobliteration area compared to the control at P10.

**Conclusion:** This study suggests that miR-125 acts as an inflammatory suppressor of activated-microglial cells protecting microvascular density during OIR. miR-125-based therapy could potentially constitute a novel anti-inflammatory therapeutic strategy to limit vascular degeneration in ischemic retinopathy and notably, during ROP.

#### W-006

**Mid-Trimester Changes in Cervicovaginal Metabolites Are Distinct in Women with and without Preterm Birth.** Megan Cavanagh<sup>†</sup>, Emmanuel Amabebe<sup>†</sup>, Neha Kulkarni<sup>†</sup>, Dilly Anumba\*. *University of Sheffield, Sheffield, United Kingdom.*

**Introduction:** The interaction between host cells and microbes in the vagina leave metabolic fingerprints that may be associated with infection and spontaneous preterm birth (sPTB, <37 weeks' gestation). In order to unravel the pathomechanism of sPTB and improve risk stratification and prediction, we investigated metabolite changes in cervicovaginal fluid (CVF) across mid-trimester in asymptomatic high risk pregnant women that deliver preterm compared to term metabolite profiles.

**Methods:** Cervicovaginal fluid was obtained from asymptomatic high risk women at gestational time point (GTP)1 (20-22 weeks, n=50) and GTP2 (26-28 weeks, n=46). Metabolomic analysis was performed using Waters Acquity UPLC coupled to a Waters Synapt G2-Si Time of Flight mass spectrometer with electrospray sample introduction. Samples were introduced directly into the mass spectrometer using the UPLC only as an automated injector without the use of chromatography. Extraction was performed and the aqueous fraction was analysed in both positive and negative modes. Differences in normalised metabolite % total ion count between term and preterm women were determined by OPLS-DA and Students' *t*-test.

**Results:** There were no significant differences in metabolites between term and preterm women at either GTPs. However, the changes in metabolites from GTP1 to GTP2 expressed as GTP2/GTP1 ratio differed between the groups. Carbohydrate derivatives (p=0.007), aminosugars and nucleotides (p=0.008), lipids, flavonoids and amines (p=0.004), fatty acids (p=0.01), steroids and vitamins (p=0.01) were altered between GTPs in different directions in preterm vs term women.

**Conclusion:** We have observed distinct metabolic changes between women with and without preterm birth from mid to late second trimester. These findings could provide predictive biomarkers for spontaneous preterm birth before symptoms of labour appear and could reveal new preterm birth associated pathways.

#### W-007

**Prostaglandin F2 $\alpha$  Does Not Induce an Inflammatory Response in Murine Macrophages and Pregnant Mouse Uterine Explants Ex Vivo.**

Madeline Snedden,<sup>1</sup> Chandrashekar Kyathanahalli,<sup>1,2</sup> Emmet Hirsch\*.<sup>1,2</sup>  
<sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States;  
<sup>2</sup>University of Chicago, Chicago, IL, United States.

**Introduction:** PGF2 $\alpha$  is thought to participate in labor by inducing uterine smooth muscle contraction. Inflammation has been implicated in the onset of labor both at term and preterm. We investigated the role of PGF2 $\alpha$  in generating inflammation in 3 pregnancy-relevant tissues cultured *ex vivo*: a) RAW-Blue<sup>TM</sup> cells (modified from the immortalized mouse macrophage cell line RAW 264.7 to express a colorimetric reporter under the control of NF- $\kappa$ B and AP1); b) peritoneal macrophages (PMs) freshly extracted from non-pregnant mice; and c) pregnant mouse uterine explants.

**Methods:** RAW-Blue<sup>TM</sup> cells were cultured (10<sup>5</sup> cells/well) with or without PGF2 $\alpha$  (1-125  $\mu$ M) or LPS (0.2 ng/ml, a positive control) in duplicate for 5h. Resident PMs obtained by lavage from virgin CD1 mice (8-16 weeks old) were cultured in triplicate with or without PGF2 $\alpha$  (5  $\mu$ M, 5h). Day 14.5 pregnant uterine explants were stimulated with or without PGF2 $\alpha$  (5  $\mu$ M) or LPS (10 ng/mL) in duplicate for 4h. To test whether estradiol (E2) influences expression of contraction-associated proteins

(CAPs), uterine explants were treated with E2 at 0 pM, 10 pM (equivalent to non-pregnant serum levels), 100 pM (physiologic pregnancy levels near term), or 1000 pM (supraphysiologic) for 8 or 24h in triplicate. The expression of interleukin (*Il-1 $\beta$* , *Il-6*, tumor necrosis factor (*Tnf*), oxytocin receptor (*Oxtr*), and prostaglandin F receptor (*Ptgfr*) were analyzed by qPCR. Western blot was performed for PTGFR, OXTR, connexin 43 (Cx43), and pERK1/2. Statistical differences were assessed by ANOVA or Kruskal-Wallis and post hoc *t*-test or Dunn's test for parametric and non-parametric data, respectively.

**Results:** As expected, LPS induced markers of inflammation in each cell type: NF- $\kappa$ B/AP1 (RAW-Blue<sup>TM</sup>), *Il-6* (PMs), *Il-1 $\beta$* , and *Tnf $\alpha$*  (explants). In contrast, PGF2 $\alpha$  did not activate NF- $\kappa$ B/AP1 in RAW-Blue<sup>TM</sup> or *Il-6* in PMs. PTGFR protein was not detected in RAW 264.7 macrophages, but was present in pregnant mouse uterus, both before and during tissue culture. Stimulation with 100 pM E2 for 24h decreased *Ptgfr* mRNA 2-fold compared to control, but E2 treatment did not alter PTGFR, OXTR, Cx43, or pERK1/2 protein levels compared to baseline. Despite the presence of PTGFR in uterine tissues, PGF2 $\alpha$  did not induce *Il-1 $\beta$* , *Tnf $\alpha$* , or *Oxtr* mRNA.

**Conclusion:** PGF2 $\alpha$  does not generate an inflammatory response in murine macrophages and pregnant uterine explants cultured *ex vivo*. In macrophages, this may be explained by absence of the prostaglandin F receptor, but this does not account for results seen in uterine tissues. In contrast to other studies, E2 failed to increase CAP expression in uterine explants. The pro-labor and uterine contractile actions of PGF2 $\alpha$  during pregnancy may not be mediated through inflammatory pathways.

#### W-008

**A Systematic Review of Prenatal Interventions in Preclinical Infection and Inflammation Preterm Birth Models.** Faith Miller, Anna L David, Ashley K Boyle<sup>†</sup>\*. *University College London, London, United Kingdom.*

**Introduction:** Preterm birth (PTB; <37 weeks gestation) affects approximately 11% of all births worldwide and is associated with adverse neonatal outcomes. Approximately 40% of spontaneous PTBs are associated with infection. Current treatment options are limited. Animal models are essential for the development and testing of new therapeutic interventions. The objective of this is systematic review is to summarise treatments for infection/inflammation-induced PTB applied in preclinical models of PTB.

**Methods:** Searches using medical subject headings (MeSH) and keywords were performed in PubMed, EMBASE and Web of Science, in accordance with PRISMA guidance. MeSH and keyword themes included "animal models", "preterm birth", "inflammation" and "therapeutics". Two independent researchers screened studies and extracted data. The inclusion criteria were formulated using the PICOS framework. Participants: animals with infection/inflammation-induced PTB. Intervention: prenatal interventions to prevent PTB. Comparison: an appropriate vehicle control for the intervention. Outcome: effect of the intervention on gestational length and maternal inflammation. Study type: Original quantitative, peer-reviewed, and controlled studies. The presence of bias in methodological design was also determined.

**Results:** Our searches identified 3,916 studies. Twenty-three studies met our inclusion criteria. All studies utilised mouse models and PTB was most commonly induced by lipopolysaccharide (LPS) or *Escherichia coli* (*E. coli*). However, the doses, serotypes and routes of delivery varied. No two interventions were the same. Gestational length was significantly prolonged in 19 of the 23 studies and markers of maternal inflammation were significantly reduced in 20 studies. All studies were assigned an unclear risk of bias.

**Conclusion:** We identified several treatments that successfully target maternal inflammation and hold promise as therapeutic agents of PTB. However, we were unable to perform a meta-analysis due to the heterogeneous nature of the data. Importantly, this systematic review highlights the poor methodological reporting of preclinical studies. Better standardisation of animal models of PTB is urgently required to improve the reproducibility of preclinical studies, to allow for meaningful comparison of the intervention efficacy and aid clinical translation.

## W-009

**Estrous Cycle-Dependent Differential Inflammatory Profiles and Responses to Bacterial Lipopolysaccharide of Mouse Peritoneal Macrophages.** Chandrashekar N Kyathanahalli,<sup>1,2</sup> Madeline Snedden,<sup>1</sup> Emmet Hirsch,<sup>1,2</sup> <sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States.

**Introduction:** Immune responses are known to be dictated in part by hormonal milieu. In the mouse, circulating estrogen levels peak prior to ovulation, which occurs during estrus, while progesterone levels rise during metestrus and diestrus. We sought to characterize the inflammatory profiles of mouse resident peritoneal macrophages (RPMs) across the estrous cycle both at baseline and after a low dose inflammatory stimulus.

**Methods:** Vaginal smears were obtained from virgin CD1 female mice (8-16 weeks) and stained with 0.1% w/v crystal violet solution to identify estrous cycle stage. Peritoneal cells were harvested by lavage with ice-cold PBS from euthanized mice in estrus, metestrus, and diestrus (n=3 each). Cells were plated 10<sup>5</sup> cells/well in 96-well plates in DMEM complete medium and incubated for 2h. To characterize basal cytokine expression, cells in triplicate wells were immediately lysed and frozen at -80°C. Adherent macrophages were cultured overnight (~18h) before stimulation with LPS (0.2 ng/ml) or control for 6h. Total RNA was extracted. qPCR was performed to quantify the expression of interleukin (*Il-6*, *Il-1 $\beta$* , *Il-10*, nitric oxide synthase 2 (*Nos2*), and 18S rRNA (housekeeping gene). Statistical differences were assessed by Kruskal-Wallis followed by Dunn's post hoc test.

**Results:** Diestrus phase was characterized by high basal levels of *Il-6* (30X) over estrus, but low *Nos2* (undetectable). No significant differences were found in expression of *Il-1 $\beta$*  and *Il-10* between estrus, metestrus and diestrus. LPS induced *Il-6*, *Nos2*, *Il-1 $\beta$* , and *Il-10* at all stages of the estrous cycle, with no differences between stages except for LPS-induced *Nos2*, whose expression was lower in estrus than in metestrus and diestrus. After 24h of culture in the absence of LPS exposure, *Il-6* levels in diestrus macrophages decreased to match those of estrus and metestrus. In contrast, in estrus macrophages, expression of *Nos2* and *Il-1 $\beta$*  increased significantly over 24h of culture (5X and 125X over diestrus). Culture-associated changes were minimal for *Il-10* at all estrous stages.

**Conclusion:** Macrophages play roles in homeostasis, antimicrobial defense, and tissue repair. Here we show that the baseline and LPS-induced inflammatory state (*Il-6*, *Il-1 $\beta$*  and *Nos2* expression) of peritoneal macrophages is lowest in estrus. Lower inflammatory responses to LPS during estrus suggest an overall blunted inflammatory milieu at this ovarian cycle stage. Prolonged (24h) culture of peritoneal macrophages significantly alters their expression profiles for inflammatory mediators.

## W-010

**The Vaginal Microbiome and the Risk of Preterm Birth: A Systematic Review and Network Meta-Analysis.** Unnur Gudnadottir<sup>†</sup>, Justine Debelius, Juan Du, Luisa W. Hugerth, Hanna Danielsson, Ina Schuppe-Koistinen, Emma Fransson\*, Nele Brusselaers\*. Karolinska Institutet, Stockholm, Sweden.

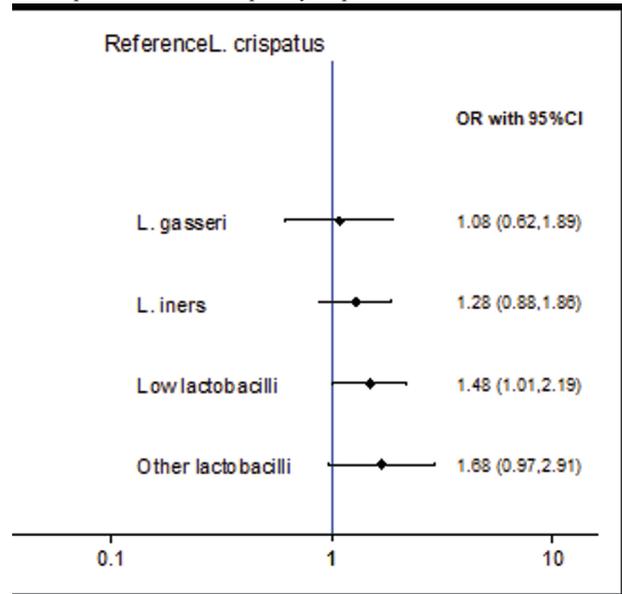
**Introduction:** Preterm birth is the major cause of neonatal mortality and morbidity worldwide. Many factors can trigger premature start of labour, including various infections and inflammation factors. Such infections of the vaginal cavity are assumed to be held at bay by the vaginal microbiome, which "normally" has low diversity and is dominated by *Lactobacillus* species. Vaginal microbiome types can be sorted into so-called community state types (CSTs) depending on which (if any) *Lactobacillus* spp is most abundant. *With this study we aim to give a clearer image on the correlation between preterm birth and the vaginal microbiota, since current literature is inconsistent.*

**Methods:** In this systematic review and network meta-analysis we looked for articles on the vaginal microbiota and preterm birth to investigate if there are specific microbiome compositions more correlated with preterm birth than others. We searched PubMed, Web of Science, Embase and Cochrane Library and selected all studies assessing the vaginal microbiome by means of non-culture dependent methods reporting 3 or more CSTs in relation to the risk of preterm birth. After compiling the data from relevant studies, the CSTs were grouped into five categories

based on dominating species: *L. crispatus*, *L. gasseri*, *L. iners*, other *Lactobacilli* and low *Lactobacillus* spp. The cumulative proportions of low *Lactobacillus* spp. in each study were pooled and weighted and presented as weighted percentages. To enable direct and indirect comparisons between all CSTs, we used a fixed network meta-analysis approach.

**Results:** Fifteen cohort studies, which were published from 2014 - 2021, were included in the meta-analysis. Although the risk of preterm birth was consistently higher among women presenting with low lactobacilli (compared to any of the three specific *Lactobacillus* spp. dominated CSTs), this association only reached statistical significance when comparing to *L. crispatus* (OR 1.48, 95% CI 1.01-2.19) (Fig 1). When comparing the different *Lactobacillus* species, the risk of preterm birth was lowest among women presenting with *L. crispatus*, but none of these associations reached statistical significance.

**Conclusion:** Our results, in combination with future larger studies, could potentially help early diagnosis and detection of women with a higher risk of preterm birth and hopefully help reduce the risk.



## W-011

**Bromodomain and Extra-Terminal (BET) Epigenetic Reader Expression and Function in Decidual Stromal Cells.** Tamás Zakár\*,<sup>1,2,3</sup> Sandeep Ajaonkar<sup>†</sup>,<sup>1</sup> Jonathan J Hirst\*,<sup>1,3</sup> <sup>1</sup>University of Newcastle, Callaghan, Australia; <sup>2</sup>John Hunter Hospital, New Lambton Heights, Australia; <sup>3</sup>Hunter Medical Research Institute, New Lambton Heights, Australia.

**Introduction:** We have reported previously [S-019, Repr Sci 26:1 (Suppl p305A)] that epigenetic chemical probes that disrupt the binding of BET proteins to their acetylated histone recognition sites block the expression of pro- and anti-inflammatory genes in decidual stromal cells. This suggests that BET-mediated epigenetic mechanisms are involved in the control of the pro- and anti-inflammatory activities of the decidua, which is critical for pregnancy maintenance and labor induction at term. Here we continued exploring the system by determining (i) the expression of BET family members in decidual stromal cells and (ii) BET protein binding and acetyl histone-3 (aH3) and -4 (aH4) levels at the promoters of the prototypical anti-inflammatory and proinflammatory genes *IDO1* and *IL6*, respectively, in decidual stromal cells treated with lipopolysaccharide (LPS) and the BET-disrupting epigenetic probe (+)-JQ1.

**Methods:** Stromal cells were isolated from decidua tissue at term, immunopurified and cultured in medium supplemented with estradiol and medroxyprogesterone acetate. Sub-confluent cultures were treated with 0.5  $\mu$ M (+)-JQ1 for 72h. LPS (1 $\mu$ M) or vehicle was added for the last 24h. Total RNA was isolated and mRNA levels encoding BET family proteins

were determined by real-time quantitative RT-PCR. Formaldehyde-fixed cells were processed for chromatin immunoprecipitation to determine BET protein binding and aH3 and aH4 levels at the *IDO1* and *IL6* promoters.

**Results:** Messenger RNAs encoding the BET family members BRD2, -3 and -4 were detected in decidual stromal cells. *BRD2* and -4 mRNA abundance were 9-fold higher than that of *BRD3*, indicating that BRD2 and -4 were dominant. LPS had no effect on *BRD* mRNA levels, but (+)-JQ1 treatment caused a 4.2-fold increase ( $P < 0.05$ ,  $N = 5$ ) of *BRD2* mRNA abundance suggestive of negative autoregulation. LPS treatment formerly shown to stimulate *IDO1* and *IL6* expression increased histone-3 and -4 acetylation and BRD4 binding at the *IDO1* and *IL6* promoters, while pre-treatment with (+)-JQ1, which blocked expression, reduced aH3, aH4 and BRD4 levels at the promoters of both genes ( $P < 0.05$   $n = 3-4$ ).

**Conclusion:** BRD4, and possibly BRD2, have critical importance in the control of pro- and anti-inflammatory gene expression in decidual cells by interacting with aH3 and/or aH4 at the promoter regions. Notably, BRD binding and histone acetylation are co-regulated at the target promoters. Genome wide studies are warranted to assess the overall importance of this epigenetic mechanism in maintaining the pregnancy-protective anti-inflammatory properties of the decidua and in promoting the switch to an inflammatory phenotype at labour.

#### W-012

**Cervicovaginal Microbiota and Immune Output: Potential Determinants of Spontaneous Preterm Birth in Black Women.** Kristin D Gerson<sup>†</sup>, Clare McCarthy, Heather H Burris, Michal A Elowitz\*, *University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.*

**Introduction:** Significant racial disparities persist in sPTB. Non-optimal cervicovaginal (CV) microbiota have been linked to spontaneous preterm birth (sPTB). Select CV immune responses appear to mitigate this microbiota-associated risk in a racially dependent manner. The prevalence of a non-optimal microbiota is higher in Black women, yet how the CV immune response might alter sPTB risk among Black women with the same non-optimal CV microbiota has not been studied.

**Methods:** This was a secondary analysis of a prospective pregnancy cohort. Term births and sPTB were selected 2:1 among Black women. Term births were included if delivered between 38-40 weeks without chorioamnionitis. CV microbiota was analyzed with 16S rRNA gene sequencing and microbial communities classified into community state types (CST) from swabs at 16-20 weeks. CST I, II, III, and V are predominated by *Lactobacillus*, while CST IV is a non-optimal community enriched in anaerobes with a relative lack of *Lactobacillus*. Cytokines were measured by Luminex, and antimicrobial peptide beta-defensin ( $\beta$ D) was measured by ELISA. Cytokine detection was stratified into high, medium and low groups.  $\beta$ D was dichotomized at the median into high and low groups. A sensitivity analysis assessed differences in the proportion of detectable samples between sPTB and term. Wilcoxon rank-sum and ANOVA tests compared cytokine abundances, and multivariate logistic regression assessed associations between significant cytokines and sPTB.

**Results:** Among the 248 Black women studied, IL-6 was increased in sPTB, while IL-2 was decreased in sPTB ( $p = 0.014$  and  $p < 0.001$ , respectively). Eleven of 14 cytokines were higher in CST IV vs non-CST IV ( $p < 0.05$  for all). After stratifying by CST and birth outcome, median cytokine abundance was higher in CST IV/sPTB and CST IV/term vs non-CST IV/sPTB and non-CST IV/term birth. Median  $\beta$ D was lower in sPTB vs term birth ( $p < 0.001$ ). There were no interactions between cytokines and CST IV, or between cytokines and  $\beta$ D, on the outcome of sPTB. In sensitivity analyses, the proportion of detectable samples differed significantly between sPTB and term births for IL-6, GM-CSF, IL-15, IL-5, and TNF- $\alpha$  ( $p < 0.05$  for all).

**Conclusion:** Detection of immune mediators in the CV space in pregnancy appears distinct from profiles outside of pregnancy. Immune response differs by optimal versus non-optimal CST. Among Black women with non-optimal CST, immune mediators may identify those at greater risk for sPTB. R01NR01478 (ME)

Table 1. Association between cytokine abundance and CST IV ( $n = 246$ )<sup>\*</sup>

Cytokine tertile	Range (units)	Unadjusted OR	aOR <sup>b</sup>	p-value
<b>IL-1<math>\beta</math></b>				
Low	0-1.62	Ref	Ref	Ref
Medium	1.66-18.82	4.16 (2.0-8.5)	5.76 (2.6-12.7)	<0.001
High	19.9-1335.6	45.59 (18.0-115.4)	71.2 (25.7-197.5)	<0.001
<b>IL-8</b>				
Low	0-270.6	Ref	Ref	Ref
Medium	277.2-1358.3	1.55 (0.8-2.9)	1.76 (0.9-3.3)	0.083
High	1395.9-2179100	2.49 (1.3-4.7)	3.07 (1.6-6.0)	0.001
<b>MCP-1</b>				
Low	0-8.7	Ref	Ref	Ref
Medium	8.7-23.2	0.95 (0.5-1.8)	0.98 (0.5-1.8)	0.959
High	24.0-4577.5	0.7 (0.4-1.3)	0.7 (0.4-1.4)	0.342
<b>IL-6</b>				
Low	0.02-0.42	Ref	Ref	Ref
Medium	0.43-0.9	2.07 (1.1-3.9)	2.11 (1.1-4.0)	0.022
High	0.92-2762.0	4.23 (2.2-8.1)	4.84 (2.5-9.5)	<0.001

<sup>\*</sup> $n = 2$  women missing  $\beta$ D

<sup>b</sup>Adjusted for low  $\beta$ D

#### W-013

**The Use of Peripheral Blood Neutrophil Counts in the Prediction of Funisitis Following Preterm Prelabour Rupture of Membranes.** Lara Budwig<sup>†</sup>,<sup>1</sup> Richard Brown,<sup>1</sup> Yun S Lee,<sup>1,2</sup> Katherine Mountain<sup>†</sup>,<sup>1,2</sup> Belen Gimeno-Molina<sup>†</sup>,<sup>1,2</sup> Malko Adan,<sup>1,2</sup> Erna Bayar,<sup>1,2</sup> David A MacIntyre,<sup>1,2</sup> Phillip R Bennett,<sup>1,2</sup> Lynne Sykes\*.<sup>2,1</sup> *<sup>1</sup>Imperial College, London, United Kingdom; <sup>2</sup>Imperial College March of Dimes PRC, London, United Kingdom.*

**Introduction:** Preterm prelabour rupture of fetal membranes (PPROM) occurs in approximately 30% of all spontaneous preterm births. It is strongly associated with the presence and/or the development of histological chorioamnionitis and funisitis. The presence of funisitis is particularly associated with adverse neonatal outcomes such as neonatal sepsis, cerebral palsy and chronic lung disease. Local neutrophil infiltration of the chorioamnion is the hallmark of histological chorioamnionitis, and hence this study set out to determine whether maternal peripheral neutrophil counts could be predictive of chorioamnionitis and funisitis at the time of delivery and/or at the time of PPRM.

**Methods:** A retrospective study was performed on 122 women who presented with PPRM between 2012-2019. Cerner<sup>©</sup> patient electronic records were used to collect maternal blood neutrophil counts, C-reactive protein (CRP) values and placental histology results both at the time of presentation following PPRM (maternal only) and at the time of delivery. Statistical analyses were carried out using the GraphPad Prism 8 software.

**Results:** Of the 122 women studied; 54 women had normal histology, 8 women had histological evidence of chorioamnionitis, and 60 women had funisitis. The median gestation of PPRM was 33<sup>+2</sup> (31<sup>+0</sup>-34<sup>+5</sup>), 24<sup>+6</sup> (23<sup>+7</sup>-31<sup>+0</sup>) and 25<sup>+5</sup> (24<sup>+1</sup>-28<sup>+0</sup>), and the median gestation of delivery was 34<sup>+2</sup> (32<sup>+6</sup>-35<sup>+1</sup>), 24<sup>+4</sup> (24<sup>+1</sup>-31<sup>+2</sup>) and 26<sup>+5</sup> (25<sup>+0</sup>-29<sup>+2</sup>) in women with normal histology, chorioamnionitis and funisitis respectively. A significant increase in neutrophil counts were seen in women who had funisitis at the time of delivery (14.45 x 10<sup>9</sup>/L compared with 10.03 x 10<sup>9</sup>/L,  $p < 0.0001$ ) and at the time of PPRM (11.12 x 10<sup>9</sup>/L compared with 8.248 x 10<sup>9</sup>/L,  $p < 0.01$ ). A significant increase was also seen in the CRP in women who had funisitis at the time of delivery (66.74 mg/L compared with 7.881 mg/L,  $p < 0.001$ ), and at the time of PPRM (26.61 mg/L compared with 5.668 mg/L,  $p < 0.001$ ). Using a neutrophil count of  $\geq 14.45$  x 10<sup>9</sup>/L and a CRP count of  $\geq 43$  mg/L at the time of PPRM, the false positive rate was 5% and the positive predictive value was 85% for both parameters to predict the presence of funisitis.

**Conclusion:** This study highlights the potential benefits of using maternal peripheral neutrophil counts, in combination with CRP, to aid clinical decisions on the timing of delivery in women with PPRM.

#### W-014

**Dampened Response to Bacterial Lipopolysaccharide by Mouse Peritoneal Macrophages during Pregnancy.** Chandrashekara N Kyathanahalli,<sup>1,2</sup> Madeline Snedden,<sup>1</sup> Emmet Hirsch,<sup>1,2</sup> *<sup>1</sup>NorthShore University HealthSystem, Evanston, IL, United States; <sup>2</sup>University of Chicago, Chicago, IL, United States.*

**Introduction:** Macrophages are thought to play important roles in establishing, maintaining, and completing healthy pregnancies. We used mouse resident peritoneal macrophages (RPMs) to study different

functions of this cell population before and during pregnancy at baseline and in response to simulated infection using bacterial lipopolysaccharide (LPS) *in vitro*.

**Methods:** Peritoneal cells were isolated by lavage with ice-cold PBS from euthanized virgin female CD1 mice (8-16 weeks old) in diestrus and from pregnant mice at gestation days (GD) 4.5, 14.5, and 18.5 (n=3 each). Cells were plated 10<sup>5</sup> cells/well in a 96-well plate in DMEM medium and incubated for 2h to allow macrophages to adhere. Medium was changed, and adherent cells in triplicate wells were immediately harvested to characterize basal cytokine expression. Remaining RPMs were cultured overnight (~18h) before stimulation with LPS (0.2 ng/ml) or control for 6h. Total RNA was extracted. qPCR was performed for interleukin (*Il*)-6, *Il*-1 $\beta$ , *Il*-10, nitric oxide synthase 2 (*Nos2*), and 18S rRNA (a housekeeping gene). Data were analyzed using the ddCt method. Statistical differences were assessed by Kruskal-Wallis followed by Dunn's post hoc test.

**Results:** Basal *Il*-6 levels in RPMs did not vary significantly between different gestation days, but all were significantly ( $p < 0.05$ ) lower in pregnancy than in diestrus. In contrast, *Nos2* was undetectable in diestrus, but increased during pregnancy, reaching significance on GD18.5. Collectively, *Il*-1 $\beta$  and *Il*-10 expression were significantly lower at GD4.5 compared to E14.5 (*Il*-1 $\beta$  and *Il*-10, 4-fold) and E18.5 (*Il*-10, 2-fold). After 24h of culture, *Il*-1 $\beta$  and *Nos2* increased, while *Il*-6 and *Il*-10 expression decreased in all samples, with highest values for all four of these transcripts found in GD 18.5 macrophages. LPS markedly enhanced the expression of *Il*-6, *Nos2*, *Il*-1 $\beta$ , and *Il*-10 in all samples. However, LPS-induced *Il*-6 expression was consistently lower in pregnancy than in diestrus. Across gestation, the magnitude of *Il*-10 induction following LPS-exposure decreased, with a significant reduction observed on GD18.5 compared to diestrus.

**Conclusion:** In this study, we characterized inflammatory responses of mouse peritoneal macrophages in the non-pregnant and pregnant states. Macrophages undergo a shift toward a diminished proinflammatory profile during pregnancy, both in the absence and the presence of an inflammatory stimulus. Prolonged (24h) culture alters the inflammatory profile of peritoneal macrophages.

## W-015

**Human Leukocytes Express Different Chemokine Receptors at Term and Preterm Labor.** Han Lee<sup>†,1,2</sup>, Nanlin Yin<sup>\*,2</sup>, Zheng Liu<sup>†,2</sup>, Lulu Wang<sup>†,2</sup>, Yuxin Ran<sup>†,2</sup>, Jenelle Chen<sup>†,1</sup>, Hongbo Qi<sup>\*,2</sup>, David Olson<sup>\*,1,1</sup>  
<sup>1</sup>University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Chongqing Medical University, Chongqing, China.

**Introduction:** Leukocytes invade the uterus at every delivery, term or preterm, to release inflammatory mediators to accelerate labor. We developed a leukocyte migration assay (LMA) that assesses changes in leukocyte migration in response to chemoattractants from the human fetal membranes (hFM). We **hypothesized** that leukocytes express different chemokine receptors at term and preterm.

**Methods:** hFM were collected at preterm labor (PL; n=3) and term labor (TL; n=6) and used to prepare whole tissue extracts at 100 mg/mL in DMEM-F12. In addition, whole blood was collected at preterm not-in-labor (PNL; n=9), PL (n=5), term not-in-labor (TNL; n=8) and TL (n=6), and leukocytes were isolated then assessed for migration in response to hFM extracts using a modified Boyden Chamber. RNA from whole blood was used to assess the mRNA abundance of CXCR1-5, CCR1-8, CX3CR1, PI3KCA/B/D/G, Rac1, RhoA, CDC42, Vav1, Arp2 and Arp3 relative to Actb in leukocytes. The migration of TL leukocytes towards TL hFM extracts was assessed in the presence of SB225002 or J113863, antagonists for CXCR2 and CCR1, respectively, and PL leukocyte migration towards PL hFM extracts was assessed in the presence of JMS17-HCl, an antagonist for CX3CR1. Correlations were tested by Pearson's correlation test and statistical differences were determined by ANOVA ( $p < 0.05$ ).

**Results:** The migration of TL leukocytes towards TL hFM extracts was positively correlated with the expression of CXCR2, CXCR3, CXCR5, CCR1, CCR3, CCR5, CCR7, PI3KCB, PI3KCD, Rac1, Vav1, Arp2 and Arp3 ( $p < 0.05-0.0001$ ) and negatively correlated with CCR4 expression ( $p < 0.05$ ). Treatment with either SB225002 or J113863 resulted in a

dose-dependent reduction in the chemotaxis of TL leukocytes towards TL extracts ( $p < 0.05-0.001$ ). The expression of CX3CR1 in leukocytes was significantly higher at PL than at PNL ( $p < 0.01$ ), and JMS17-HCl inhibited migration ( $p < 0.05$ ).

**Conclusion:** Fourteen chemokine receptors in TL leukocytes and one in PL were associated with leukocyte migration. This key distinction may guide both the diagnosis and treatment of preterm birth. Supported by CIHR and NSFC.

## W-016

**Impact of Mild Restraint Stress on Placental Pathology in Murine Model.** Ethelin Cammock<sup>†</sup>, Jennifer J Barr<sup>†</sup>, Abigail Combs<sup>†</sup>, Mauro Schenone<sup>\*</sup>, Giancarlo Mari<sup>\*</sup>. University of Tennessee Health Sciences Center, Memphis, TN, United States.

### Introduction:

Preterm birth is one of the leading causes of infant mortality in the United States. Maternal stress has been correlated with preterm birth in several population studies. However, the mechanism is poorly understood. Multiple studies have demonstrated that maternal stress leads to an increase in intrauterine inflammation which has been associated with changes in placental pathology (Chen et al., 2020). Investigation of pathological changes in the placenta may increase understanding of the link between maternal stress and preterm birth. Our objective is thus to assess the impact of 1 hour of daily antepartum restraint stress on placental stress in the murine model.

### Methods:

Nulliparous mice (n=16) underwent timed breeding at 8-10 weeks of age. Dams were then randomly assigned to either stress or control groups. The mice assigned to stress underwent daily 1-hour periods of restraint in a ventilated clear 50-mL conical centrifuge tube from E1-E15. Mice were sacrificed on day E17. Uteri were immediately harvested and fixed in 10% formalin. Histopathology was examined for 4 placentas from each specimen and graded in a semi-quantitative fashion using a scale from 0-4 for each outcome.

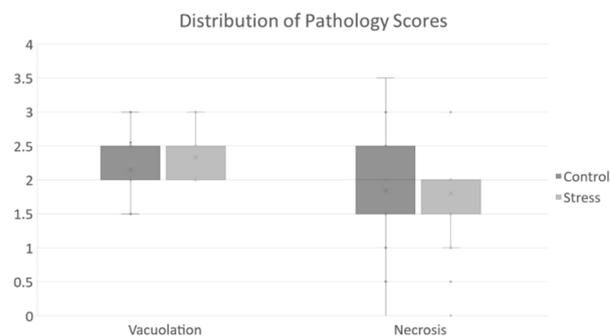
The primary outcome was the fraction of placentas scoring greater than 2/4 on the vacuolation scale (corresponding to moderate vacuolation). The secondary outcome was the fraction of placentas scoring greater than 2/4 on the necrosis scale (corresponding to coalescing foci of necrosis). Difference in vacuolation and in necrosis, considered separately, were evaluated with Fisher's exact test with a significant p-value of .05.

### Results:

Distribution of pathology scores is shown in Figure 1. There was no statistically significant difference between the degree of placental vacuolar degeneration ( $p=0.22$ ) or necrosis ( $p=0.22$ ) between controls and mice exposed to restraint stress.

### Conclusion:

One hour of daily restraint stress does not produce significant changes in placental vacuolar degeneration or necrosis in murine model. Further study is needed to determine if more severe stress stimuli will induce these changes in placental pathology.



**W-017**

**Phenotypic Analysis of Human Choriodecidua in Relation with Parturition.** Léa Chicoisne†, Vaarany Karunanithy\*, Céline Bertholle\*, Brigitte Izac\*, Franck Letourneur\*, Daniel Vaiman\*, Francisco Miralles\*, Muriel Andrieu\*, Céline Mehats\*. *Institut Cochin, Paris, France.*

**Introduction:** The timely onset of labor and birth is a critical determinant of perinatal outcome. Both preterm birth (before 37 weeks of gestation) and postterm pregnancy (after 40 weeks) are associated with an increased risk of adverse pregnancy events. A better understanding of the mechanisms involved in the onset of labor may enable to predict, even control, preterm and/or term delivery. The choriodecidua is a maternal-fetal interface constituted of the maternal decidual stromal and immune cells -mostly T cells and antigen-presenting cells (APC) in late pregnancy- directly in contact with the fetal chorionic trophoblasts of the fetal membranes. This choriodecidual interface shows the highest gene expression variability in labor among gestational tissues. To examine cell state changes, single nucleus RNA-sequencing (snRNA-seq) approach was performed as it achieves comparable gene detection to single cell RNA-seq, with reduction of cell dissociation bias and stress response. Our aim is to gain knowledge in the cell populations present in the choriodecidua at term pregnancy before and after the onset of labor.

**Methods:** Choriodecidua samples were obtained at term pregnancy from 7 non-laboring women delivered by caesarean section and from 7 women who delivered spontaneously after labor. Nuclei are isolated from frozen tissues using dounce homogenization in lysis buffer and sorted by flow cytometry using a pan-nuclear pore antibody. Further encapsulation and snRNA-seq were performed using the 10X Genomics technology. Data computational analyses were processed using 10X Cell Ranger software and Seurat v.4 pipelines.

**Results:** Transcriptomic profiles of 62,752 nuclei were obtained. We identified the expected cell populations, ranked from the most to the less abundant: trophoblasts, stromal cells, immune cells -myeloid and lymphoid subtypes-, endothelial cells -lymphatic and vascular- and epithelial endometrial cells. We could also identify a small population of proliferative cells. For the first time, we are able to define five distinct subsets of trophoblasts in chorion. Mesenchymal cells comprise decidual stromal cells and a new population of fibroblasts, yet uncharacterized. The largest number of deregulated genes with labor was observed in these fibroblasts, followed in a less extent by lymphoid cells and then trophoblasts. As expected, an upregulation of inflammatory genes was associated with labor, and this, in all the different cell populations.

**Conclusion:** Our preliminary results suggest a broader degree of cellular heterogeneity in trophoblasts and stromal cells than reported before. Our unbiased snRNA-seq approach holds the promise of a detailed molecular characterization of the distinct cell types and their changes with labor.

**W-018**

**Human Fetal Membranes Secrete Proinflammatory Cytokines Upon Toll-Like Receptor 9 and Cell-Free Fetal DNA Stimulation.** Samantha P Oetjen†, Chelsea A Saito Reis†, Claire E Kendal-Wright\*. <sup>1,2</sup> *Chaminade University of Honolulu, Honolulu, HI, United States;* <sup>2</sup> *John A. Burns School of Medicine University of Hawaii, Honolulu, HI, United States.*

**Introduction:** The biological trigger that initiates normal parturition remains unclear, although it is thought that inflammation plays a role in fetal membrane weakening. Our lack of knowledge is in part due to the unique nature of some of these processes in humans, and the logistical and ethical issues of studying human pregnancy. We recently demonstrated that all ten of the innate immune system Toll-like Receptors (TLR) are expressed in the amnion, with TLR9 at high levels. TLR9 detects both viral and bacterial CpG rich DNA fragments, eliciting an inflammatory response. However, its endogenous ligand is unknown. Cell-free fetal DNA (cffDNA) in the mom's circulation that originates from the placenta activates TLRs. As cffDNA is also found in the amniotic fluid, we hypothesized that it would activate TLR9 in the amnion causing the secretion of pro-inflammatory cytokines.

**Methods:** Fetal membranes were collected from repeat cesarean sections at Kapi'olani Medical Center for Women and Children with IRB approval. Amnion epithelial cells (AECs) were isolated by consecutive trypsin

(0.2%) digestion. Cultured AECs were treated with 5µM of TLR9 ODNs (2006, 2395, 2216) and their respective controls (Invivogen, CA) for 6 hours. Cytokine secretion (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-14, IL-17A, IFNγ, TNFα, and GM-CSF) was measured by MultiAnalyte ELISArray (Qiagen, CA), and the data normalized to total protein. Fetal membrane explants were cut and secured to a modified transwell insert. These were treated with cffDNA on the amnion side. Conditioned media was collected from both sides of the explant. IL6 (R&D Systems, MN) secretion measured by ELISA. Data were normalized to the weight of the explant and total protein secretion into the media.

**Results:** The three ODNs increased different cytokines. ODN 2006 increased cytokine secretion for IL-6 and GM-CSF (6.48 and 0.75 fold respectively). ODN 2216 increased IL-1α, IL-6, IL-8, and IL-10 (0.52, 1.90, 2.56, and 1.37 fold respectively). ODN 2395 increased IL-2 and IL-17A (0.58 and 0.98 fold respectively) (n=3). cffDNA caused significantly (p=0.0324) more secretion of IL-6 apically (6.27 fold, p=0.0142) compared to basally (1.86 fold, p=0.0081) (n=5).

**Conclusion:** The ODN data shows that TLR9 is functional and able to increase cytokine secretion in AECs, although each tested elicited a different response. cffDNA evoked both an autocrine apical increase in IL-6 secretion, as well as a signal to the basal side of the explant. This suggests that cffDNA may be an endogenous ligand that can initiate inflammation in the fetal membranes, adding to the inflammatory load in this tissue at term. TLR9 knockdown experiments are ongoing to confirm cffDNA action through this receptor.

**W-019**

**Progesterone (P4) Is Locally Induced by Inflammatory Signals at the Maternal-Fetal Interface in Fetal Membranes.** Robert Moore\*, Deepak Kumar\*, Joseph Mansour\*, Brian Mercer\*, Sam Mesiano\*, John Moore\*. *Case Western Reserve University, Cleveland, OH, United States.*

**Introduction:** We use an *in-vitro* human fetal membrane (FM) explant model system to study the human FM weakening pathway, a prerequisite for FM rupture and pPROM. In this model both TNFα, modeling inflammation/infection, and thrombin, modeling decidual bleeding/abruption (the leading associations with pPROM), cause dose dependent FM weakening and concomitant tissue changes that mimic the natural weak zone seen in fresh FM at term. The choriodecidua (CD) is the primary target for both TNFα and thrombin induced weakening in this model. Also, GM-CSF is a critical intermediate in the pathway, its induction being both necessary and sufficient for TNFα and for thrombin induced FM weakening. GM-CSF ultimately induces many MMPs and other proteases, and suppresses many TIMPs and other protease inhibitors in FM. As the fetal-maternal interface is barraged with inflammatory and bleeding challenges, FM integrity would be threatened unless an inhibitory system was also in play. We have previously shown exogenous P4 inhibits inflammation induced weakening due to TNF, thrombin and even GM-CSF in our system. We hypothesized P4 produced within FM might inhibit inflammation induced FM weakening. We found evidence that P4 is part of a previously undescribed, inducible negative feedback system at the fetal-maternal interface, which inhibits inflammation induced FM weakening.

**Methods:** FM fragments from term uncomplicated repeat CS deliveries were mounted in Transwell inserts and cultured with control media and increasing GM-CSF (0-200 ng/ml), 10nM RU486 (to block P4 action), or 1µM trilostane +/- 10<sup>-7</sup>M P4 (to block P4 production). P4 and rupture strength were determined. Also immortalized trophoblast cells (BeWo) were cultured with control media or increasing concentrations of GM-CSF. P4 production was determined by ELISA and 3βHSD by Western Blot.

**Results:** GM-CSF induced P4 production in both FM explant cultures and BeWo cells in a concentration dependent manner (p < .001). GM-CSF also increased 3βHSD protein in BeWo cells in a concentration dependent manner (p<.05). Incubation of FM explants with RU486, blocking P4 action, or trilostane, blocking P4 production, each caused FM weakening. Exogenous P4 with Trilostane restored FM strength.

**Conclusion:** In addition to its role in inflammation induced FM weakening, GM-CSF induces P4 production in FM and trophoblastic cells. Blockade of P4 action with RU486 or inhibition of P4 production with Trilostane cause FM weakening. The weakening due to inhibition

of P4 production with Trilostane is reversed by exogenous P4. These data are consistent with the existence of a tissue level negative feedback system whereby P4 is induced by GM-CSF generated as a critical part of inflammation induced FM weakening. The P4 then functions to inhibit both GM-CSF production and its downstream action with resultant preservation of FM integrity.

## W-020

**Cigarette Smoke Condensate Exposure Induces RAGE-Dependent Sterile Inflammation in Amniotic Epithelial Cells.** [Helena Choltus<sup>†,1</sup>](#), [Corinne Belville<sup>1</sup>](#), [Denis Gallot<sup>1,2</sup>](#), [Régine Minet-Quinard<sup>1,2</sup>](#), [Julie Durif<sup>3</sup>](#), [Loïc Blanchon<sup>1</sup>](#), [Vincent Sapin<sup>\\*,1,2</sup>](#) <sup>1</sup>Clermont Auvergne University, Clermont-Ferrand, France; <sup>2</sup>Clermont-Ferrand Hospital, Clermont-ferrand, France; <sup>3</sup>Clermont-Ferrand Hospital, Clermont-Ferrand, France.

**Introduction:** Maternal tabagism is a well-known risk factor of preterm prelabor rupture of the fetal membranes (pPROM), an obstetrical complication responsible for one third of preterm births. Cigarette consumption during pregnancy is described for inducing inflammation or oxidative stress, mechanisms both implicated in fetal membranes weakening. Nevertheless, data about cell signaling cascades implied in such mechanisms are still poorly understood. Our proposed hypothesis is that the Receptor for Advanced Glycation End-products (RAGE) and its ligands are ones of the important actors of the cigarette-dependent inflammation associated with extracellular matrix degradation in fetal membranes.

**Methods:** Human fetal membranes explants and primary amniotic epithelial cells (pAECs) were treated with Cigarette Smoke Condensate (CSC) combined or not with RAP, a competitive inhibitor of RAGE (n=5). Cell suffering was evaluated by lactate dehydrogenase release. HMGB1 (a RAGE ligand) cellular media secretion by amnion and choriodecidua explants was determined using Western-Blot. Inflammation status was evaluated by checking transcription and release of inflammatory cytokines (IL6, IL8, TNF $\alpha$ , IL1 $\beta$ ) by RT-qPCR and protein quantification. Induction of NF $\kappa$ B pathway, a cellular effector of the RAGE activation was determined using a luciferase reporter assay. Finally, gelatinase (MMP2 and 9) activity was assessed using zymography assay. Statistical analysis were performed by non parametric tests using GraphPad PRISM software.

**Results:** CSC induced cell suffering and HMGB1 media release only for amnion explants and those are directly associated to a RAGE-dependent inflammatory response (as attested by the use of RAP inhibitor). Thus, pAECs, derived from amnion, are also sensible to CSC treatment regarding the induction of inflammation (cytokines release, NF $\kappa$ B pathway activation) through RAGE engagement. In addition, CSC activated metalloproteases activity, reflecting an increase of extracellular matrix (ECM) degradation.

**Conclusion:** For human amnion, RAGE-NF $\kappa$ B axis appears implicated in inflammatory response induced by CSC exposure in explants and pAECs. This inflammation associated with an increase of gelatinase activity could explain a pathological earlier fetal membranes weakening possibly implied in pPROM. Our work establishes a new molecular pathway to understand negative effects of maternal smoking on amniotic membranes integrity leading to potential advances in terms of diagnosis and prevention of pPROM.

## W-021

**Initial Validation of Cervix Microstructure Imaging Using Quantitative Histology.** [Wenjie Wu<sup>†,1</sup>](#), [Zhexiong Sun<sup>†,1</sup>](#), [Hui Wang<sup>1</sup>](#), [Xiao Ma<sup>†,1</sup>](#), [Hansong Gao<sup>†,1</sup>](#), [Sicheng Wang<sup>†,1</sup>](#), [Zichao Wen<sup>†,1</sup>](#), [Qing Wang<sup>1</sup>](#), [Peinan Zhao<sup>1</sup>](#), [Pamela K Woodard<sup>1</sup>](#), [Hannah R Krigman<sup>1</sup>](#), [Alison G Cahill<sup>2</sup>](#), [Yong Wang<sup>\\*,1</sup>](#) <sup>1</sup>Washington University School of Medicine, Saint Louis, MO, United States; <sup>2</sup>Dell Medical School, University of Texas, Austin, TX, United States.

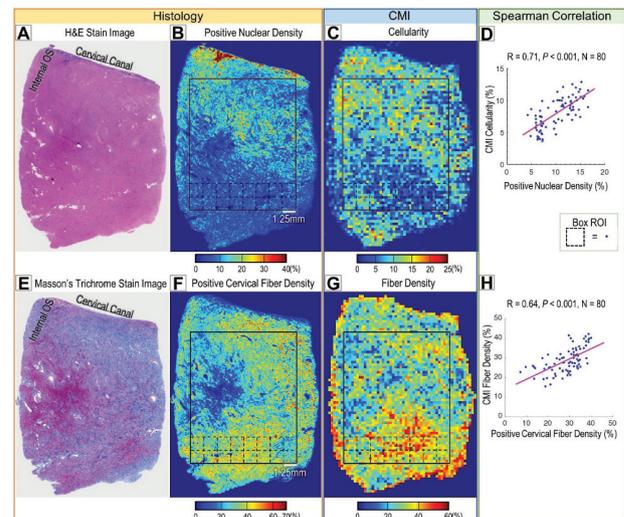
**Introduction:** Cervix Microstructure Imaging (CMI) is a novel imaging tool to noninvasively quantify features like cellular and fiber architectures, by modeling their diffusion signatures using magnetic resonance imaging

(MRI). We employed high-resolution *ex vivo* MRI of human cervical tissue to test the hypothesis that the CMI-derived cellularity and fiber density correlates with quantitative histology.

**Methods:** A 15 × 20 × 3 mm section of cervix was dissected from the posterior midline of a fresh total hysterectomy specimen and imaged with a Varian 11.7T MRI, using spin-echo sequence with 20-direction diffusion encoding (max b = 3900 s/mm<sup>2</sup>, voxel = 0.25 × 0.25 × 1 mm). It was later fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections (5  $\mu$ m) were stained with H&E (Fig. 1A) and Masson's trichrome (Fig. 1E). Whole slide images of both were digitized at 20 $\times$  magnification. A positive nuclear density map (Fig.1B) was obtained by calculating the density of hematoxylin-stained nuclei. A positive cervical fiber density map (Fig. 1F) was obtained by segmenting cervical fibers on the trichrome stained slide and calculating their density. The H&E and trichrome images were registered to the MR images by initial manual alignment and non-rigid registration with their delineated contours. The registration was then applied to all histology maps. We used in-house CMI software to generate a cellularity map (Fig. 1C) from MR images by computing the fraction of restricted isotropic diffusion ( $\leq 3 \times 10^{-4}$  mm<sup>2</sup>/s) in each image voxel and a fiber density map (Fig. 1G) by computing the fraction of anisotropic diffusion (radial diffusivity 1 - 5  $\times 10^{-4}$  mm<sup>2</sup>/s), which could model the water diffusion near and between collagen and muscle fiber.

**Results:** In the center region (bolded box), 80 evenly spaced regions of interest (ROIs, 1.25 × 1.25 mm) were drawn in the H&E-, Trichrome-, and CMI-derived maps. Correlation plots revealed that CMI-derived cellularity correlates with positive nuclei density (R = 0.71, P < 0.001), and CMI-derived fiber density correlates with positive cervical fiber density (R = 0.64, P < 0.001) in each ROI.

**Conclusion:** The CMI-derived cellularity and fiber density significantly correlate with histological quantifications. Our findings support CMI's validity of quantifying cervical microstructure properties in future human studies.



**Figure 1:** Correlation between histologically and CMI-derived cellularity and fiber density. (A) Brightfield image of H&E stained section. (B) Positive nuclear density map derived from the H&E image in A. (C) CMI-derived cellularity. (D) Spearman's correlation plot of positive nuclear density and CMI-derived cellularity. (E) Brightfield image of Masson's trichrome stained section. (F) Positive cervical fiber density derived from the trichrome image in E. (G) CMI-derived fiber density. (H) Spearman's correlation plot of positive cervical fiber density and CMI-derived fiber density. Each blue dot in the correlation plots represents respective data in a ROI of 1.25 × 1.25mm shown in B, C, F and G.

## W-022

**Gardnerella Vaginalis Is Associated with Increased Tryptophan Metabolism in the Cervicovaginal Space: A Potential Role for Microbial Metabolites in Spontaneous Preterm Birth.** [Kristin D Gerson<sup>†</sup>](#), [Clare McCarthy](#), [Heather H Burris](#), [Michal A Elovitz<sup>\\*</sup>](#). University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.

**Introduction:** Non-optimal cervicovaginal (CV) microbiota, including *Gardnerella vaginalis* (GV), are associated with spontaneous preterm birth (sPTB). *Lactobacillus crispatus* (LC) constitutes a component of a healthy CV ecosystem and has been shown to mitigate sPTB risk associated with non-optimal microbiota. We recently determined that metabolomic output

differs among Black women with non-optimal microbiota who have sPTB, noting an increase in tryptophan (Trp) metabolites. This study sought to determine associations between relative abundances of select microbial species and Trp metabolites with birth outcome.

**Methods:** This is a nested case-control study of 40 Black women. CV microbiota was characterized by 16S rRNA gene sequencing and classified into community state types (CSTs) from swabs at 20-24 weeks. Four groups (n=10/group) were compared: 1) women in CST IV, a non-optimal community characterized by anaerobes such as GV, who delivered at term; 2) women in CST IV who had sPTB; 3) women in CST I, a healthy ecosystem dominated by LC, who delivered at term; and 4) women in CST IV who had sPTB. We dichotomized GV and LC relative abundances at the median into high and low groups. Metabolomic analyses generated relative abundances of Trp metabolites, kynurenate (KY) and tryptamine (TY). Kruskal-Wallis tests and Wilcoxon rank-sum tests were used to establish significance ( $p < 0.05$ ).

**Results:** Trp metabolite detection differs by GV abundance and birth outcome as shown in Table 1. Among women with high GV, median KY is higher in those with sPTB, while lowest levels of KY are noted among women with low GV regardless of birth outcome ( $p = 0.035$ ). TY is highest among women with high GV and term birth ( $p < 0.001$ ). Among women with sPTB, KY and TY are greater in those with high GV ( $p = 0.015$  and  $p = 0.007$ , respectively). As shown in Table 2, KY and TY are higher among women with low vs high LC ( $p = 0.048$  and  $p = 0.007$ , respectively).

**Conclusion:** Relative GV and LC abundances are associated with differential Trp metabolism and birth outcome. KY and TY have been implicated in the regulation of epithelial and mucosal barrier integrity in other biologic systems, suggesting that these microbial metabolites may play a similar role in the CV space. Incorporation of relative bacterial and microbial metabolite abundances into future studies examining host-microbe interactions in sPTB is warranted. R01NR01478 (ME)

Table 1. Metabolite detection by GV abundance and birth outcome (n=40)<sup>a</sup>

Metabolite	High GV (n=18)		Low GV (n=22)		p
	sPTB (n=9)	Term (n=9)	sPTB (n=11)	Term (n=11)	
Kynurenate	3.2 (1.0-4.4)	0.6 (0.3-0.7)	0.3 (0.3-1.1)	0.4 (0.3-0.5)	0.035
Tryptamine	0.2 (0.2-2.0)	0.8 (0.04-1.5)	0.03 (0.03-0.03)	0.03 (0.03-0.03)	<0.001

Data presented as median (IQR)

<sup>a</sup>GV dichotomized at median abundance among women with present GV in cohort (n=36); 0.056 pg/mL

Table 2. Metabolite detection by LC abundance and birth outcome (n=40)<sup>a</sup>

Metabolite	High LC (n=19)		Low LC (n=21)		p
	sPTB (n=11)	Term (n=8)	sPTB (n=9)	Term (n=12)	
Kynurenate	0.3 (0.3-1.3)	0.3 (0.3-0.5)	1.8 (1.0-3.9)	0.5 (0.3-0.9)	0.048
Tryptamine	0.03 (0.03-0.03)	0.03 (0.03-0.03)	0.2 (0.03-2.0)	0.3 (0.0-1.2)	0.007

Data presented as median (IQR)

<sup>a</sup>LC dichotomized at median abundance among women with present LC cohort (n=38); 0.024 pg/mL

## W-023

**Interrogation of Collagen Degradation Pathways for Cervical Extracellular Matrix (ECM) Remodeling through Pregnancy.** Mariano Colon-Caraballo<sup>†</sup>, Mala Mahendroo. *UT Southwestern Medical Center, Dallas, TX, United States.*

**Introduction:** During pregnancy, the structure and mechanical function of the cervix is regulated by precise changes in processing, assembly, and composition of the extracellular matrix (ECM). Recent transcriptomic studies demonstrate a constant expression of genes encoding fibrillar collagens and ECM molecules required for collagen assembly. In contrast, expression of collagen-degrading proteases was reduced in pregnancy time points, with a few notable exceptions - MMP14 (constant expression at all time points) and fibroblast activation protein (FAP-induced expression on gestation days 15 and 18). Recent proteomics studies demonstrate a remarkably high turnover rate for fibrillar collagens in the cervix of both nonpregnant (NP) and pregnant mice. These findings suggest continuous collagen degradative pathways must be in place to ensure cervical ECM

homeostasis. Therefore, we hypothesize that previously unrecognized extracellular and intracellular collagen degradative pathways are required to ensure collagen homeostasis in the cervix. Understanding ECM remodeling processes that determine a successful pregnancy is essential to understand how this process can go awry and lead to a premature birth.

**Methods:** Studies were carried out in the mouse cervix. Immunohistochemistry (IHC) was used to evaluate the cell-specific expression of the proteases MMP14 and FAP in the non-pregnant (NP) and pregnant (d6-d18) mice cervix. MMP14 collagenolytic activity was determined by in situ-zymography (ISZ). UPARAP-Endo180 receptor protein levels were assessed by Western blot in the NP and pregnant cervix.

**Results:** IHC revealed cell-type specific expression of MMP14 in the cervix. Strong immunostaining for MMP14 was observed in the epithelial compartment in the NP and D6 pregnant cervix with a decline in late pregnancy. However, strong immunostaining of MMP14 in the stromal compartment was observed in the D15 and D18 pregnant cervix compared to early gestational time points. ISZ experiments demonstrate MMP14-dependent collagenolytic activity that can be pharmacologically inhibited with a MMP14 inhibitor. Consistent with its gene expression, FAP1 protein expression was increased in the stromal ECM in the late pregnant cervix (d15 and d18). UPARAP-Endo 180 receptor protein expression was highly expressed in the NP and pregnant (days 6-18) cervix.

**Conclusion:** Our results suggest that constant collagen turnover in the cervical ECM is critical to sustain the structural and mechanical changes that occur during pregnancy under physiological conditions. Importantly, the expression and activation of MMP14, FAP, and UPARAP observed during pregnancy support the involvement of both extracellular and intracellular collagen degradative pathways to facilitate cervical ECM remodeling in preparation for parturition.

## W-024

**Stretch Preconditioning of the Uterine Unfolded Protein Response Promotes Uterine Quiescence and Prevents Preterm Labor: In Vivo Observations in the Non-Human Primate.** Chandrashekara Kyathanahalli<sup>1</sup>, Arren Simpson<sup>1</sup>, Judith Ingles<sup>1</sup>, Miranda Li<sup>2</sup>, Hazel Huang<sup>2</sup>, Jeff Munson<sup>2</sup>, Lakshmi Rajagopal<sup>2</sup>, Mark R Johnson<sup>3</sup>, Pancharatnam Jeyasuria<sup>1</sup>, Kristina M Adams Waldorf<sup>2</sup>, Jennifer C Condon<sup>\*1</sup>. <sup>1</sup>Wayne State University, Detroit, MI, United States; <sup>2</sup>University of Washington, Seattle, WA, United States; <sup>3</sup>Imperial College London, London, United Kingdom.

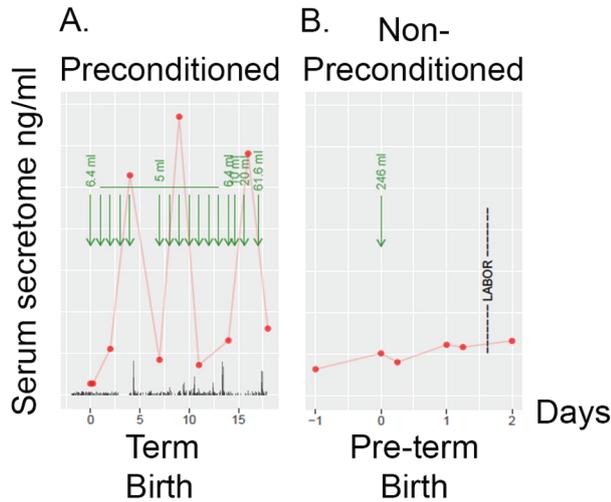
**Introduction:** Preconditioning refers to sub-lethal insults which induce tolerance to subsequent more damaging stressors. We've previously demonstrated preconditioning promotes uterine quiescence in an anti-apoptotic, anti-inflammatory manner in the pregnant mouse and is regulated at the level of the cellular unfolded protein response (UPR). Utilizing an *in-vivo* uterine stretch model of preconditioning in the pregnant pig-tailed macaque, we examined the levels and action of serum GRP78, a component of the uterine secretome released during preconditioning, its relationship to circulating cytokine and prostaglandin levels, uterine contractility and the timing of labor.

**Methods:** Chronically catheterized pregnant pigtail macaques at 118-125 days of gestation received either chorio-decidual and intra-amniotic saline infusion (n=7) or varying protocols of intra-amniotic balloon infusion with saline (n=5). Maternal blood was sampled frequently before and after balloon inflation. Intra-amniotic balloon inflation rates varied to produce a slow (preconditioning) or rapid (non-preconditioning) rise in biomechanical uterine stretch. Amniotic cavity pressure was measured to quantitate contraction frequency and intensity. GRP78, cytokine and prostaglandins were examined by ELISA. *In vitro* siRNA and lentiviral analysis in the HTERT-HM and THP-1 cell line examined the action of GRP78.

**Results:** Pregnant uterine stretch preconditioning generated a temporal, robust and recurring increase in circulating GRP78 levels, which was associated with suppressed cytokine and prostaglandin responses and prolonged gestation. Non-preconditioned animals, failed to generate a surge in GRP78 and consequently demonstrated increased cytokine,

prostaglandin levels and preterm labor. The anti-inflammatory action of GRP78 in the context of the uterine myocyte and macrophage cell was confirmed *in vitro*.

**Conclusion:** Uterine UPR preconditioning acts as a signaling mechanism whereby normal stressors experienced across gestation, act to promote uterine quiescence through the generation of a specific preconditioned secretome. Components of which may serve as non-invasive biomarkers for preterm birth.



A. Incremental uterine distension promotes a preconditioned response resulting in increased serum secretome levels which capacitate uterine quiescence in an anti-inflammatory manner.

B. Rapid uterine distension disables the preconditioning response resulting in the onset of preterm labor.

#### W-025

**A Role for Mirabegron in the Management of Uterine Contraction.** Hazik Asif†, Scott Barnett†, Buxton Iain\*. *University of Nevada, Reno, Reno, NV, United States.*

**Introduction:** Preterm labor (PTL) is the leading cause of infant morbidity and mortality. The  $\beta_3$  adrenergic receptor ( $\beta_3$ AR) is present in the myometrium making it a potential therapeutic target in PTL. Although downstream effects of the  $\beta_3$ AR are known in some cell types, there is still much that is unknown. We hypothesized that mirabegron, a selective  $\beta_3$ AR agonist FDA approved for overactive bladder, can be used to mediate relaxation in contracting human myometrial tissue, and that  $\beta_3$ AR expression in human myometrium varies in disparate states of pregnancy. We hypothesized that the  $\beta_3$ AR is present in both smooth muscle and endothelial cell compartments of the myometrium.

**Methods:** Myometrial tissue from term nonlaboring (TNL) patients (n=4) were dissected into strips (5 x 2 x 1-2 mm (l.w.d)). The strips were secured in a horizontal tissue bath system (Danish Myo Technology) suspended in oxygenated Krebs buffer (NaCl 118 mM, KCl 4.75 mM, KH<sub>2</sub>PO<sub>4</sub> 1.2 mM, Na<sub>2</sub>HCO<sub>3</sub> 25 mM, glucose 20 mM, MgCl<sub>2</sub> 1.2 mM, CaCl<sub>2</sub> 1.8 mM, at a pH of 7.4) at 37°C. Strips were exposed to 60 mM KCl (3min) followed by washout and oxytocin (8 nM, 30 min). Tissues were then exposed to mirabegron (1, 3, 10, 30, or 100uM drug or DMSO control for 60 min), followed by washout. Analysis was performed *via* LabChart (ADInstruments). Western blot was performed to determine the relative expression of the  $\beta_3$ AR in non-pregnant (NP, n=13), TNL (n=12), term labor (n=12), preterm nonlabor (PTNL, n=6), and preterm laboring (PTL, n=6) tissue samples prepared in MAPK lysis buffer including protease and phosphatase inhibitors.  $\beta_3$ AR expression was normalized to GAPDH. Human myometrial cells were derived by enzymatic expression and grown in primary culture. The primary cells were resuspended with

CD31(+) conjugated microbeads and magnetically separated and then cultured into CD31<sup>-</sup> (myocyte) and CD31<sup>+</sup> (endothelial) cell populations. Cells were stained with anti- $\beta_3$ AR, wheat germ agglutinin (WGA), and DAPI antibodies and imaged via immunofluorescence. DAPI and WGA established cellular membranes and nuclei.

**Results:** Mirabegron relaxed contracting human myometrium tissue strip with an EC<sub>50</sub> of 32.5uM, with contractions returning after washout. Western blot analysis showed that the  $\beta_3$ AR was found to increase in all states of pregnancy when compared to the NP state (p<0.0001). There was no difference in the  $\beta_3$ AR concentration throughout pregnancy (p>0.05). Imaging revealed that the  $\beta_3$ AR is present in both myocyte and endothelial cells of the human myometrium.

**Conclusion:** The upregulation of the  $\beta_3$ AR during disparate states of pregnancy further support the significance of the role it may play in mediating uterine quiescence in pregnancy. The presence of the  $\beta_3$ AR in both myocyte and endothelial cells suggest that it has specific functions in each cellular compartment that contribute to the agonist response. Mirabegron shows promise as a tocolytic strategy for PTL patients.

#### W-026

**Pharmacological Chaperones Sensitize Cells to Oxytocin Treatment.** Manasi Malik†, Yingye Fang†, Michelle Roh†, Antonina I. Frolova, Princess I. Imoukhuede, Sarah K. England\*. *Washington University in St Louis, St Louis, MO, United States.*

**Introduction:** Oxytocin is administered to ~50% of laboring patients for induction and augmentation, and to almost all patients for prevention of post-partum hemorrhage. However, response to oxytocin varies widely between patients. Inadequate oxytocin response can result in cesarean delivery, uterine atony, and post-partum hemorrhage. Thus, identifying patients at risk for poor oxytocin response, and developing strategies to enhance oxytocin response, could have clinical utility. We hypothesized that genetic variants in the oxytocin receptor (*OXTR*) impair trafficking of the receptor to the cell surface, thus decreasing oxytocin response. Further, we investigated whether pharmacological chaperones could mobilize *OXTR* to the cell membrane and enhance cellular response to oxytocin.

**Methods:** Experiments were performed in HEK293T cells transfected with wild type (WT) and variant *OXTR* and in hTERT-immortalized human myometrial (hTERT-HM) cells. Missense genetic variants for study were selected from the gnomAD database based on prevalence and a functional screen. *OXTR* was tagged with an N-terminal HA tag to enable detection of cell surface and total *OXTR* by quantitative flow cytometry. Oxytocin response was measured by using fluorescence-based calcium flux assays and inositol monophosphate (IP1) accumulation assays.

**Results:** We found that two of the four variants analyzed, V281M and E339K, impaired receptor trafficking from the endoplasmic reticulum and Golgi body to the cell surface. V281M and E339K decreased cell surface *OXTR* localization by 49% and 36%, respectively, compared to WT *OXTR*. Accordingly, these variants reduced maximal oxytocin signaling by 23% and 22%. Next, we investigated whether small molecule modulators of *OXTR* could act as pharmacological chaperones, increasing the trafficking of *OXTR* from intracellular stores to the cell membrane. Overnight (16 hour) treatment with three different modulators increased the cell surface localization of transfected V281M *OXTR* to above WT levels. These same modulators increased cell surface localization of the endogenous (WT) *OXTR* in hTERT-HM cells by three-fold. Finally, pre-treatment with one modulator increased oxytocin-induced IP1 accumulation in hTERT-HM cells by 12%.

**Conclusion:** Genetic variants present in the human population impair trafficking of *OXTR*, which can decrease oxytocin response. Pharmacological chaperones may be a useful treatment strategy for patients predicted to have poor oxytocin response due to genetic or other factors.

## W-027

**Combination Tocolysis of Dysregulated Myometrial Pathways for the Treatment of Preterm Labor.** Scott D Barnett<sup>†</sup>,<sup>1</sup> Mitchell Anderson,<sup>2</sup> Hazik Asif<sup>†</sup>,<sup>1</sup> Iain L.O. Buxton\*.<sup>1</sup> <sup>1</sup>University of Nevada, Reno School of Medicine, Reno, NV, United States; <sup>2</sup>University of Nevada, Reno, Reno, NV, United States.

**Introduction:** Approximately 10-12% of US births are preterm, a number that has declined little in decades. Tocolytics currently in use are ineffective because (1) most are 'generalized' smooth muscle modifiers not designed to target myometrial-specific pathways, and (2) conservative dosing schedules must be employed to avoid adverse effects on the fetus. We seek to develop novel therapeutic approaches that leverage pathways specific to the myometrium through 'combination tocolysis' to delay birth. We have recently determined that by targeting two dysregulated proteins in the myometrium, connexin-43 (Cx43) and the  $\beta_3$  adrenergic receptor ( $\beta_3$ AR), with low doses of 18 $\beta$ -glycyrrhetic acid and nebitivolol, respectively, their tocolytic effects are additive, all but eliminating uterine contractions. Here we expand upon that finding and hypothesize that 'combination tocolysis' using the Cx43 gap junction channel (GJC) specific inhibitor, 18 $\alpha$ -glycyrrhetic acid (18 $\alpha$ -GA), and atosiban, an oxytocin receptor (OXTR) antagonist which is not an effective tocolytic when used alone, will produce an additive effect when co-administered.

**Methods:** Myometrial strips (n=3 term non-laboring) were attached to a force transducer and isometrically stretched in an organ bath containing Krebs buffer. Tissues were maintained at 37°C with balanced oxygen (95% O<sub>2</sub>, 5% CO<sub>2</sub>). Tissues were then sequentially challenged with KCl (60 mM) and oxytocin (8 nM) then treated with either 18 $\alpha$ -GA (100-300  $\mu$ M), atosiban (10-300 nM), or both, as accumulative doses in 15-minute intervals. Data were analyzed with LabChart®.

**Results:** 18 $\alpha$ -GA decreased 'area under the curve' (AUC) (10 $\mu$ M n.s., 30 $\mu$ M n.s., 100 $\mu$ M 17.5%, 300 $\mu$ M 59.8%) and 'contractions per unit time' (CPUT) (23.9% decrease over control at max dose), but not peak tension. Similarly, the atosiban decreased AUC (10nM n.s., 30nM 3.3%, 100nM 45.3%, 300nM 49.5%) and CPUT (33.3% decrease over control at max dose over control. When co-administered, the negative inotropic effects were amplified over individual dosing schedules for both AUC (18 $\alpha$ -GA:atosiban - 10 $\mu$ M:nM 5.9%, 30 $\mu$ M:nM 38.1%, 100 $\mu$ M:nM 61.5, 300 $\mu$ M:nM 92.9%) and CPUT (73.4% decrease over control at max dose over control).

**Conclusion:** These data indicate that 'combination tocolysis' using the Cx43 GJC inhibitor, 18 $\alpha$ -GA, and the OXTR antagonist, atosiban, produce additive negative inotropic effects over the use of individual drugs, which may allow for effective tocolysis at reduced concentrations. These findings, in concert our recent work which confirmed a similar trend using a non-specific Cx43 inhibitor (GJC/hemichannel) with a  $\beta_3$ AR (eNOS) agonist, highlight the robust potential for 'combination tocolysis' of dysregulated and myometrial-specific contractile pathways to treat preterm labor.

## W-028

**Micro-RNA 203 Regulates Myometrial Smooth Muscle Cell Expression of the Transient Receptor Vanilloid 4 Channel and Contractility.** Lihua Ying, Cristina M Alvira, David N Cornfield\*. Stanford University, Stanford, CA, United States.

**Introduction:** Expression of the transient receptor vanilloid 4 (TRPV4) channel increases in myometrial smooth muscle cells (mSMC) during pregnancy and activation contributes uterine contractility. In chondrocytes, micro-RNA 203 (miR-203) represses TRPV4 expression. Whether pregnancy associated changes in miR-203 expression contributes to the heightened expression of TRPV4 in the pregnant myometrium remains unknown. **Objective:** We hypothesis that decreases in miR-203 across gestation indirectly facilitates increases in TRPV4 expression in the pregnant myometrium.

**Methods:** Uterine miR-203 and TRPV4 expression were measured from pregnant and nonpregnant mice via qPCR and western blot, in whole uterine tissue of PG and NP mice via immunofluorescent and *in situ* hybridization, as well as in human and mouse mSMC transfected with miRNA-203 mimetic or inhibitor. Contractility of mSMC was assessed

in the presence and absence of miR-203 transfection. To determine whether TRPV4 is a direct target of miR-203, we performed a dual luciferase activity assay using a Gluc reporter construct containing the 3'UTR of TRPV4 (GeneCopoeia). To validate target site-specificity, we generated a site-specific TRPV4 mutant and a non-specific (control) using a site-directed mutagenesis approach (New England BioLabs). Wild type (wt) or mutant (mut) TRPV4 3'UTR were co-transfected with miR-203 mimetic or control miRNA using EndoFectin Max Transfection Reagent (GeneCopoeia). Relative luciferase activity was normalized to secreted SEAP reporter.

**Results:** Uterine miR-203 expression was decreased in PG compared to NP mice. Treatment of human or mouse mSMC with the miR-203 mimetic decreased, while miR-203 inhibitor increased both TRPV4 RNA and protein. In mSMC transfected with miR-203 mimetic, the contractile response to oxytocin was attenuated. In dual-luciferase reporter assays using human mSMC co-transfected with reporter constructs containing either (i) wt + control miRNA (1.07 $\pm$ 0.29); (ii) wt + miR-203 mimetic (0.46 $\pm$ 0.11); (iii) mut + control miRNA (1.07 $\pm$ 0.28) and (iv) mut + miR-203 mimetic (1.02 $\pm$ 0.17). The miR-203 mimetic co-transfected with wt TRPV4 3'UTR exhibited the lowest luciferase activity (0.46 $\pm$ 0.11) [P<0.05, vs. mut TRPV4 3'UTR (1.02 $\pm$ 0.17); N=6]. These results demonstrated that miR-203 regulates TRPV4 via directly targeting 3'UTR of TRPV4. Estradiol (E2) increases TRPV4 and decrease miR-203 expression in uterus mSMC.

**Conclusion:** In mSMC, miR-203 expression is dynamic and regulates TRPV4 expression. During pregnancy, miR-203 expression decreases. Given that TRPV4 is a direct target of miR-203, we conclude that the increase in TRPV4 expression in mSMC during pregnancy is determined, at least in part, by the decreasing miR-203 expression. miR-203 represents a novel target to suppress uterine contractility, prolong pregnancy and prevent preterm delivery.

## W-029

**M<sup>6</sup>A Posttranscriptional Modification Allows for an Adaptable Fluid Myometrial Proteome during Pregnancy.** Jenkins Lindsay,<sup>1</sup> Simpson Arren,<sup>1</sup> Jennifer Condon,<sup>2</sup> Jeyasuria Pancharatnam\*.<sup>1</sup> <sup>1</sup>Wayne State University School of Medicine, Detroit, MI, United States; <sup>2</sup>Michigan, Detroit, MI, United States.

**Introduction:** We have previously shown that alternative spliced estrogen receptor  $\alpha$  (ESR1) isoforms and endoplasmic reticulum stress (ERSR) were implicated in the control of contractility of the myometrium. We further believe that the ERSR is crucial to regulating an alternate proteome necessary for stage related gestational functions. In this study we link the aforesaid phenomena to m<sup>6</sup>A methylation of mRNA. M<sup>6</sup>A methylation is the most frequently used epitranscriptomic modification and is performed by MettL3, a methyl transferase that is part of the m<sup>6</sup>A writer complex. We believe that m<sup>6</sup>A posttranscriptional modification of pre-mRNA and mRNA, controls alternative splicing and stress induced cap-independent translation during gestation allowing for an elastic proteome that is reactive to changes in the developing uterus. We hypothesized that the generation of ERSR will induce stress-dependent m<sup>6</sup>A activity by upregulating MettL3 (m<sup>6</sup>A writer) and will regulate hnRNPs and YTH proteins (m<sup>6</sup>A readers) levels.

**Methods:** The levels of m<sup>6</sup>A readers, hnRNPG and hnRNPC, and the m<sup>6</sup>A writer, MettL3, were measured using western blotting techniques after a tunicamycin (TM, an ER stress inducer) treatment in a myometrial cell line (hTERT<sup>HM</sup>). MettL3, hnRNPC, hnRNPG, YTHDC1 and ALKBH5 protein expression levels and m<sup>6</sup>A methylation of RNA were analyzed using western blot and EpiQuik<sup>TM</sup> m<sup>6</sup>A RNA Methylation Quantification Kit in a mouse uterine gestational series.

**Results:** Proteins HnRNPC1/C2 and MettL3 were significantly upregulated with 0.1  $\mu$ g/mL TM ERSR induction at both the 12 and 48-hour intervals when compared to the control. HnRNPG was also significantly upregulated after induction with 0.1  $\mu$ g/mL TM ERSR at 12, 48 and 72-hour intervals when compared to control. The strong correlation between ERSR and the increase in MettL3, HnRNPG and HnRNPC1/C2, signify not only an increase in m<sup>6</sup>A methylation associated with ERSR but a reader associated change in the functional proteome. MettL3 was

gestationally regulated with levels peaking at mid gestation and decreasing with the onset of labor. A similar expression pattern was seen in the reader proteins hnRNPG, hnRNPC, YTHDC1 and m<sup>6</sup>A labeled RNA. In contrast the eraser protein ALKBH5 was upregulated with the onset of labor.

**Conclusion:** Our current data together with our previously published changes in alternative splicing and the up regulation of ERSR during pregnancy support a role for m<sup>6</sup>A methylation in the generation of a gestationally regulated myometrial proteome. Overall, as we understand the progression of alternative splicing, ERSR and m<sup>6</sup>A methylation of the myometrium during normal gestation, we can therapeutically target specific molecular pathways in order to prevent preterm birth.

### W-030

**Uterine Stimulation Index: A Promising Technique for Personalized Use of Oxytocin.** Ponnilla S Marinescu<sup>†</sup>,<sup>1</sup> Roger C Young,<sup>2</sup> David A Adair,<sup>3</sup> Braxton Hern,<sup>3</sup> Evelina Galas,<sup>3</sup> Eva K Pressman,<sup>1</sup> Neil S Seligman\*.<sup>1</sup> <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>PreTeL, Inc., Chattanooga, TN, United States; <sup>3</sup>University of Tennessee College of Medicine, Chattanooga, TN, United States.

**Introduction:** We identified novel uterine electromyography (uEMG) signals named uterine oscillatory signals (uOS); uOS are intermittently expressed between contractions and are characterized by regular periods and amplitudes. Frequency and duration of uOS are longer during oxytocin exposure and may be an indicator of the degree of uterine stimulation. Distribution and duration of uOS can be combined to calculate a “uterine stimulation index” (uSI). Our objective is to evaluate the relationship between uSI and oxytocin dosing.

**Methods:** 4 term subjects receiving oxytocin were studied at a single academic center. Oxytocin doses were increased by 2 mU/min every 20 min per institutional protocol. 6-channel uEMG recordings were acquired using directional sensors. Presence of uOS was assessed by computer for each channel. uSI was calculated as the fraction of time uOS was observed within a 10-min moving window, averaged over all channels. If all 6 channels expressed uOS continuously throughout the window, the uSI would be maximal at 1; if no channel displayed any uOS, uSI would be 0.

**Results:** All subjects showed cervical change after initiation of oxytocin, with doses ranging from 4-12 mU/min. When observed, peak uSI values occurred following a rise in uSI and successively increased to a max of 0.48. Following each peak, uSI decreased; however, mean inter-peak uSI successively increased (Figure). Peak uSI at each dose of oxytocin per patient are reported (Table). uSI did not exceed 0.15 at oxytocin doses < 6mU/min. Oxytocin > 6mU/min was required to achieve uSI values above 0.2.

**Conclusion:** Transient rises in uSI, observed with each incremental increase in oxytocin dose, suggest that uSI reflects the degree of uterine stimulation by oxytocin. uSI may offer a personalized approach to oxytocin titration rather than reliance on generic protocols using contraction frequency and/or cervical change. This tool has the potential to optimize oxytocin efficiency and shorten the duration of time to delivery.

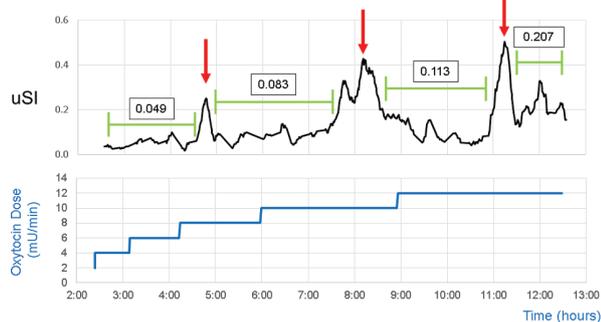


Figure. An example of uSI plotted over time with oxytocin dose increases (Subject 1). uSI peaks are identified by red arrows. In between peaks, mean uSI values are shown, calculated over the time period designated by corresponding green bars. Peak uSI and mean uSI values are both seen to successively increase with increasing oxytocin doses.

Table. Peak uterine stimulation index (uSI) at increasing doses of oxytocin.

	Oxytocin Dose (mU/min)						
	0 (Pre-Oxytocin)	2	4	6	8	10	12
Subject 1	X*	X*	0.03	0.08	0.22	0.42	<b>0.48†</b>
Subject 2	0	0.05	0.04	<b>0.05†</b>			
Subject 3	X*	0.12	0.08	0.13	<b>0.36†</b>		
Subject 4	0.9	0.13	<b>0.11†</b>				

\* No uterine electromyography reading obtained at corresponding dose  
† Values in bold indicate where dose increases were stopped

### W-031

**Identification of Mundulone and Mundulone Acetate as Natural Products with Tocolytic Efficacy in Mono and Combination Therapy with Current Tocolytics.** Shajila Siricilla<sup>†</sup>,<sup>1</sup> Christopher J Hansen,<sup>1</sup> Jackson H Rogers,<sup>1</sup> Carolyn L Simpson,<sup>1</sup> Stacey L Crockett,<sup>1</sup> Jeff Reese,<sup>1</sup> Bibhash C Paria,<sup>1</sup> Jennifer L Herington\*.<sup>1,2</sup> <sup>1</sup>Vanderbilt University Medical Center, Nashville, TN, United States; <sup>2</sup>Vanderbilt University, Nashville, TN, United States.

**Introduction:** Currently, there are no FDA-approved tocolytics for the management of preterm labor due to the off-target side effects and short duration of benefit of current off-label drugs. High-throughput screening of oxytocin induced Ca<sup>2+</sup> mobilization in uterine myometrial cells identified mundulone and mundulone acetate (MA) as hit antagonists. The aim of this work was to 1) examine the uterine selectivity of mundulone and MA by counterscreening vascular smooth muscle cells (VSMC)-the major off-target limiting the use of current tocolytics, 2) determine cytotoxic effects, 3) identify synergistic combinations of mundulone (and MA) with current tocolytics to increase efficacy and/or potency to decrease off-target side effects and 4) examine *ex vivo* tocolytic effects and confirm uterine selectivity at the tissue level by evaluating their effect on constriction of fetal ductus arteriosus (DA), a major off-target of known tocolytics

**Methods:** Primary human myometrial cells were isolated from tissue collected at the time of cesarean delivery from women at term (≥39 weeks) pregnancy. A phenotypic high-throughput Ca<sup>2+</sup> mobilization assay was used to compare concentration-response curves (CRC; 10 point, 3 fold dilutions) between myometrial and aorta VSMC. The above assay was adapted for combination screening with current tocolytics (atosiban, indomethacin and nifedipine) in 8x8 concentration-response matrix format. Bliss, HSA and Loewe models were used to determine synergy using Combenefit software. Cytotoxicity was determined using WST1 cell viability assay on myometrial cells and to calculate a safety index (SI; ratio of IC<sub>50</sub> in cell viability assay to IC<sub>50</sub> in Ca<sup>2+</sup> assay). *Ex vivo* organ bath studies using day 19 mouse myometrial tissue and fetal DA vessels were performed to examine CRC on contractility and vessel diameter, respectively

**Results:** MA was found to display selectivity towards myometrial cells compared to aorta VSMCs. Fold change (FC) in efficacy and potency was 13.5 and >4.5 for MA, respectively, while FC in potency for mundulone was >2.3. Mundulone displayed synergism with atosiban and nifedipine, while MA displayed synergistic efficacy with only nifedipine. Mundulone affected the viability of myometrial cells while MA and the mundulone+atosiban combination demonstrated a SI >10. Mundulone, MA and the combination of mundulone + atosiban showed concentration dependent inhibition of uterine contractions but did not affect fetal DA vasoreactivity

**Conclusion:** Based on differences in uterine-selectivity and tocolytic efficacy between mundulone and MA, this natural product could benefit from medicinal chemistry efforts to study structural activity relationship

## W-032

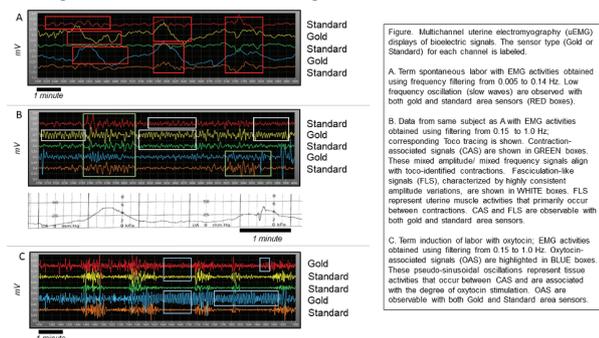
**Novel Gold Electromyographic Area Sensors Detect Uterine Bioelectric Activity as Well as Standard Hydrogel-Silver Area Sensors.** Ponnala Sunderi Marinescu†, Roger C Young, Pulin Wang, Eva K Pressman, Neil S Seligman\*. <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>PreTeL, Inc., Chattanooga, TN, United States; <sup>3</sup>Stretch Med, Inc., Austin, TX, United States; <sup>4</sup>University of Rochester Medical Center, Rochester, NY, United States.

**Introduction:** Uterine electromyography (uEMG) is an emerging technology for non-invasively assessing uterine activity. Standard uEMG area sensors contain silver tape and adhesive hydrogel, which limits their application for long-term monitoring because of gel drying and associated altered signal acquisition. We have developed a novel gold uEMG area sensor with no adhesive hydrogel that may allow continuous monitoring for days or weeks. Here, we seek to compare the bioelectrical signal quality observed using gold and standard sensors.

**Methods:** Observational cohort study of 6 subjects at  $\geq 37$  weeks with uncomplicated, singleton pregnancies in active labor. Two gold and 2 standard uEMG sensors were applied in varying configurations over the gravid abdomen. Multichannel uEMG was performed for 60 minutes. Uterine frequencies were assessed between 0.005-1.0 Hz. Primary outcome was the difference in bioelectrical signal quality, defined as signal-noise ratio, between gold and standard sensors. A signal-noise ratio  $>2.5$  was considered acceptable for reliable signal detection. The bioelectric signals assessed were as follows: contraction-associated signals (CAS), fasciculation-like signals (FLS), oxytocin-associated signals (OAS), and low frequency oscillations (slow waves).

**Results:** Both gold and standard sensors were able to detect all four signal types (CAS, FLS, OAS, and slow waves; Figure). There was no significant difference in CAS or slow wave signal-noise ratios between gold and standard sensors (CAS: gold= $3.8 \pm 2.1$  vs standard= $7.2 \pm 3.3$ ,  $P=.058$ ; slow waves: gold= $6.3 \pm 3.1$  vs standard= $7.9 \pm 2.7$ ,  $P=.369$ ). OAS and FLS were too infrequent to assess differences in signal-noise ratio; however, only 3 subjects received oxytocin, and FLS are uncommonly observed during labor. Motion artifacts were more common in gold sensors.

**Conclusion:** We demonstrate that our novel gold sensors can be used for uEMG analysis. Gold sensors have the ability to detect bioelectrical signals with signal-noise ratios equivalent to standard hydrogel-silver sensors. While gold sensors can remain applied to the skin for long periods of time, motion artifacts limit their application to periodic, short recordings; alternative technologies will need to be evaluated to accomplish the goal of long-term, continuous monitoring.



## W-033

**Effects of Monocytes on Contractile Function and Inflammatory Response of UtSM Cells under Hypoxia.** Binsh Wu†, Xiaoyan Sha\*, Huishu Liu†. Guangzhou Women & Children Medical Center, Guangzhou, China.

**Introduction:** Uterine muscle is smooth muscle and has the common characteristics — contractile function of vascular smooth muscle and other visceral smooth muscle. Contractile function is one of the most important functions of pregnancy uterus, uterine contraction is the core factor that determines delivery. Abnormal uterine contraction during labor can lead to prolonged labor, fetal distress, postpartum uterine

inertia. At present, it is considered that normal pregnancy is a low-grade “inflammatory response” process, and the inflammatory response during delivery is enhanced, but the regulatory mechanism of inflammatory response during delivery is not clear. The expression of chemokines and cell adhesion factors in the myometrium of late pregnancy was up-regulated, a large amount of leukocyte infiltration was recruited, abundant inflammatory mediators were released into local tissues, and signaling pathways such as prostaglandin synthesis or activation of nuclear factor  $\kappa$ B were promoted. Positive feedback recruited more white blood cells, further released inflammatory mediators, enlarged inflammatory responses, increased prostaglandin synthesis and increased uterine contraction. The study shows that the human uterus is in the environment of 3%-5% oxygen volume fraction. Hypoxia can also stimulate oxidative stress in cells. Culture in vitro hypoxic environment more in line with the body environment.

**Methods:** The primary human uterine smooth muscle cell culture system and the primary human peripheral blood monocyte culture system will be established, and the co-culture system of the two cells will be established; The activity of UtSM cells under hypoxic co-culture system will be analyzed by CCK8, the gel contraction test will detect the effect of hypoxic cells on UtSM cells contractile function; Western Blot the effect of hypoxic cells on the expression of UtSM cells contractile related proteins will be detected; The effects of hypoxic monocytes on the secretion of UtSM cells inflammatory factors were detected by ELISA, and the related signaling pathway of hypoxic monocytes on the inflammatory response of UtSM cells was detected.

**Results:** Under hypoxia, the activity of UtSM cells increased, the secretion of inflammatory factors increased, and the expression of contractile related proteins increased. Under the treatment of hypoxia and monocytes, the activity of UtSM cells increased significantly, the secretion of inflammatory factors proteins increased.

**Conclusion:** Under hypoxia, contractility of UtSM cells inflammatory factors increased. Hypoxia and monocytes played a synergistic role in the expression of UtSM cells inflammatory factors and contractility.

## W-034

**Leveraging Human Genomics to Accelerate Next-Generation Female Contraceptive Drug Discovery.** Karen Hunter Cohn, Caterina Clementi, Genevieve Galarneau, Piraye Yurttas Beim\*. Celmatix Inc, New York, NY, United States.

**Introduction:** Unwanted side effects, including heavy and irregular menstrual bleeding, are a barrier for hormonal contraceptive drug utilization by many women. Though dozens of products and formulations are available, they all rely on common mechanisms of action (MoA) through estrogen and/or progesterone. There is, therefore, an unmet need for next-generation female contraceptive drugs that unlock novel MoAs. Here, we report our efforts to identify novel contraceptive drug targets through genomic analyses aimed at surfacing molecular pathways and genes associated with ovulatory function in humans.

**Methods:** We began by structuring multiple data streams for identification and algorithmic ranking of putative drug targets. These included Literature Mined Genetic Evidence (literature-based association with an ovulatory phenotype, scored on the quantity and strength of evidence with guidelines adapted from ClinGen), Animal Genetic Evidence (genotype-phenotype data from the Mouse Genetic Informatics database, curated for relevance to ovulation/reproduction), and Tissue Expression Specificity (expression in ovary/reproductive tissues using GTEx project data). Genetic analyses were completed using genomic and deep phenotypic data from our Personalized Reproductive Medicine (PRem) cohort of infertility clinic patients. These included GWAS in patients of European ancestry for phenotypes such as basal antral follicle count and anti-Mullerian hormone levels, controlling for patient age. We also identified rare functional variant carriers using Ensembl Variant Effect Predictor on variants with a minor allele frequency  $< 1\%$  in gnomAD and located within RefSeq gene boundaries.

**Results:** Algorithmic ranking of these data streams allowed us to prioritize putative contraceptive drug targets with multiple lines of evidence associating them with ovulatory function in humans. Notably,

genes encoding receptors for estrogen and progesterone (*ESR1*, *ESR2*, and *PGR*) were among the most highly ranked genes using the approach. Further analysis of the most highly ranked putative targets also allowed us to identify rare, highly penetrant genetic variants in women who have failed multiple rounds of fertility treatments.

**Conclusion:** We have demonstrated that genomic analysis can reveal and help prioritize putative contraceptive drug targets. Furthermore, identification of rare, highly penetrant genetic variants in otherwise healthy women who have been unable to conceive spontaneously and/or who have failed multiple rounds of fertility treatments simultaneously reveal targets that are promising from an efficacy and safety perspective.

### W-035

**Mass Cytometry Reveals Unique Clusters of Monocytes in Blood and Decreased Phagocytic Capacity of Eutopic Endometrial Macrophages in Women with Endometriosis.** *Júlia Vallvé-Juanico*†, Sushmita Sen†, Ashley F George†, Kim Chi Vo\*, Juan C Irwin\*, Alexis Combes\*, Nadia Roan\*, Linda C Giudice\*. *University of California San Francisco, San Francisco, CA, United States.*

**Introduction:** We previously described that eutopic endometrial macrophages of women with endometriosis display a pro-inflammatory phenotype by RNASeq. Thus, our objective was to deep phenotype and identify differences in myeloid populations in eutopic endometrium and blood in women with endometriosis.

**Methods:** A total of 31 endometrial biopsies and 26 blood samples in proliferative and secretory phases of the menstrual cycle from women with (Endo, n=35) and without endometriosis (Ctrl, n=22) were obtained from the UCSF/NIH Human Endometrial TissueBank under IRB approval (IRB#10-02786). Written informed consent was obtained from all participants. A 40-marker panel for mass cytometry (CyTOF) was designed. Samples were processed, barcoded, stained, and run on CyTOF@2 instrument. The obtained fcs. files were normalized, debarcoded and imported into FlowJo, for manual gating. Supervised analyses were done using the manually gated populations. Differential abundance (2-way ANOVA) and expression analyses (t-test (pVal<0.05) and Benjamini-Hochberg (FDR<0.05)) were performed. As we found significant differences between markers in myeloid populations, unsupervised clustering analyses were performed only on these populations. Association between cluster identity and associations with markers, disease status and cycle phase are currently underway.

**Results:** In the supervised analyses pipeline, no significant differences in abundance of populations were found between Endo and Ctrl at any phase of the cycle. However, markers were differentially expressed. Endometriosis eutopic endometrial macrophages overexpressed *SIRPα*, a phagocytosis inhibitor. In blood, dendritic cells, intermediate and non-classical monocytes overexpressed *CD80*, an activation marker, in disease. Unsupervised analyses showed unique clusters in blood from endometriosis, corresponding to intermediate and non-classical monocytes.

**Conclusion:** Our results show decreased phagocytic capacity of endometrial macrophages in endometriosis, consistent with defective clearance of endometrial cells shed during menses having implications in the pathophysiology of the disease. In addition, higher activation and unique clusters of intermediate and non-classical monocytes present in blood of endometriosis patients indicate a systemically aberrant function of the myeloid system therein. Functional studies and increased sample size are planned for further validation. **Grant:** NIH P50 HD055764.

### W-036

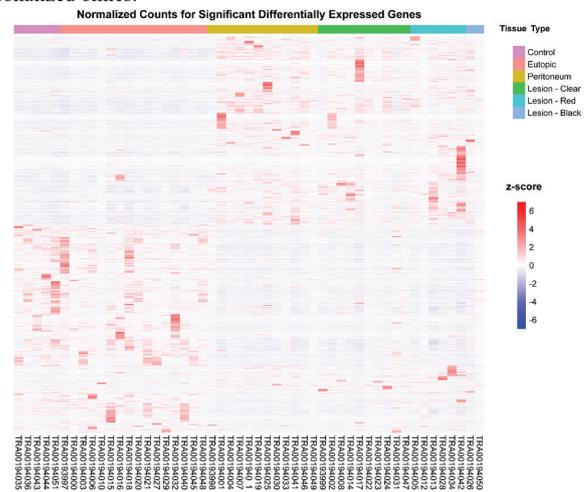
**Whole Transcriptome Sequencing of an Endometriosis Cohort and Generation of iPSC-Derived Models for Functional Screening.** *Jeremy Y Huang*†, Songlei Liu, Li Li, Ian N Waldman, Raymond M Anchan, George M Church\*. *<sup>1,2</sup>Blavatnik Institute, Harvard Medical School, Boston, MA, United States; <sup>3</sup>Wyss Institute for Biologically Inspired Engineering, Boston, MA, United States; <sup>3</sup>Brigham and Women's Hospital, Boston, MA, United States.*

**Introduction:** Endometriosis is hypothesized to be the ectopic growth of endometrial tissue, with retrograde menstruation as the most likely etiology. Frustratingly, there is a reported average of an 8-year diagnostic delay from symptom onset (pain and infertility), which is further compounded by the lack of treatment options and palliation of symptoms with treatment. New insights into the characteristic differences between healthy and diseased tissue and new models for in vitro research and development are needed.

**Methods:** We present a whole-transcriptome sequencing dataset from 20-patient diseased and 5-patient control cohorts with accompanying surgical and phenotypic metadata. Notably, 10 patients out of the diseased cohort had high-quality tissue sequenced from three different sites: eutopic endometrium, peritoneal membrane, and endometriosis lesions allowing for intra-patient transcriptional comparison. Additionally, we present recent efforts to generate endometrial and decidual-like cells from human induced pluripotent stem cells through transcription-factor overexpression.

**Results:** We examine differential gene expression in sequenced tissues such as inflammatory-related genes, *CXCL2*, *CCL18* and *CCL19* in red versus clear lesions, or cancer-related genes *DCSTAMP*, *ASCL2*, and *TRPM1* in red lesions versus peritoneum and discuss potential implications for disease classification and treatment. Transcriptional profiling of the candidate synthetic cell lines suggest similarity with in vivo patient tissues and existing endometrial single-cell RNA sequencing datasets.

**Conclusion:** Comparative gene expression analysis of endometriosis patient tissues reveals new markers and drug targets for endometriotic lesions. Synthetic endometrial-like cells may enable high-throughput in vitro drug screening future patient-specific treatment options through personalized omics.



**Fig 1 | Comparison of differentially expressed genes using DESeq2.** Rows are hierarchically clustered by gene. Columns indicate individual tissue samples.

### W-037

**The Role of Estrogen Receptor Alpha in Mediating Progesterone Resistance and Disease Progression in Endometriosis.** *Valerie A. Flores*†, Tran Dang, Joshua Huttler, Hugh S Taylor\*. *Yale School of Medicine, New Haven, CT, United States.*

**Introduction:** Endometriosis is a chronic, gynecologic disease affecting 1 in 10 reproductive-aged women. It is an estrogen and progestin responsive disorder, however some endometriosis is resistant to progestin-based therapies. We have previously demonstrated that progesterone receptor

(PR) levels are low in progestin-resistant disease. However, the role of estrogens acting through estrogen receptor alpha (ER $\alpha$ ) in modulating PR expression and response to progestin-based therapy has not been fully characterized. Here we aimed to determine if aberrant ER $\alpha$  expression correlates with response to progestin based therapy and regulates disease progression.

**Methods:** Endometriotic lesions were obtained from 48 subjects undergoing surgical evaluation in this retrospective cohort study. Matched eutopic endometrium was obtained from 11 subjects. Immunohistochemistry (IHC) was performed using a rabbit polyclonal IgG for detection of ER $\alpha$ , and the H-score was used to quantify ER $\alpha$  expression. Two investigators blinded to patient response independently scored IHC specimens. Data regarding hormonal therapy status, PR status in eutopic and matched ectopic lesions, and response to progestin-based therapy were determined from review of the electronic medical record. A Mann-Whitney U and Spearman's rank correlation were used for statistical analysis.

**Results:** ER $\alpha$  levels were significantly lower in non-responders compared to those who responded to progestin-based therapy (4 vs 13, respectively;  $p = 0.02$ ). There was a correlation between ER $\alpha$  levels and PR levels in ectopic lesions ( $p=0.001$ ,  $r=0.68$ ). There was no correlation between ER $\alpha$  levels in matched ectopic and eutopic lesions ( $n=11$ ). ER $\alpha$  expression did not correlate with stage of disease, or medication use.

**Conclusion:** ER $\alpha$  expression was lower in women with progestin-resistant compared to progestin responsive endometriosis. As low ER $\alpha$  correlated with low PR expression, ER $\alpha$  is likely required to induce PR and allow progestin sensitivity. Existing medical therapies rely on lowering estradiol levels or inducing decidualization of lesions, yet each has associated failure rates. Maintenance of ER in endometriosis therapy may allow adequate PR to allow for progestin based therapies.

#### W-038

**Tofacitinib Alters STAT3 Signaling and Leads to Endometriosis Lesion Regression.** Alexander Kotlyar<sup>†</sup>, Ramanaiah Mamillapalli, Valerie Flores, Hugh Taylor. *Yale University, New Haven, CT, United States.*

**Introduction:** Endometriosis is a wide-spread gynecologic condition affecting up to 15% of reproductive age women. The JAK/STAT3 pathway is upregulated in endometriosis and a therapeutic target. Here we sought to determine the effect of Tofacitinib, a Janus kinase-inhibitor in widespread clinical-use, on JAK/STAT signaling in endometriosis and lesion growth.

**Methods:** Cells were cultured with Tofacitinib at a therapeutic and subtherapeutic dosage. Mice with surgically-induced endometriosis were treated with Tofacitinib versus vehicle. Endometriosis lesion size and adhesion burden were assessed in mice treated with Tofacitinib versus vehicle. HIF1 $\alpha$  and VEGF expression levels in human cells were assessed by qPCR. STAT3 and phospho-STAT3 expression from Tofacitinib-treated and untreated cells was performed via western blotting and densitometry.

**Results:** Using a mouse model of surgically-induced endometriosis ( $n=20$ ), daily Tofacitinib treatment led to lesion regression ( $p<0.01$ ) and reduced adhesion burden ( $p<0.001$ ). STAT3 phosphorylation was significantly decreased in both lesions and uteri of mice treated with Tofacitinib versus vehicle ( $p<0.05$ ). In cell-culture, Tofacitinib reduced HIF1 $\alpha$  and VEGF mRNA levels at 12 and 24hrs of treatment. Following 24 hrs of exposure, Tofacitinib effectively reduced STAT3 phosphorylation in Ishikawa cells ( $p<0.05$ ), and patient-derived stromal ( $p<0.01$ ) and epithelial cells ( $p<0.01$ ) from eutopic endometrium. Expression of HIF1 $\alpha$  was significantly reduced with Tofacitinib treatment of control endometrial stromal and epithelial cells, but not in patients with endometriosis.

**Conclusion:** This study suggests that inhibition of JAK/STAT signaling using Tofacitinib may be a viable method for the treatment of endometriosis. By reducing STAT3 phosphorylation, lesion size and adhesive disease are both reduced.

#### W-039

**Modeling the Adenomyotic Phenotype Using Endometrial Organoids and a Fully Defined Synthetic Extracellular Matrix.** Juan S Gnecco<sup>†</sup>, Kira Buttrey<sup>†</sup>, Alex Brown<sup>†</sup>, Clara Ives,<sup>1</sup> Megan Loring,<sup>2</sup> Keith Isaacson,<sup>3</sup> Linda Griffith\*.<sup>1</sup> *MIT, Cambridge, MA, United States;* <sup>2</sup>*NWH, Newton, MA, United States.*

**Introduction:** Adenomyosis is a gynecological disorder characterized by the histologic presence of endometrial glands and stroma within the myometrium of the uterus and is associated with subfertility, pelvic pain and abnormal uterine bleeding. However, its etiology is poorly understood due to a lack of phenotypic models. Recent advancements in 3 dimensional (3D) imaging and organoid cell cultures derived from primary human tissues provide an avenue to investigate endometriotic disease *in vitro*. Herein, we developed a fully defined and tunable synthetic polyethylene glycol (PEG) hydrogel to culture human endometrial epithelial organoids (EEOs). and investigate the role of extracellular matrix (ECM) in promoting disease pathogenesis.

**Methods:** PEG hydrogels were prepared by reacting a multi-arm PEG-vinyl sulfone macromer with specific peptides designed to promote cell adhesion by targeting integrins expressed by endometrial cells. ECM stiffness was tuned to mimic the bulk stiffness of the native endometrium (~300 Pa) and the myometrium (>2 kPa) by increasing macromer concentration. EEO morphology, efficiency and hormonal response was characterized using immunofluorescent imaging compared to Matrigel. The contribution of stromal cell was assessed as a co-culture aggregate spheroid. Transcriptomic analysis was performed using qPCR. Cytokines and matrix proteinase (MMPs) profiles were quantified using multiplexing Luminex assays.

**Results:** Direct encapsulation single cell EEOs in the PEG-based hydrogels resulted in formation of organoids in stiff and soft PEG hydrogels with no evident difference in efficiency or size. However, the EEO morphology was drastically altered in stiffer ECMs that mimicked the myometrial stiffness and mimicked ectopic lesion morphology as demonstrated by *in situ* tissue clearing and light-sheet imaging. Specifically, the organoids lost their architecture and demonstrated an invasive phenotype. We demonstrated these EEOs demonstrated many of the hallmarks of endometriotic disease which included reduced hormone expression, increased inflammatory cytokine expression, and invasive behavior mediated by epithelial-mesenchymal transition (EMT).

**Conclusion:** A common feature of endometriotic (endometriosis and adenomyosis) disease is a stiff environment. We utilized an innovative synthetic hydrogel platform and identified that increased extracellular stiffness promotes an EMT-driven invasive endometriotic phenotype *in vitro*. This model provides an avenue to understand the biophysical origins of adenomyotic disease and drug screening. Next generation models will introduce additional cellular and molecular components of the lesion microenvironment.

#### W-040

**Clinical Presentation of Women with Adenomyosis Alone and Concurrently with Uterine Fibroids.** Nawras Zayat, Anthony Filipovic, Brittany Dey, Victor Mniarji, Cassandra Charles, Serin Seekin, Ozgul Muneeyirci-Delale. *SUNY Downstate Medical Center, Brooklyn, NY, United States.*

**Introduction:** Uterine adenomyosis is a condition in which endometrial glands and stroma break through the myometrium, causing dysmenorrhea, pelvic pressure, heavy menses, abnormal uterine bleeding and dyspareunia with a prevalence of 2% in women aged 15-50 years. Uterine fibroids can also cause similar symptoms, however the symptom profile of having both pathologies present has not been well documented in the literature.

**Methods:** An IRB-approved retrospective chart review was conducted using the electronic medical records of 375 women aged 18 years and older seen between 2011 and 2019 at SUNY Downstate's Outpatient OB/GYN Suite. Women were excluded if they were pregnant as well as those who were diagnosed with malignancy, HIV, renal failure, psychiatric illness and those using hormone therapy or history of steroid medication use. Diagnosis of adenomyosis and uterine fibroids were determined by a gynecologist. Data collected included demographics, BMI, menstrual and

obstetric history as well as ultrasound findings and laboratory parameters including CBC, CMP, HbA1C and lipid profile. Women were categorized as adenomyosis alone (AA), adenomyosis concurrent with uterine fibroids (AF) and controls without either adenomyosis or uterine fibroids. Data were then analyzed for group differences between AA vs controls and AA vs AF using Wilcoxon Rank Sum and Fisher's exact test.

**Results:** Women were mostly Black (64.8%) with median BMI of 27.0 kg/m<sup>2</sup>. AA women were significantly older than controls (42 vs 36 years, respectively;  $p = 0.01$ ). AA women had a significantly higher prevalence of dysmenorrhea compared to controls (70.6 vs 29.2%, respectively,  $p = 0.002$ ), endometriosis (17.2 vs 6.2% respectively;  $p = 0.045$ ) and heart disease (13.3 vs 4.1%, respectively;  $p = 0.05$ ). AA women were also more likely to have had a myomectomy than controls (20.7 vs 2.9%, respectively,  $p < 0.001$ ). There were no clinically significant differences in laboratory values between the two groups. The subset of women with adenomyosis (AA & AF) were similar in age ( $p = 0.9577$ ) and BMI ( $p = 0.1353$ ). AF women were significantly less likely to have PCOS compared to AA women (2.8 vs 50.0%, respectively;  $p < 0.0001$ ). AF women compared to AA women were noted to have significantly thicker endometrium on ultrasound (7.6 vs 2.9 mm, respectively;  $p = 0.005$ ) as well as significantly lower levels of hemoglobin (11.9 vs 12.8 g/dL, respectively;  $p = 0.03$ ) and HbA1C (5.5 vs 5.7%, respectively;  $p = 0.04$ ).

**Conclusion:** Women with adenomyosis reported having dysmenorrhea at a higher rate than controls. They were also more likely to have a history of endometriosis, heart disease and previous myomectomy. Women with concurrent fibroids and adenomyosis were more likely to have thickened endometrium as well as lower levels of hemoglobin and HbA1C. A history of myomectomy was associated with the presence of adenomyosis.

#### W-041

**The Role of Tfp2c in Endometriosis with Infertility.** Juan Yin<sup>†</sup>,<sup>1</sup> Kosina Wong,<sup>2</sup> Radu Apostol,<sup>2</sup> Huan Yang\*,<sup>2</sup> Anping Lin.<sup>3</sup> <sup>1</sup>The Ninth People's Hospital of Chongqing, Chongqing, China; <sup>2</sup>Coney Island Hospital, Brooklyn, NY, United States; <sup>3</sup>The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China.

**Introduction:** Impaired decidualization of eutopic endometrium is a key contributor to infertility in women with endometriosis. Our previous work identified a series of transcriptional factors, which might contribute to the development of endometriosis. Tfp2c was possible one of them. However, and its mechanism is still unknown.

**Methods:** Eutopic endometrial samples were collected from 14 endometriosis patients with infertility and 16 healthy who underwent tubal sterilization were confirmed to be free of endometriosis laparoscopically. Endometrial tissues were digested for cell culture. PCR and western blot were used to verify Tfp2c expression. The expression of Tfp2c in primary ESCs was depleted by transfection with Tfp2c siRNA. Then, MTT assay, Flow cytometry, and wound healing assay were used to identify the effect of down-regulated Tfp2c on proliferation, apoptosis, and migration in ESCs.

**Results:** The expression of Tfp2c was reduced in eutopic endometrium from patients with endometriosis compared to control ( $p < 0.05$ ). Tfp2c siRNA transfected in ESCs successfully confirmed by Western blot. Cell viability of ESCs with transfected Tfp2c siRNA increased significantly, whereas the apoptosis rate decreased (both  $p < 0.05$ ). Transfection of Tfp2c siRNA promoted ESCs migration ( $p < 0.05$ ).

**Conclusion:** Our results suggested Tfp2c deficiency promotes cell proliferation and suppresses apoptosis in eutopic endometrium in patients with endometriosis, which might contribute to infertility. Additionally, cell migration enhancement of eutopic endometrium may be responsible for the formation of ectopic endometriosis sites.

#### W-042

**Should I Stay or Should I Go? The Effect of Ovarian Endometrioma on Ovarian Reserve and Pregnancy Outcomes.** Caroline Peschansky<sup>†</sup>, Safina Usmani, Sarah Dynia, Sonia Patel, Jawaria Amir, Royi Lynn, Kayla Vitale, Lauren Grimm, Erica Loudon, Roohi Jeelani, Angie Beltsos. *Vios Fertility Institute, Chicago, IL, United States.*

**Introduction:** Endometriosis is one of the most common reproductive disorders among women. Up to 44% suffer from ovarian endometriomas, which are often asymptomatic but can be large enough to cause pain, discomfort, and inhibit normal ovarian function. The standard surgical management for endometriomas is an ovarian cystectomy, however, for those seeking to conceive this has been associated with upwards of a 50% decrease in ovarian function, lower Anti-Müllerian hormone (AMH) levels, and difficulty achieving pregnancy. For those already experiencing infertility, this compounding effect can be devastating. While this data is well recognized, there is little data showing the implications of endometriomas on fertility prior to cystectomy. Thus, we sought to better understand how the presence and size of endometriomas may impact ovarian reserve in women that have not yet had surgical intervention, and their effects on controlled ovarian hyperstimulation for in-vitro fertilization (IVF) treatment.

**Methods:** Retrospective chart review at a private fertility clinic. All patients 46 yo and younger undergoing IVF between Aug 2016 and Nov 2020 with a history of endometriosis were included. Baseline characteristics, including estradiol (E2) and AMH levels at time of menses, and endometrioma sizes were measured and IVF cycle statistics recorded. IVF outcomes were characterized as the number of oocytes retrieved, oocyte maturation and fertilization rate, and blastocyst utilization rate. Blastocyst utilization was defined as any embryo suitable for transfer or cryopreservation. Linear regression, T-tests, and chi-square analysis were used to analyze the data using SPSS (SPSS Inc., Chicago, IL, USA).

**Results:** 75 patients were identified to have at least one endometrioma present on ultrasound at baseline, cycle day 2-5. Baseline characteristics were noted, with an average age being 37 years, average antral follicle count (AFC) of 8.98, and average AMH of 1.78. 72% of the cohort analyzed were noted to have more than one endometriomas. The presence and size of endometriomas did not significantly affect AMH or AFC, with  $R^2 = 0.14$  and  $0.01$  respectively. Cycle outcomes including average oocyte maturation rate, fertilization rate, and blastocyst utilization rate were 78%, 95%, and 38% respectively, similar to national data for this age group.

**Conclusion:** Our results indicate that the size and presence of ovarian endometriomas prior to cystectomy do not have a significant negative effect on ovarian reserve and IVF outcomes. These results are encouraging for physicians and patients, as those struggling to conceive or suffering from painful ovarian endometriomas can now be recommended for oocyte or embryo cryopreservation prior to endometrioma removal without fear of further implications on their reproductive health.

#### W-043

**Is Endometriosis Associated with Congenital Uterine Anomalies? A Systematic Review.** Bo Peng\*,<sup>1</sup> Erroll I. Byer\*,<sup>2</sup> Michael Moretti\*.<sup>2</sup> <sup>1</sup>American University of the Caribbean, School of Medicine, Cupecoy, Netherlands Antilles; <sup>2</sup>The Brooklyn Hospital Center, Department of Obstetrics and Gynecology, Brooklyn, NY, United States.

**Introduction:** Endometriosis is a benign endometrial proliferation outside of the uterus and affects about 1/3 of infertile women. Congenital uterine anomalies (CUA) include different types of uterine malformations associated with adverse pregnancy outcomes such as miscarriage, preterm birth, and infertility. Previous studies yielded controversial results on the link between endometriosis and CUA, and the prevalence of endometriosis in women with CUA is not clear. The aim of our study is to conduct a systematic review of published literature to investigate the association between endometriosis and CUA.

**Methods:** Research articles and high-quality abstracts on MEDLINE, CINAHL, Web of Science, and Embase from data inception date through January 31<sup>st</sup> 2021 that studied of the prevalence of endometriosis in women with or without CUA were included. Data were analyzed, and the

pooled prevalence was presented with percentage and 95% confidence interval (CI). Odd ratio (OR) with 95% CI were used to present the risk of endometriosis in CUA. Heterogeneity was analyzed with  $I^2$ .

**Results:** A total of 21 studies were included in our systematic review. The pooled prevalence of endometriosis in CUA is 35.9% (95% CI 17.9 to 53.9,  $I^2=98\%$ ). Endometriosis is presented in 30.1% (95% CI 13.7 to 46.6,  $I^2=98\%$ ) of women diagnosed with septate uterus, a subtype of CUA. The prevalence of endometriosis in other subtypes unicornuate uterus, bicornuate uterus, and uterus didelphys is 28.8% (95% CI 15.5 to 42.2,  $I^2=85\%$ ), 31.2% (95% CI 13.2 to 49.2,  $I^2=83\%$ ), and 34.5% (95% CI 19.2 to 49.7,  $I^2=78\%$ ), respectively. No significant differences were observed between the risk of endometriosis in women with or without CUA (OR=0.88, 95% CI 0.46 to 1.66,  $I^2=80\%$ ). Additionally, in women diagnosed with endometriosis, the prevalence of CUA is 12.9% (95% CI 5.6 to 20.2,  $I^2=97\%$ ).

**Conclusion:** Our systematic review suggested that the pooled prevalence of endometriosis in women with CUA and distinct subtypes are between 25-40% based on available published data. Despite the previous report of the coexistence of endometriosis and CUA, the risk of endometriosis does not differ between women with or without CUA, however, heterogeneity between studies is significant. Nevertheless, our study may provide clinicians a current understanding of the epidemiology of endometriosis in women with CUA. Future studies with larger sample sizes and controls may assist us to elicit the association between these conditions.

#### W-044

**Simvastatin Inhibits Progesterone Receptor Signaling in Uterine Leiomyoma Cells.** Sadia Afrin†, Malak El Sabeh, Mariko Miyashita-Ishiwata, Mostafa Borahay\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Uterine leiomyoma is the most common benign tumors in the female reproductive tract and its growth is partly mediated by steroid hormones. Several progesterone (P4)-related aberrations have been identified in leiomyomas, including extensive extracellular matrix (ECM) deposition, upregulation of growth factors and their signaling pathways, epigenetic alterations, as well as the overexpression of the progesterone receptors (PRs). Thus, due to the involvement of P4 and PR in leiomyoma pathobiology, the objective of this study was to test the hypothesis that simvastatin, an anti-hyperlipidemic drug, would alter the P4-induced proliferation and PR signaling pathways in leiomyoma cells.

**Methods:** Primary leiomyoma cells were treated with simvastatin (0.001-1  $\mu$ M) and P4 (100 nM), alone or in combination, for 48 h to examine cell viability by MTT assay. The expression of PR-A/B was examined using western blot and immunofluorescence assay after simvastatin treatment. Next, we quantified the protein expression levels of ECM (collagen 1, fibronectin, versican), MAPK (ERK1/2 and JNK), mTOR, and PGMRC1 in response to simvastatin treatment. To investigate the role of simvastatin in PR transcriptional activities, we measured PRE-Luc activity through luciferase assay. The student's t-test was used to determine statistically significant differences ( $p<0.05$ ).

**Results:** Simvastatin treatment for 48 h decreased cellular proliferation in a dose-dependent manner compared to the vehicle control (DMSO). Treatment with P4 (100 nM) alone for 48 h increased proliferation by 18%, while the combination with simvastatin decreased this effect at all tested concentrations. Simvastatin concentrations of 0.01 to 1  $\mu$ M significantly suppressed the protein levels of PR-B by 1.78-3.22-fold, but not PR-A. Immunofluorescence analysis further demonstrated that simvastatin treatment decreased the immunostaining intensity of PR-B in leiomyoma cells compared to the control. The expression levels of collagen 1, fibronectin, and versican were decreased by 2.17-fold, 2.63-fold and 3.57-fold after simvastatin treatment. Simvastatin treatment remarkably suppressed the expression of non-genomic PR signaling, p-ERK/ERK by 2.38-fold, p-JNK/JNK by 3.33-fold, p-mTOR1/mTOR1 by 2.30-fold, and PGMRC1 by 2.78-fold, respectively. When leiomyoma cells were transfected with PR-B, there was a significant 15-fold P4-dependent induction of PRE-Luc activity, and simvastatin significantly decreased reporter gene activity up to 10-fold.

**Conclusion:** We conclude that simvastatin suppressed both genomic and non-genomic PR actions in uterine leiomyoma cells. The results reveal the effectiveness of simvastatin in treating uterine leiomyoma and highlights simvastatin as a unique candidate for further mechanistic and therapeutic investigations. *Supported by NIH grant 1R01HD094380*

#### W-045

**The Mediator Kinase-Dependent Myometrial Stem Cell Phosphoproteome.** Lindsey Barron, Subash Khadka, Thomas G Boyer\*. *University of Texas Health San Antonio, San Antonio, TX, United States.*

**Introduction:** Recurrent somatic mutations in the RNA polymerase II transcriptional Mediator subunit MED12 are dominant drivers of uterine fibroids (UFs), accounting for 70% of these clinically significant lesions. Within the multiprotein Mediator, MED12, along with MED13, CycC, and CDK8 (or its paralog CDK19) collectively comprise a 4-subunit kinase module. Previously, we and others have shown that MED12-dependent activation of CycC-CDK8/19 is required nuclear transduction of signals instigated by multiple oncogenic pathways. Mechanistically, we showed that MED12 activates CycC-CDK8 through direct stabilization of the CDK8 activation (T)-loop and, notably, that UF driver mutations in MED12 alter CDK8 T-loop stabilization and impair Mediator kinase activity. Accordingly, we hypothesize that Mediator kinase disruption and altered signaling as a consequence of UF driver mutations in MED12 is a precipitating force in UF formation. However, the identity of Mediator kinase substrates and their pathogenic contribution to UF development have not been established.

**Methods:** Herein, we used SILAC-based quantitative phosphoproteomics coupled with a highly specific chemical inhibitor of CDK19 to identify Mediator kinase substrates in myometrial stem cells (MM SCs), the presumptive cell of origin for UFs. Quantitative assessment of high-confidence Mediator kinase targets was determined using an Empirical Bayes analysis. Orthogonal validation of CDK8/19 targets was achieved by vitro kinase assays featuring reconstituted 4-subunit Mediator kinase modules comprising WT or G44D mutant MED12, the latter corresponding to the most frequent MED12 UF driver mutation.

**Results:** 306 phosphosites in 166 different proteins were significantly decreased ( $\geq 2$ -fold;  $p < 0.05$ ) upon CDK8/19 inhibition, including 110 phosphosites in 71 nuclear proteins that represent high-confidence CDK8/19 substrates. Network analyses linked these putative substrates with RNA splicing and transport, chromatin organization and gene expression, and DNA repair and mitotic cell cycle. Orthogonal validation confirmed a protein subset, including STAT1, p53BP1, FOXK1, NELFB, and CUX1, to be direct targets of MED12-dependent CDK8 phosphorylation in vitro in a manner abrogated by the most frequent UF driver mutation (G44D) in MED12, implicating these substrates in disease pathogenesis. Among validated substrates, FOXK1 with established links to myogenic stem cell fate, was found to be coordinately enriched along with Mediator kinase module, but not core Mediator, components in MM SCs cells compared to differentiated MM cells.

**Conclusion:** Altogether, these studies identify a new catalog of patho/biologically relevant Mediator kinase targets for further study in the pathogenesis and treatment of MED12-mutation positive UFs, and further suggest a biochemical basis to link Mediator kinase activity with FOXK1 in MM SC cell fate.

#### W-046

**Pathological Reprogramming of Epitranscriptomics via METTL3 in Uterine Fibroids.** Qiwei Yang,<sup>1</sup> Karthigayan Shanmugasundaram,<sup>2</sup> Chuan He,<sup>3</sup> Ayman Al-Hendy,<sup>1</sup> Thomas G Boyer.<sup>2</sup> <sup>1</sup>University of Chicago, OB/GYN, Chicago, IL, United States; <sup>2</sup>University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; <sup>3</sup>University of Chicago, Chemistry, Chicago, IL, United States.

**Introduction:** Uterine fibroids (UFs) are benign monoclonal neoplasms of the myometrium (MM) and represent the most common tumors in women worldwide. So far, no long-term, non-invasive treatment option currently exists for UFs, and deeper mechanistic insight into tumor etiology is key to develop newer targeted therapies. The epitranscriptome is an emerging frontier in molecular medicine owing to its vast potential as an

additional highly dynamic layer of gene regulation above and beyond the epigenome. Among >160 different chemical modifications in RNA, N6-methyladenosine (m<sup>6</sup>A) is the most pervasive, abundant, and conserved modification within eukaryotic mRNAs. However, the role of m<sup>6</sup>A and its regulation have not been identified in UFs. In this study, we characterize the role of the key m<sup>6</sup>A writer METTL3 in UFs.

**Methods:** *MED12* mutation-positive and -negative UFs (n=12 each) along with matching MM (n=12) from the same patient uteri were collected at The University of Texas. Immunoblots were used to measure the METTL3 levels in tissues as well as in UF and MM cell lines. METTL3-specific shRNAs were introduced into a UF cell line to determine the role of METTL3 in cell growth (MTT assay), survival (apoptotic markers), and epigenomic changes (active or repressive Histone marks). The Student's t-test was used to determine the significant difference.

**Results:** Quantitative immunoblot analysis revealed a significant upregulation of the m<sup>6</sup>A writer METTL3 (~3-fold, p<0.001) in UFs (independent of *MED12* mutation status) compared to MM, which is consistent with upregulation of METTL3 in UF cells compared to uterine smooth muscle cells. Knockdown of METTL3 in UF cells exhibited altered morphological features, reduced proliferation and enhanced sensitivity to the apoptosis-inducing agent Staurosporine, concomitantly with upregulation of the pro-apoptotic BAX and downregulation of the anti-apoptotic BCL2 proteins (p<0.05). Unexpectedly, we also observed that METTL3 knockdown led to global upregulation of transcriptionally repressive histone methylation (H3K9me2, H3K9me3, and H3K27me3), suggesting that METTL3-dependent RNA methylation may promote a transcriptionally permissive chromatin environment in UF cells.

**Conclusion:** Together, these data suggested that m<sup>6</sup>A writer METTL3 is aberrantly upregulated in UFs, representing an initial paradigm for pathological dysregulation of the m<sup>6</sup>A machinery in UFs. METTL3 depletion in UF cells triggers global alterations in the m<sup>6</sup>A modification landscape along with reduced cell proliferation, increased death and upregulation of repressive histone methylation marks. These studies establish the RNA methylation machinery to be a viable target for therapeutic intervention in UFs.

#### W-047

**Tryptophan Catabolism Is Dysregulated in Leiomyomas.** Tsai-Der Chuang, Derek Quintanilla, Drake Boos, Omid Khorram\*. *The Lundquist Institute at UCLA Medical Center, Torrance, CA, United States.*

**Introduction:** To determine the expression and functional roles of indoleamine 2,3-dioxygenase (IDO1) and tryptophan 2,3-dioxygenase (TDO2) in leiomyoma and the effects of their inhibitors on extracellular matrix (ECM).

**Methods:** Leiomyoma and matched myometrial tissue samples were collected from patients (N=58) including Caucasians (N=12), African Americans (N=25) and Hispanics (N=21). The menstrual cycle phase was determined by histologic analysis with 27 specimens being identified as in the proliferative phase and 14 specimens in the secretory phase. The *MED12* (Mediator of RNA polymerase II transcription, subunit 12 homolog) mutation status was determined by PCR amplification and Sanger sequencing. Of the specimens sequenced, 40 fibroids had the *MED12* mutations (68.9%). Kynurenine concentration in paired leiomyoma and myometrium homogenates was measured using the Human Kynurenine ELISA kit. MSMC (myometrium smooth muscle cells) and LSMC (leiomyoma smooth muscle cells) were isolated for culture and the cell proliferation and the gene expression analysis were using three-dimensional spheroid culture system determined by CellTiter-Glo 3D Cell Viability Assay and western blotting analysis. Results were analyzed by Student's t-tests and one-way ANOVA with Tukey's HSD for post hoc analysis.

**Results:** Leiomyoma as compared with matched myometrium expressed significantly higher levels of IDO1 and TDO2 mRNA and protein, and kynurenine, a marker of enzyme activity. The expression of TDO2 but not IDO1 mRNA was significantly higher in fibroids from African American as compared with Caucasian and Hispanic patients. TDO2 but not IDO1 protein and mRNA levels were more abundant in fibroids bearing the *MED12* mutation as compared with wild type leiomyomas. Treatment of

LSMC and MSMC spheroids with the TDO2 inhibitor, 680C91 but not the IDO1 inhibitor, Epacadostat significantly repressed cell proliferation and the expression of collagen type I (COL1A1) and type III (COL3A1) in a dose-dependent manner; these effects were more pronounced in LSMC as compared with MSMC spheroids.

**Conclusion:** These results underscore the physiological significance of tryptophan degradation pathway in the pathogenesis of leiomyomas and the potential utility of anti-TDO2 drugs for treatment of leiomyomas.

#### W-048

**Simvastatin Induces the Expression of Matrix Metalloproteinase in Human Leiomyoma Cells.** Malak El Sabeh†, Sadia Afrin†, Mostafa Borahay\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Uterine leiomyoma (UL) is the most common benign tumor in the female reproductive tract. UL arises from cellular proliferation and aberrant extracellular matrix deposition. One of the key proteins involved in regulating extracellular matrix is matrix metalloproteinases (MMPs) that are responsible for extracellular matrix degradation, among other functions. MMP levels are elevated after ulipristal acetate treatment, and high MMP activity correlated with reduced leiomyoma size. Simvastatin is a 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor and is commonly used for the treatment of hypercholesterolemia. In vivo and in vitro studies have shown promising effects of simvastatin as a treatment for uterine leiomyomas by inducing apoptosis, reducing proliferation, and altering extracellular matrix deposition. The aim of this study was to test the hypothesis that simvastatin increases the expression of MMPs in UL tissue.

**Methods:** Primary and immortalized leiomyoma cells were treated with simvastatin concentration (0.001, 0.01, 0.1, and 1 μM) for 48 hours. The effect of simvastatin on the mRNA and protein expression of several MMPs was examined after 48 hours treatment by using RT-qPCR and western blot, respectively. To confirm this effect, primary leiomyoma cells were stained with anti-MMP2 and anti-MMP9 for immunofluorescence. The student's t-test was used to determine statistically significant differences (P<0.05).

**Results:** Simvastatin significantly increased the mRNA expression of MMP-2, MMP-7, and MMP-14 in a dose-dependent manner, with the highest increase seen with MMP-9 with a 9.2-fold increase at the highest concentration (1 μM). Simvastatin also significantly induced the protein expression of MMP-2 in a dose-dependent manner with a 6.1-fold increase at the highest concentration (1 μM). These results were also confirmed through immunocytochemistry using anti-MMP-2 and anti-MMP-9 antibodies.

**Conclusion:** Simvastatin induces the expression of MMPs in human leiomyoma cells, altering the sensitive balance of extracellular protein degradation, the main component in uterine leiomyoma development.

#### W-049

**Targeting Hippo Signaling by Epigallocatechin Gallate (EGCG) Reduces Cell Growth, Fibrosis, Inflammation, and Angiogenesis in Uterine Fibroid Cells.** Md Soriful Islam, Kamaria C Cayton Vaught, Joshua T. Brennan, James H Segars. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Uterine fibroids are mechanically stiff, fibrotic tumors. Recently, we reported that the Hippo signaling effector YAP was overexpressed in fibroids, possibly contributing to cell growth, fibrosis, inflammation and angiogenesis. Epigallocatechin gallate (EGCG) is a natural compound isolated from green tea that reduced fibroid growth in vitro and in a randomized clinical trial. Here, we tested the hypothesis that the reduction in cell growth, fibrosis, inflammation, and angiogenesis caused by EGCG would involve Hippo signaling.

**Methods:** To assess cell viability, human myometrial (P51M) and fibroid (P51F) cells were treated with EGCG at different doses (1, 10, 50, 100 and 200 μM) for 24 hrs. To measure mRNA and protein expression, cells were treated with EGCG at 100 μM for 24 hrs. Several genes or proteins were evaluated. For cell growth: cyclin D1 (CCND1); for fibrosis: versican (VCAN), fibronectin (FN1), activin-A (INHBA), transforming growth factor β1 (TGFB1), TGFB2, and plasminogen activator inhibitor-1 (PAI-

1); for inflammation: interleukin-11 (IL-11), IL-1B, and high mobility group box 1 (HMGB1); for angiogenesis: endothelin 1 (EDN1), vascular endothelial growth factor C (VEGFC), and platelet-derived growth factor C (PDGFC), and for Hippo signaling: non-phospho-YAP, baculoviral IAP repeat containing 5 (BIRC5), and connective tissue growth factor (CTGF). T-test was used for data analysis and  $p < 0.05$  was considered significant. **Results:** We found that viability of fibroid cells was slightly (5%) reduced by EGCG treatment at 100  $\mu$ M, while myometrial cells growth increased (18%). In fibroid cells, the mRNA and protein levels of proliferative gene, cyclin D1, were reduced by EGCG treatment at 100  $\mu$ M. Several fibrotic factors including *VCAN*, *FNI*, *INHBA*, *TGFBI*, *TGFB2*, and *PAI-1* were found to be highly expressed at mRNA in fibroid cells which were downregulated by EGCG treatment. Notably, the protein expression of FN1 and PAI-1 was also reduced by EGCG treatment. We noticed that EGCG treatment significantly decreased mRNA levels of inflammatory mediators, *IL-11*, *IL-1B*, and *HMGB1*. The gene expressions of key mediators of angiogenesis such as *EDN1*, *VEGFC*, and *PDGFC* were also reduced by EGCG treatment. Mechanistically, EGCG treatment reduced protein expression of a transcriptional effector of Hippo signaling, YAP. We also found reduced transcript or protein levels of YAP-responsive genes, *BIRC5* and *CTGF*.

**Conclusion:** EGCG treatment altered Hippo signaling and was associated with reduced levels of genes involved in cell proliferation, fibrosis, inflammation, and angiogenesis in uterine fibroid cells. These results support the beneficial effect of green tea for human health and can be a useful natural compound for fibroid treatment.

#### W-050

**Evidence for Homeotic Transformation in Uterine Fibroids: A Novel Paradigm.** Jaime A Roura-Monllor<sup>†</sup>,<sup>1</sup> Minnie Malik,<sup>1</sup> Paul H Driggers,<sup>1</sup> Joy Britten,<sup>1</sup> Anthony M DeAngelis<sup>†</sup>,<sup>2</sup> Erin F Wolff,<sup>1,3</sup> William H Catherino<sup>\*,1,2</sup> <sup>1</sup>Uniformed Services University of the Health Sciences, Bethesda, MD, United States; <sup>2</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, MD, United States; <sup>3</sup>Pelex Med, McLean, VA, United States.

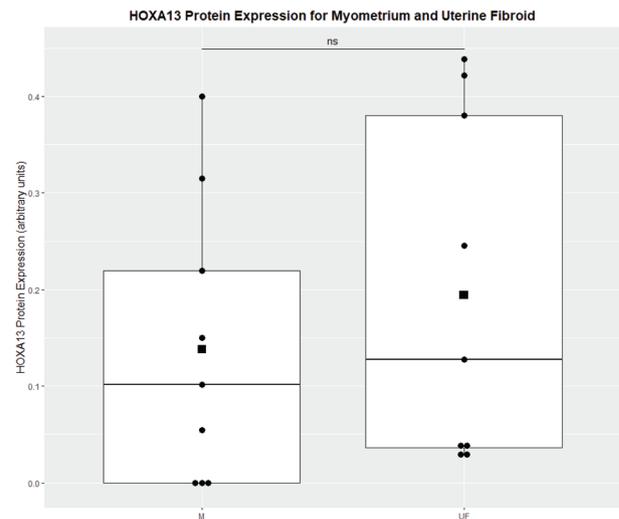
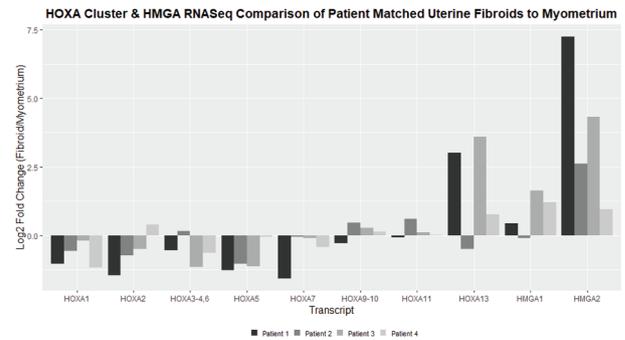
**Introduction:** A novel paradigm of uterine fibroid (UF) development was recently reported that UFs undergo homeotic transformation by suppressing rostral and overexpressing caudal HOXA genes in UF subtypes with high HMGA2 expression, but not in those with high HMGA1 expression. HOXA13 gene expression, which is normal in the cervix and vagina, was reported to be increased in UFs compared to myometrium (M) in UFs with HMGA2 upregulation. Even so, it is not known if this transformation translates into altered HOXA13 protein expression. We hypothesize that UFs will have increased HOXA13 protein when compared to myometrium.

**Design:** Laboratory study.

**Methods:** RNASeq was performed on patient matched M and UF tissue samples to assess a homeotic transformation gene expression pattern. Gene expression was confirmed with quantitative polymerase chain reaction (qPCR) and western blot analyses. Results are shown as mean  $\pm$  standard error of the mean.

**Results:** Using RNAseq, we found HMGA2 upregulation in all 4 patients. We confirmed a pattern of decreased HOXA1-HOXA7 expression and increased HOXA10-HOXA13 expression, with HOXA13 highest in UF compared to patient matched M in 3 out of 4 patients (Fig 1). At the protein level, HOXA13 was upregulated in 4 out of 9 patients in a confirmatory cohort (Fig 2). The HOXA13 protein expression data exhibited significant variation (UF  $0.19 \pm 0.06$ , M  $0.14 \pm 0.05$ ,  $p = 0.6$  for the difference UF - M).

**Conclusion:** Many UFs with HMGA2 upregulation exhibit a pattern of homeotic transformation at the RNA level. In a confirmatory cohort, HOXA13 protein expression was increased in some, but not all UF. Cell culture studies are underway to address the variability between patient samples as are studies to further characterize the relation between HOXA13 protein expression and HMGA1/2 changes.



#### W-051

**Using Mendelian Randomization to Understand Uterine Leiomyomata and Its Associated Clinical Phenome.** Jacqueline A Piekos<sup>†</sup>,<sup>1</sup> Jacklyn N. Hellwege<sup>\*,2</sup> Nikhil K. KhanKari,<sup>2</sup> Samantha Greenblatt<sup>†</sup>,<sup>1</sup> Todd L. Edwards<sup>\*,2</sup> Digna R Velez Edwards<sup>\*,3</sup> <sup>1</sup>Vanderbilt University, Nashville, TN, United States; <sup>2</sup>Vanderbilt University Medical Center, Nashville, TN, United States; <sup>3</sup>Vanderbilt University Medical Center, Nashville, TN, United States.

**Introduction:** Uterine leiomyomata (UL) are the most common pelvic tumor in women affecting around 70% of European ancestry (EA) women and 80% of African ancestry (AA) women. ULs are highly heritable with genetic inheritance as high as 33%. We previously developed and validated an EA race specific polygenic risk score (PRS) for UL. Our objective in this study is to use the PRS in Mendelian Randomization (MR) analyses to better understand the causal relationships between ULs and other health conditions.

**Methods:** Using our previously constructed and validated EA PRS developed within Vanderbilt's Biorepository (BioVU) and the Electronic Medical Records and Genomics (eMERGE) Network we conducted MR analyses evaluating the relationship between our UL PRSs and candidate novel clinical phenotypes and known risk factors from the literature. Phenotypes evaluated included endometriosis, ovarian cysts, uterine polyps, dysmenorrhea, and optic atrophy identified in our prior phenotype-wide analyses of UL PRS and body mass index (BMI), type 2 diabetes, and blood pressure traits from published literature. To conduct analyses, we used a two-sample MR approach that used our previously developed genome-wide association study (GWAS) data to build our PRSs as the instrument for UL and race-specific GWAS summary statistics for candidate novel and known clinical phenotypes from the UK Biobank (UKBB).

**Results:** The MR Egger model was significant ( $p = 0.018$ ) for the EA PRS and endometriosis. The intercept of the model was significant

( $p=5.4 \times 10^{-3}$ ) indicating some of the variants in the EA PRS directly influence endometriosis risk, independently of UL risk. MR analyses between the EA PRS and the phenotypes ovarian cysts, BMI, and systolic blood pressure revealed no horizontal pleiotropy between the phenotypes. **Conclusion:** MR analysis identified potential horizontal pleiotropy between the EA PRS and endometriosis. Horizontal pleiotropy between the two phenotypes suggests there are shared pathways involved in their etiology. Through this study, we will gain insights into the genetic origin of UL. Future MR analyses will be conducted on the remaining phenotypes to understand their relationships with UL.

#### W-052

**Cardiovascular Risks Factors and Uterine Leiomyoma: Is There an Association?** Serin Seckin, Cassandra Charles, Fadi Yacoub, Shukla Minakshi, Anthony Filipovic, Vanessa Pinard, Ozgul Muneyyirci-Delale. *SUNY Downstate Health Sciences University, Brooklyn, NY, United States.*

**Introduction:** Uterine leiomyomas (UL) are the most common tumors affecting women with prevalence ranging over 70% by the onset of menopause. Many different risk factors, both modifiable and non-modifiable, have been associated with the development of UL. Recent studies have indicated an association between uterine fibroids and several cardiovascular disease (CVD) risk factors such as hypertension, obesity and abnormal serum lipids. It has also been reported that estrogen levels are inversely related to cholesterol levels. As fibroids are estrogen-related tumors, an inverse association between hyperlipidemia and the risk for fibroids should be observed.

**Methods:** An IRB-approved retrospective chart review was conducted using the electronic medical records of 888 women aged 18 years and above seen at the State University of New York (SUNY) Downstate Health Sciences University's Outpatient OB/GYN Suite. Women who were pregnant were excluded as well as those who were diagnosed with HIV or renal failure, had history of psychiatric illness, and were on hormone therapy for reasons other than UL. Data extracted included demographics, social history, medical history, BMI as well as laboratory parameters specifically glucose, Hemoglobin A1C (HbA1C), total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. Initial analysis revealed a group difference in age. Age matching was completed using a propensity score analysis to match on exact age, using a greedy algorithm. The matched data were then analyzed for group differences using t-tests, Chi-square and Fisher's exact test.

**Results:** A total of 444 women were included in the age-matched analysis: 222 women with UL and 222 without UL as controls. Women were predominantly Black (86%) with mean age of  $42.1 \pm 11.3$  years and BMI of  $31.2 \pm 7.6$  kg/m<sup>2</sup>. Majority of the women were currently non-smokers (89%) who denied current alcohol use (81%). There was no difference between the groups in terms of BMI ( $p=0.7908$ ), current smoking ( $p=0.0589$ ) and current alcohol use ( $p=0.2956$ ). Women with UL were more likely to have a history of hypertension than controls (UL= 33.6 % vs. controls= 23.6%;  $p=0.0213$ ). There was no difference between the groups in history of diabetes mellitus ( $p=0.4798$ ), heart disease ( $p=0.9835$ ) and dyslipidemia ( $p=0.2326$ ). There was no difference between the groups in mean glucose levels ( $p=0.3127$ ) and mean HbA1C levels ( $p=0.0995$ ). Mean lipid profile parameters were all within normal range and there was no difference noted between the groups for any those parameters.

**Conclusion:** In our population of predominantly Black women assessed for cardiovascular risk factors, women with UL were more likely to have a history of hypertension than those without UL. There was no association between presence of UL and other risk factors such as history of diabetes mellitus, heart disease and dyslipidemia.

#### W-053

**Effectiveness of Microwave Endometrial Ablation Combined with Transcervical Resection in Treating Submucous Uterine Myoma.** Toshiyuki Kakinuma, Kakinuma Kaoru, Kaneko Ayaka, Kagimoto Masataka, Yanagida Kaoru, Matsuda Yoshio, Takeshima Nobuhiro, Ohwada Michitaka. *International University of Health and Welfare Hospital, Tochigi, Japan.*

**Introduction:** Background/Objective: Submucous uterine myomas can be reliably and effectively treated by microwave endometrial ablation (MEA), yet additional treatment is often required due to postoperative recurrence. This study investigated the therapeutic efficacy of MEA when combined with transcervical resection (TCR).

**Methods:** This retrospective study analyzed 32 women who had been treated for submucous uterine myoma(s) by MEA in combination with TCR ("MEA+TCR") at the International University of Medicine and Welfare Hospital between January 2016 and June 2020, and had been followed up for a minimum of six months after the procedure. Their medical records were retrospectively reviewed for information on the procedure's effectiveness in reducing heavy menstrual flow (menorrhagia/hypermorrhoea), secondary effects in reducing menstrual cramps (dysmenorrhoea), and complications. Efficacy was assessed in terms of pre- and post-treatment hemoglobin (Hb) levels and patients' visual analog scale (VAS) ratings of hypermenorrhoea and dysmenorrhoea severities as well as treatment satisfaction (maximum: 10 pts). Results are reported as mean $\pm$ SD (range).

**Results:** The study population consisted of 32 women (6 nulliparous, 26 multiparous) of mean age  $45.2 \pm 4.3$  (36-52). Fibroids were single and multiple in 10 and 22 cases, respectively, with a major diameter of  $26.3 \pm 12.3$  mm and protrusion rate of  $51.3 \pm 11.3\%$ . The procedure lasted  $45.5 \pm 21.0$  (19-110) min and involved  $7.0 \pm 1.3$  (5-11) ablation cycles on average; patients stayed in hospital for  $2.5 \pm 0.5$  (2-3) d. Patients regarded their hyper- and dysmenorrhoea as very severe at 10 months before the procedure, uniformly giving VAS scores of 10/10 pts for both items. Their ratings of menorrhagia severity had significantly improved by 3 and 6 months post-surgery, dropping to  $1.2 \pm 1.3$  (0-5) and  $0.9 \pm 1.3$  (0-5) pts, respectively (both  $p < 0.001$ ). Similarly, VAS scores for dysmenorrhoea severity markedly improved over the same time frames, falling to  $1.3 \pm 1.8$  (0-7) and  $1.3 \pm 1.8$  (0-5) pts, respectively (both  $p < 0.001$ ). Circulating Hb significantly improved from  $8.7 \pm 1.9$  (5.1-12.5) g/dl before the procedure to  $13.5 \pm 1.1$  (11.3-15.2) g/dl after it ( $p < 0.001$ ). No surgical complications were observed. The mean follow-up length was  $33.8 \pm 16.8$  (6-60) months: while 10 women (31.3%) developed amenorrhoea during this period, no one experienced recurrence of hypermenorrhoea. Patients were highly satisfied with MEA+TCR's ability to relieve menorrhagia (mean VAS score:  $9.5 \pm 0.8$  (7-10) pts).

**Conclusion:** MEA+TCR can reliably and effectively treat hypermenorrhoea resulting from submucous myomas by reducing the size of uterine fibroids. The procedure's effectiveness is further supported by patients' high levels of satisfaction with it.

#### W-054

**The Kinase Inhibitor Nintedanib Regulates Multiple Key Targets of Inflammation, Fibrosis, and Angiogenesis Involved in Uterine Fibroid Pathogenesis.** Md Soriful Islam, Sadia Afrin, Christina N Cordeiro Mitchell, Mostafa A Borahay, James H Segars\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Uterine fibroids feature dysregulation of major physiological events leading to fibrosis, inflammation, and neo-angiogenesis. These processes are regulated by growth factors, including PDGF, FGF, VEGF, and activin A. Nintedanib is an FDA-approved receptor tyrosine kinase inhibitor used for the treatment of idiopathic pulmonary fibrosis. Here, we tested the hypothesis that nintedanib would alter expression of genes involved in fibrosis, inflammation, and angiogenesis in uterine fibroid cells.

**Methods:** Human myometrial (P51M) and fibroid (P51F) cells were treated with nintedanib at different concentrations (0.1, 0.5, 1, 5, and 10  $\mu$ M) for 24hrs to examine cell viability. After 24hrs serum starvation, both cell types were treated with nintedanib at 5  $\mu$ M for 24hrs with media

supplemented with 10% serum. Then we measured mRNA and/or protein expression of fibronectin (FN1), versican (VCAN), plasminogen activator inhibitor-1 (PAI-1), and activin A (INHBA) (fibrosis), interleukin 8 (IL-8; inflammation), and endothelin 1 (EDN1; angiogenesis). Differences were considered significant at  $p \leq 0.05$ .

**Results:** Myometrial and fibroid cells proliferated at lower concentrations (0.1–1  $\mu$ M) of nintedanib, and viability of fibroid cells exceeded  $\geq 80\%$  at 5  $\mu$ M concentration. As expected, mRNA levels of the major extracellular matrix components *FN1* and *VCAN* were elevated 1.84 fold  $\pm$  0.07 and 2.80 fold  $\pm$  0.24, respectively in fibroid cells vs myometrial cells. Nintedanib treatment significantly reduced levels of both transcripts in both cell types. Western blots confirmed the reduction in FN1 protein levels in fibroid cells after nintedanib exposure. The profibrotic growth factor *INHBA* was found to be higher (3.07 fold  $\pm$  0.15) in fibroid cells at baseline and nintedanib treatment significantly reduced mRNA levels of *INHBA*. Nintedanib treatment greatly reduced both mRNA and protein levels of *PAI-1*, a downstream target of activin A-mediated signaling in both cell types. The basal mRNA levels of the inflammatory molecule *IL-8* were significantly higher (4.60 fold  $\pm$  0.33) in fibroid cells, as compared to myometrial cells while nintedanib treatment significantly reduced *IL-8* levels in both cell types. Furthermore, basal levels of angiogenic factor *EDN1* were found to be higher (3.22 fold  $\pm$  0.32) in fibroid cells vs myometrial cells, whereas nintedanib significantly reduced *EDN1* levels.

**Conclusion:** Treatment of uterine fibroid cells with nintedanib reduced expression of transcripts and proteins of key genes involved in fibrosis, inflammation, and angiogenesis. These results support further research efforts to examine the effect of nintedanib on growth factor-mediated signaling pathways for *in vitro* and *in vivo* models of uterine fibroid development.

#### W-055

**Extracellular Matrix Gene Expression in At-Risk Human Myometrial Stem Cells Align with Fibroid Tumor-Initiating Cells and Is Distinct from Normal Myometrial Stem Cells.** *Maria Victoria Bariani\**,<sup>1</sup> Mohamed Ali,<sup>2</sup> Sandra L. Grimm,<sup>3</sup> Cristian Coarfa,<sup>3</sup> Qiwei Yang,<sup>1</sup> Ayman Al-Hendy.<sup>1</sup> <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>Ain Shams University, Cairo, Egypt; <sup>3</sup>Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Uterine fibroids (UF) are monoclonal benign tumors that develop from the uterus smooth muscle tissue. It has been shown that UF originates from pathologically transformed myometrial stem cells (MMSCs). However, the mechanisms underlying this conversion remain unknown. This work aimed to identify drivers of UF by identifying pathways and genes differentially regulated in MMSCs isolated from human myometrium without fibroids (MyoN), myometrium adjacent to UF (MyoF), and UF using RNA-seq approach.

**Methods:** Fresh MyoN, MyoF, and UF myometrial tissues (n=5 each) from consented women were collected at the time of hysterectomy and subjected to MMSC and UF stem cell (SC) isolation using dual Stro-1 and CD44 surface markers. Whole-genome RNA-sequencing was performed in MMSCs to compare the differential gene expression profiles between groups.

**Results:** Genome-wide RNA-seq showed 185 upregulated and 365 downregulated genes in MyoF-MMSCs compared to MyoN-MMSCs. The over-representation analysis of Enriched Reactome pathways demonstrated that among the top 20 pathways, 10 belonged to the extracellular matrix (ECM) signaling. We observed that 41 genes involved in ECM composition and organization were differentially expressed between MyoF-MMSCs and MyoN-MMSCs. Specifically, we found that several collagen genes (*COL5A3*, *COL6A1/A2/A3*, *COL7A1*, *COL14A1*, and *COL16A1*) related to ECM primary structural element, were significantly upregulated in MyoF-MMSCs compared to MyoN-MMSCs. In addition, we found that Biglycan, Decorin, and Syndecan-2, proteoglycans that support fibrous elements, showed the same trend. Moreover, the gene expression of the metalloproteinases MMP1 and MMP2 were upregulated on MyoF-MMSCs compared to MyoN-MMSCs, while MMP15 and ADAMTS3/5 expression were downregulated. Also, we found that laminins LAMC2, LAMA3/5 were downregulated in

MyoF-MMSCs in comparison with MyoN-MMSCs. Integrins, the key players on cell-ECM communication, showed deregulated expression with upregulation of *ITGA11* and downregulation of *ITGA3/6*, and *ITGB4*. Furthermore, Fibronectin, a central participant in establishing the architecture of ECM, was upregulated in MyoF-MMSCs compared to MyoN-MMSCs. Interestingly, ECM-related gene expression profile in MyoF-MMSCs aligned with UF-SC pattern, indicating a similarity between these MMSCs.

**Conclusion:** Our results suggest that alterations of ECM components related to their organization and architecture, participate in the pathological transformation of MMSCs, which eventually give rise to UF.

#### W-056

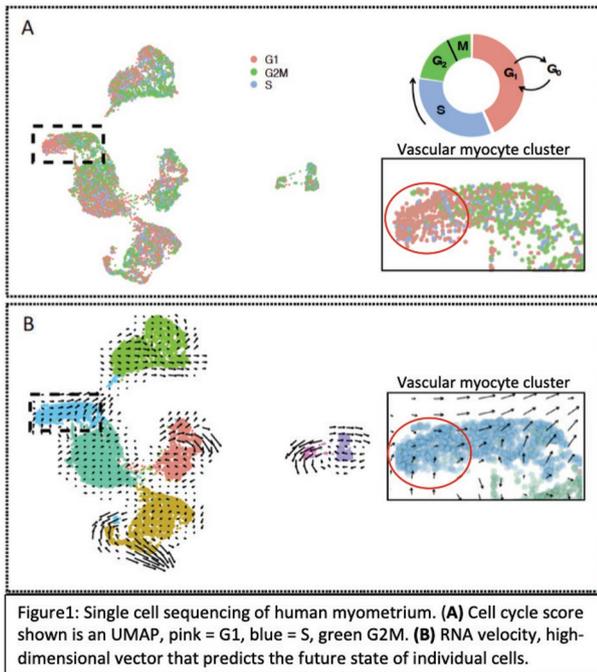
**Myometrial Stem Cell Enrichment to Understand Uterine Fibroid Etiology.** *Emmanuel N. X. Paul†*, Tyler J. Carpenter, Joshua A. Grey, Jose M. Teixeira\*. *Michigan State University, Grand Rapids, MI, United States.*

**Introduction:** Uterine fibroids are benign tumors of the myometrium. They are the most common reproductive tract tumors in women with an incidence up to 80% by age 50. Common symptoms are heavy menstrual bleeding, pelvic pain, and dysmenorrhea. Currently, hysterectomy is the only cure for these symptoms, and accounts for about 50% of all hysterectomies performed in the US. Understanding the disease etiology will lead to alternative and less invasive therapies. Uterine myometrium has the unique property to significantly enlarge in size during pregnancy to accommodate the developing fetus, suggesting the existence of myometrial stem cells, which we predict will have a mesenchymal stem cell (MSC) phenotype. Moreover, uterine fibroids are almost always clonal, strongly suggesting a single, dysregulated cell origin that we hypothesize is the MSC. We have used next generation sequencing, such as 1) standard RNAseq and 2) single cell RNAseq (scRNAseq) to identify markers capable of significantly enriching MSCs for experimentation in order to better understand the disrupted mechanisms that lead to fibroid development.

**Methods:** Uterine myometrial samples were collected (n=5) and digested. Live cells were separated by flow cytometry using a MSC surface marker, SUSD2. Total RNA from SUSD2+/- cells was submitted for RNA-seq. Cells (n=7,000) from each patient were submitted for scRNA-seq on the 10X Genomics Chromium platform. A Uniform Manifold Approximation and Projection plot was generated from the scRNA-seq and combined with the RNA-seq signatures from SUSD2+/-cells.

**Results:** 2,531 genes were enriched in the SUSD2+ cell population, including known MSCs markers such as *MCAM*, *PDGFRB* and *CSPG4*. Seven cell clusters were found within the myometrium and the vascular myocyte cluster (a common niche for MSCs) was enriched for MSC markers (*MCAM*, *PDGFRB*, *CSPG4* and *SUSD2*). We delimited the MSCs within the vascular myocyte cluster (Fig 1, red circle) by using stem cell characteristics, such as the quiescent cell cycle state (G0) and the transcriptional dynamics of individual cells (velocity). Indeed, a smaller cell population expressed gene markers for the quiescent G0/G1 phase (Fig 1A) and have low RNA velocity (Fig 1B). We are using these markers to enrich for these myometrial cells and confirm stemness using assaying typical stem cell activities.

**Conclusion:** We have found a population of myometrial cells with characteristic MSC markers that can be used to enrich for myometrial stem cells for continued studies and better understand the etiology of uterine fibroids.



W-057

**Discovery of Novel Molecular Mechanisms Underlying the Pathophysiology of Uterine Fibroids and Associated Heavy Menstrual Bleeding.** Chen-Yi Wang,<sup>1</sup> Marina Maritati,<sup>1</sup> Darragh P O'Brien,<sup>1</sup> Kavita S Subramaniam,<sup>1</sup> Adam Cribbs†,<sup>1</sup> Jessica Malzahn,<sup>1</sup> Thomas M Zollner,<sup>2</sup> Bianca De Leo,<sup>2</sup> Maik Obendorf,<sup>2</sup> Joerg Mueller,<sup>2</sup> Martin Fritsch,<sup>2</sup> Benedikt M Kessler,<sup>1</sup> Krina T Zondervan,<sup>1</sup> Adrian Harris,<sup>1</sup> Christian M Becker,<sup>1</sup> Udo Oppermann,<sup>1</sup> Martin Philpott\*.<sup>1</sup> <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Bayer AG, Berlin, Germany.

**Introduction:** Uterine fibroids (UFs) are benign tumours affecting up to 80% of women of reproductive age. Approximately 30% of fibroid patients suffer severe symptoms including painful heavy menstrual bleeding (HMB). Although mutations in MED12 or HMGA2 account for the majority of UF occurrence, the processes by which these lead to UF and HMB remain poorly understood. Using a systems biology approach, we undertake a comprehensive study of the pathomechanisms underlying UF and associated HMB.

**Methods:** Fibroid, endometrium, myometrium, pseudocapsule, and healthy control samples were obtained from patients undergoing surgery at the John Radcliffe Hospital, Oxford, UK. All tissue samples were obtained under IRB approval and informed consent. Cycle phase was established by histology of the endometrium. Gene and protein expression was investigated by RNA-Seq and quantitative proteomics. Genotyping was performed at the genome wide SNP level and by sequencing of the entire MED12 locus. MED12 chromatin-occupancy is being investigated by CUT&Tag. Laser capture microdissection of endothelial cells from fibroid pseudocapsule followed by RNA-Seq and proteomics along with single cell RNA-seq from the same tissues, is being used to investigate the role of aberrant vasculature in fibroid pathology.

**Results:** To date, tissues have been collected from 137 donors, with transcriptomics, proteomics and genotyping complete for 91. Systems level analysis has revealed altered biological pathways between fibroid patients and controls and between fibroid patients with and without HMB, identifying potentially novel mechanisms which drive growth of UFs and HMB.

**Conclusion:** Using our multi -omics approach, we have gained novel insight into the pathomechanisms of UFs and associated HMB and identified novel mechanisms which might support the development of new therapies.

W-058

Abstract Withdrawn

W-059

**Metastatic Crohn's Disease Involving the External Female Genitalia: A Review and Analysis of Published Cases.** Rachel L Leib†,<sup>1</sup> Allison M Parrill†,<sup>1</sup> Melissa A DeViney†,<sup>1</sup> Ryan Raffel†,<sup>2</sup> David Adelstein\*,<sup>1</sup> Bo Peng\*,<sup>1</sup> Lisa Eng\*,<sup>3</sup> Pierre Hindy\*,<sup>4</sup> Aruna Mishra\*.<sup>5</sup> <sup>1</sup>American University of the Caribbean School of Medicine, Cupecoy, Netherlands Antilles; <sup>2</sup>Nassau University Medical Center, East Meadow, NY, United States; <sup>3</sup>The Birthing Center of NY, Brooklyn, NY, United States; <sup>4</sup>Gastroenterology Associates of Brooklyn, Brooklyn, NY, United States; <sup>5</sup>BronxCare Health System, Bronx, NY, United States.

**Introduction:** Crohn's disease (CD) is a chronic gastrointestinal inflammatory condition that rarely metastases to female genitalia (MCD). Our objective is to conduct a comprehensive review and analysis of published cases of female genital MCD to investigate the demographic data, signs and symptoms, and treatment to educate providers and prevent misdiagnosis.

**Methods:** Literature searches were conducted using the search terms: "vulvar Crohn's disease", "vaginal Crohn's disease", "genital Crohn's disease" and "genital granulomatosis". Case reports and case series from journal inception date to November 20th, 2020 in PubMed, CINAHL, Web of Science, and OVID databases were retrieved.

**Results:** A total of 89 articles reporting 105 female cases of genital MCD were included. The median ages of admission, CD diagnosis, and onset of genital symptoms were 31, 20, and 29 years, respectively. About 26.2% of the women presented with genital symptoms before diagnosis of CD. The common genital symptoms of MCD were vulvar edema, pain, ulcers, fistulas, masses, and infections, see Table 1. The majority of the cases (n=93) were diagnosed by the presence of non-caseating granuloma via genital biopsy. Around 83.8% of women received medical and surgical management as listed in Table 2. About 71.4% of women included achieved partial improvement (n=34) or complete resolution (n=41).

Symptom	Number of cases (n=105)	Percentage (%)
vulvar edema	85	81.0
pain	62	59.0
ulcer	44	41.9
fistula	25	23.8
mass	13	12.4
infection	10	9.5

Treatments	Medical (n=81, 92%)					Surgical (n=13, 14.8%)	
	Corti-costeroids	Anti-biotics	Immuno-modulators	Bio-logics	Anti-Inflam-matory	Resec-tion	Drainage
Number of cases (n=88)	50	45	29	24	22	9	5
Percentage (%)	56.8	51.1	32.9	27.2	25	10.2	5.7

**Conclusion:** Genital metastasis is a rare and commonly misdiagnosed complication of CD in women. The presentations of vulva edema, pain, and ulcers warrant investigation and careful management of genital MCD via medical and surgical intervention. To our knowledge, our study is by far the most thorough review of published cases, which may increase provider awareness, improve patient outcomes, and offer preventative measures.

**W-060**

**Adipose Tissue Inflammation-Stimulated Adrenomedullin (ADM) Overexpression Contributes to Lipid Dysfunction in Diabetic Pregnancy.** Yuanlin Dong\*, Ancizar Betancourt, Michael Belfort, Chandra Yallampalli. *Baylor College of Medicine, Houston, TX, United States.*

**Introduction:** Gestational diabetes mellitus (GDM) is one of the most common complications of pregnancy whose pathophysiology to date has not been completely clarified. Emerging evidence suggest an important involvement of adipose tissues (AT) in insulin resistance (IR), but the profile of adipokines involved in inflammation and lipid metabolism in GDM remain obscure. Present study was designed to investigate if omental AT (OMAT) from GDM women express greater levels of proinflammatory and lipolytic molecules compared to subcutaneous AT (SCAT), and if so, whether the regional differences in AT have implications for lipid homeostasis.

**Methods:** Paired samples of OMAT and SCAT were excised from women during scheduled Cesarean sections at term in pregnant women with normal glucose tolerance (NGT) with either normal weight NW, BMI<25 kg/m<sup>2</sup> or obese (OBS, BMI>30 kg/m<sup>2</sup>) and GDM (BMI>30 kg/m<sup>2</sup>) (n=4 in each group). The mRNA expressions for proinflammatory and lipid metabolic molecules were determined by quantitative RT-PCR, and their cellular localizations in AT were visualized by immunofluorescent staining. Human adipocyte cell line and human AT were cultured and glycerol release was measured to assess the lipolytic status.

**Results:** (1) Human AT express mRNA for proinflammatory molecules of RBP4 and TLR-4, and adipogenic molecules of ChREBP and CEL, but the differences between OMAT and SCAT and among NW, OBS, and GDM groups were not significant (P>0.05). (2) The mRNA of monocyte chemoattractant protein, MCP-1, macrophage marker CD68, and IL-6, IL-8, and TNF- $\alpha$  were significantly increased in OMAT from GDM women compared to OMAT and SCAT in NW and OBS women (P<0.05). (3) Immunostaining intensity per adipocyte show that ADM and its receptor components CRLR, RAMP2 and RAMP3, were significantly higher in OMAT compared to SCAT in all segments tested (P<0.05). (4) Glucose stimulates adipocyte MCP-1 mRNA expression, and TNF- $\alpha$  enhances ADM and its receptor component mRNA in adipocytes in culture in a dose-dependent manner. (5) In both OBS and GDM women basal glycerol release was higher in OMAT compared to SCAT (P<0.01), and OMAT from GDM releases more glycerol than OMAT from NW and OBS subjects (P<0.01), and (5) ADM dose-dependently stimulates glycerol release by OMAT, but not by SCAT, in women with NW, and ADM-induced increases in glycerol were reduced by pre-incubation of AT with ADM receptor blocker, ADM22-52.

**Conclusion:** Excessive ADM and its receptor expressions by OMAT, but not by SCAT appear to contribute substantially to the lipid dysregulation in GDM women, and the increased proinflammatory molecules in OMAT may play a major role in stimulating ADM and its receptor expressions. Therefore, detailed understanding of the role of ADM in AT may provide new insights into GDM pathophysiology and open new possibilities for its prevention and treatment.

**W-061**

**McDonald versus Shirodkar Cerclage Technique in the Prevention of Preterm Birth: A Systematic Review and Meta-Analysis.** Liam McAuliffe,<sup>1</sup> Ashad Issah,<sup>2</sup> Rosanna Diacci,<sup>1</sup> Kimberley P Williams,<sup>2</sup> Anne-Marie Aubin,<sup>2</sup> Jason Phung,<sup>2</sup> Carol Wang,<sup>2</sup> Alexander Maouris,<sup>3</sup> Sebastian Leathersich,<sup>4</sup> Panos Maouris,<sup>5</sup> Craig E Pennell\*.<sup>2,1</sup> *University of Newcastle, The Junction, NSW, Australia;* <sup>2</sup>*University of Newcastle, Newcastle, NSW, Australia;* <sup>3</sup>*Sir Charles Gairdner Hospital, Perth, WA, Australia;* <sup>4</sup>*King Edward Memorial Hospital, Subiaco, Western Australia, Australia;* <sup>5</sup>*Obstetrics and Gynaecology, Subiaco, Western Australia, Australia.*

**Introduction:** Cervical cerclage has been used for decades to decrease rates of preterm birth. The Shirodkar and McDonald cerclage are the two most commonly used cerclage techniques with no current consensus on the preferred technique. **Objective:** To compare the efficacy of the two techniques.

**Methods: Search strategy:** Studies were sourced from six electronic databases and reference lists. **Selection criteria:** Studies including women with a singleton pregnancy, requiring a cervical cerclage, using either the Shirodkar or McDonald technique that ran comparative analyses between the two techniques. **Data collection and analysis:** The primary outcome was preterm birth before 37 weeks, with sub analyses at 28, 32, 34 and 35 weeks. Secondary data was also collected on neonatal, maternal and obstetric outcomes.

**Results:** Seventeen papers were included - analysis showed the Shirodkar group had significantly less chance of preterm birth before 37 weeks (RR 0.91, 95% CI 0.85-0.98). This finding is reinforced by statistically significant reduction in rates of preterm birth before 37, 35, 34 and 32 weeks, PPRM (RR 0.87, 95% CI 0.77 - 0.99), difference in cervical length (mean difference 5.25, 95% CI 4.68-5.83), cerclage to delivery interval (mean difference 10.79, 95% CI 8.20-13.38), and an increase in birthweight (mean difference 348 grams, 95% CI 291-406) in the Shirodkar group

**Conclusion:** Shirodkar cerclage leads to a significant reduction in preterm birth and delivers better maternal and neonatal outcomes when compared to McDonald cerclage.

**W-062**

**Perivable Birth in the North Carolina Triad: Do Outcomes Vary by Birth Etiology?** Melissa L Kozakiewicz†, Kathleen V Ferry†, Jeff M Denney\*. *Wake Forest University School of Medicine, Winston Salem, NC, United States.*

**Introduction:** Since our group began offering interventions of antenatal corticosteroids and antenatal magnesium sulfate for fetal neuroprophylaxis along with cesarean section as early as viability is now defined at 22 weeks, our group sought to evaluate recent perivable birth (PVB) outcomes.

**Methods:** Descriptive study of all women delivering between 20 and 25 6/7 wks at a Tertiary Care Center for a 22-county area in the North Carolina Triad for 2 years (1/1/2017 to 12/31/2018). All deliveries were screened to identify eligibility. Medical records were reviewed and outcomes were tracked. Physician panel categorized birth etiology for stratification. Intrapartum death was defined as fetal death in labor/during cesarean section (CD). Univariate and multivariate analysis were used where appropriate.

**Results:** 113 PVB (2.0% of 5587 deliveries of  $\geq 20$  completed weeks) were identified. 19 occurred 20w0d-21w9d with zero survivors, 6 antepartum IUFD's occurred, and 5 fetal anomalies/aneuploidy were excluded from analysis. Characteristics of the 83 remaining non-anomalous PVB were stratified by birth etiology from 22 0/7-25 6/7 weeks in **Table 1**. Birth etiology was 67/83 (80.7%) SPTB, 6/83 (7.2%) IUGR, and 10/83 (12.0%) preeclampsia/HELLP. CD risk was high (52.2%) and 100% live births required NICU admission. Of the 54 SVD that occurred 38/54 (70.4%) of these were due to spontaneous PTL, and 20/54 (37%) survived  $\geq 30$  days. The smallest and earliest SCD survivor was 520g at 22w3d. Of deliveries 22wks-25 6/7 wks, 59 CD were performed with earliest performed at 22w1d and earliest survivor at 22w3d and 360g. 45/59 (76%) fetuses delivered by CD lived  $\geq 30$  days. Having IUGR or preeclampsia increased delivery by CD compared to SPTB (16/16 vs. 38/54; OR 25.3 95 CI 1.5,438.9).

**Conclusion:** Few deliveries (2%) occur prior to 26 wks. Indicated deliveries 16/5587 (0.3%) are much rarer than SPTB <26 wks even in a referral center. Outcomes vary by birth etiology with respect to mode of delivery and rate of intrapartum fetal death. Our data may prove useful for patient-doctor discussions when appropriate in similar populations in the United States and abroad.

**W-063**

**A Randomized, Placebo-Controlled, Proof-of-Concept Trial of Ebopiprant for the Treatment of Spontaneous Preterm Labor (PROLONG).** Ben W Mol,<sup>1,2</sup> Anh Nguyen\*,<sup>3</sup> Ildar Fatkullin\*,<sup>4</sup> Hynek Heřman\*,<sup>5</sup> Antonín Pařízek\*,<sup>6</sup> Petr Janků\*,<sup>7</sup> Tuong Ho\*,<sup>8</sup> Tal Biron-Shental\*,<sup>9</sup> Andrew Humberstone\*,<sup>10</sup> Michel Brethous\*,<sup>10</sup> Jean-Pierre Gotteland\*,<sup>10</sup> Elizabeth Garner\*.<sup>11</sup> <sup>1</sup>Monash University Monash Medical Centre, Melbourne, Australia; <sup>2</sup>University of Aberdeen, Aberdeen, United Kingdom; <sup>3</sup>Hanoi Obstetrics and Gynecology Hospital, Hanoi, Viet Nam; <sup>4</sup>Kazan State Medical University, Kazan, Russian Federation; <sup>5</sup>The Institute for the Care for Mother and Child, Prague, Czech Republic; <sup>6</sup>Charles University and General Faculty Hospital in Prague, Prague, Czech Republic; <sup>7</sup>Masaryk University, Brno, Czech Republic; <sup>8</sup>My Duc Hospital, Ho Chi Minh City, Viet Nam; <sup>9</sup>Tel Aviv University, Tel Aviv, Israel; <sup>10</sup>ObsEva SA, Geneva, Switzerland; <sup>11</sup>ObsEva Inc., Boston, MA, United States.

**Introduction:** Ebopiprant (OBE022) is a novel, oral and selective prostaglandin F2α (PGF2α) receptor antagonist being developed to delay preterm birth. The PROLONG trial (NCT03369262) was a double-blind, randomized, placebo controlled, parallel group study, to assess the efficacy, safety and pharmacokinetics of ebopiprant in patients with spontaneous preterm labor at a gestational age (GA) of 24 to 34 weeks.

**Methods:** Participants had to have ≥ 4 contractions/30 mins, cervical dilatation of 1 to 4 cm, and ≥ one other sign of preterm labor (cervical length ≤ 25 mm, progressive cervical change, or a positive test for preterm labor) and be receiving atosiban infusion for 48 h. After informed consent, participants were randomised to ebopiprant or placebo, starting with 1000 mg, administered ≤ 24 h after starting atosiban, then 500 mg twice a day for 7 days. Efficacy endpoints (including delivery ≤ 48 h or 7 d) were exploratory. Maternal, fetal and neonatal safety were assessed up to 28 d after delivery.

**Results:** Between Jan 2019 and Mar 2020, 113 pregnant women (83 singletons, 30 twins) were randomized and treated. Overall, 7/56 (12.5%) women delivered ≤ 48 h with ebopiprant vs 12/55 (21.8%) with placebo (OR 0.52 95% CI 0.22, 1.23), Table 1). This difference was observed for singletons (12.5% vs 26.8%) but not for twins (12.5% vs 7.1%). At 7 days after randomisation, no differences were observed, except for singletons, ≤ 30 weeks GA (Table 1). The incidence of maternal, fetal and neonatal adverse events were comparable between the treatment groups (Table 1).

Table 1: Delivery within 48 h and 7 days and maternal, fetal and neonatal adverse events, birthweight.

	Ebopiprant + atosiban	Placebo + atosiban	OR (95% CI)
<b>All women</b>	N=56	N=55	
Delivery ≤ 48 h – n (%)	7/56 (12.5)	12/55 (21.8)	0.52 (0.22, 1.23)
GA 24 to 30 wks at BL	3/25 (12.0)	5/24 (20.8)	1.05 (0.20, 5.43)
GA 30 to 34 wks at BL	4/31 (12.9)	7/31 (22.6)	0.77 (0.21, 2.89)
Delivery ≤ 7 days – n (%)	15/56 (26.8)	15/55 (27.3)	1.00 (0.49, 2.04)
GA 24 to 30 wks at BL	5/25 (20.0)	6/24 (25.0)	1.07 (0.28, 4.18)
GA 30 to 34 wks at BL	10/31 (32.3)	9/31 (29.0)	1.41 (0.52, 3.87)
<b>No. of subjects with ≥ 1 adverse event – n (%)</b>			
Maternal and fetal	24/58 (41.4)	23/55 (41.8)	NE
Neonatal	44/72 (61.1)	41/69 (59.4)	NE
Neonatal death – n (%)	2/72 (2.8)	3/69 (4.3)	NE
Mean (SD) birthweight – g	2309 (795)	2321 (835)	NE
<b>Singletons only</b>	N=40	N=41	
Delivery ≤ 48 h – n (%)	5/40 (12.5)	11/41 (26.8)	0.39 (0.15, 1.04)
GA 24 to 30 wks at BL	2/21 (9.5)	5/21 (23.8)	0.34 (0.08, 1.49)
GA 30 to 34 wks at BL	3/19 (15.8)	6/20 (30.0)	0.44 (0.12, 1.62)
Delivery ≤ 7 days – n (%)	11/40 (27.5)	13/41 (31.7)	0.81 (0.36, 1.83)
GA 24 to 30 wks at BL	3/21 (14.3)	5/21 (23.8)	0.53 (0.14, 2.01)
GA 30 to 34 wks at BL	8/19 (42.1)	8/20 (40.0)	1.09 (0.37, 3.18)
Neonatal death – n (%)	0 (0)	2/41 (4.9)	NE
<b>Twins only</b>	N=16	N=14	
Delivery ≤ 48 h – n (%)	2/16 (12.5)	1/14 (7.1)	2.05 (0.23, 18.1)
Delivery ≤ 7 days – n (%)	4/16 (25.0)	2/14 (14.3)	1.87 (0.36, 9.79)
Neonatal death – n (%)	2/32 (6.3)	1/28 (3.6)	NE

**Conclusion:** The PROLONG trial provides initial evidence that ebopiprant, when administered with atosiban infusion to women with preterm labour, reduces the probability of delivering within 48 h.

**W-064**

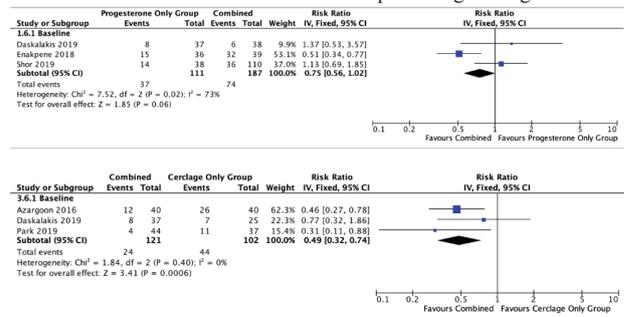
**Combined Vaginal Progesterone and Cervical Cerclage in the Prevention of Preterm Birth: A Systematic Review and Meta-Analysis.** Anne-Marie Aubin†,<sup>1</sup> Kimberley P Williams†,<sup>1</sup> Liam McAuliffe†,<sup>1</sup> Ashad Issah†,<sup>1</sup> Rosanna Diacci†,<sup>1</sup> Jason Phung\*,<sup>2</sup> Carol Wang\*,<sup>2</sup> Craig Pennell\*,<sup>2</sup> <sup>1</sup>University of Newcastle, Newcastle, Australia; <sup>2</sup>Hunter Medical Research Institute, Newcastle, Australia.

**Introduction:** Vaginal progesterone and cervical cerclage are both effective interventions for reducing preterm birth (PTB). It is currently unclear whether combined therapy offers superior effectiveness compared to single therapy. Further, there is a lack in evidence-based guidelines on the recommended type of intervention. This study aims to determine the efficacy of combining cervical cerclage and vaginal progesterone in the prevention of PTB.

**Methods:** A systematic review of the literature and meta-analysis of studies comparing progesterone and cerclage was conducted following PROSPERO guidelines. The primary outcome was birth <37-weeks. Secondary outcomes included birth <28-, <32- and <34-weeks, gestational age at delivery (GA), days between intervention and delivery, Preterm Premature Rupture of Membranes, caesarean section, neonatal mortality, Neonatal Intensive Care Unit admission, intubation and birthweight.

**Results:** Following title and full-text screening, ten papers were included in the final analysis. Three studies had low risk of bias and seven studies had moderate-to-critical risk of bias. Combined therapy was associated with lower risk of PTB <37-weeks compared to cerclage alone (RR 0.49, 95% CI 0.32-0.74) or progesterone alone (RR 0.70, 95% CI 0.51-0.97). Compared to cerclage only, combined therapy was associated with reduced risk of PTB <32-weeks, reduced risk of PTB <34-weeks, increased birthweight, increased GA and a longer interval between intervention and delivery. Compared to progesterone alone, combined therapy was associated with reduced risk of PTB <32-weeks, reduced risk of PTB <28-weeks and increased GA. There were no differences in any other secondary outcomes.

**Conclusion:** Combined treatment of cervical cerclage and vaginal progesterone achieves a greater reduction in PTB compared to single therapy. Further well-conducted and adequately powered randomized controlled trials are needed to assess these promising findings.



**W-065**

**Enhancing Contraction Signals in Uterine Electromyography by the Wavelet-Based Denoising Algorithm SureShrink: Application in EMMI.** Zichao Wen, Hui Wang, Sicheng Wang, Yong Wang\*. Washington University School of Medicine, St. Louis, MO, United States.

**Introduction:** Uterine electromyography (EMG) is an electrophysiological signal from uterine contractions. A Butterworth band-pass filter (BBF) is usually used to process raw recordings to obtain desired frequency band but it cannot reject interfering noises with overlapping spectra. In addition to a BBF, we propose to use a wavelet-based algorithm, Sureshrink, to further remove background noises. We validate the effectiveness in the state-of-the-art electromyogram imaging (EMMI) technology, where we denoise EMGs recorded on body surface and derive EMGs on entire uterine surface (uEMG) with EMMI software. We show that Sureshrink significantly increases the SNR of uEMG and thus improves EMMI.

**Methods:** Data from EMMI human studies are used. We applied <192 BioSemi electrodes to abdomen and lower back encasing the uterus and

collected data on body surface at a 2048 Hz sampling rate. Data collection is done in a delivery room a few hours before labor, along with the pressure recording from a Tocodynamometer (TOCO) contraction monitor. After a BBF of (0.34-1Hz), we perform SureShrink in Matlab to further exclude interfering noises. It involves a discrete wavelet transform to decompose signals to wavelet coefficients (WC) and the inverse transform to synthesize WCs back to signals. In between, WCs are shrank by an optimized soft threshold rule, which is nonlinear, adapted and level/time-dependent. We then obtain 320-channel uEMGs by EMMI software and compare Signal-to-noise ratio (SNR) with/without SureShrink. For each channel, we define  $SNR=20 \log_{10}(A_{con}/A_{base})$ , where  $A_{con}$  and  $A_{base}$  denote the root mean square of segments of contraction and baseline confirmed by TOCO respectively.

**Results:** 1280 uEMGs from 4 EMMI cases are analyzed. The change of mean  $\pm$  standard deviation of SNRs are: Case 1, from  $6.27 \pm 3.88$  to  $9.85 \pm 6.34$ ; Case 2, from  $3.26 \pm 2.47$  to  $8.23 \pm 4.90$ ; Case 3, from  $4.65 \pm 2.70$  to  $10.26 \pm 5.90$ ; Case 4, from  $5.52 \pm 1.46$  to  $9.26 \pm 2.99$ ; all together, from  $4.93 \pm 2.98$  to  $9.40 \pm 5.24$ .  $P < 1.4e-69$  in the t-tests of all comparisons.

**Conclusion:** SureShrink significantly improves the SNR of EMG and thus enhance the signals of contractions in EMMI. This method could be adopted in other EMG applications requiring high SNR, such as onsite detection of contractions and preterm prediction using EMG-derived features.

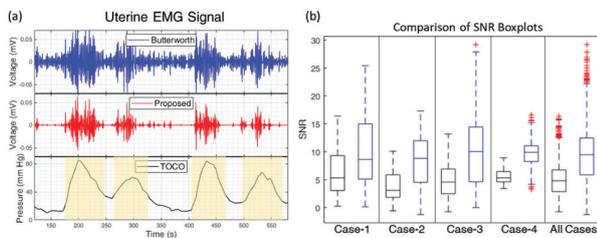


Figure 1. (a) Denoised uEMG: BBF only (blue); with SureShrink (red). TOCO (black) determines the segments of contraction (yellow area) and baseline. (b) Boxplots of SNR: BBF only (black); with SureShrink (blue).

## W-066

**Differential Distribution of T Cell Subtypes at the Maternal-Fetal Interface May Contribute to Preterm Labor.** Lu Gao\*, Jianqiang Yuan†, Yuanyuan Liu†, Hongping Liu†. *Second Military Medical University, Shanghai, China.*

**Introduction:** The incidence of preterm labor is about 12% world-wide, and remains the leading cause of neonatal mortality and morbidity, bringing heavy emotional and financial burdens to both family and society. Parturition is a complicated maternal-fetal interactive process, coordinately regulated by hormones, nutrition, metabolites and the immune system. Our previous studies revealed the signaling mechanisms and regulation of maternal-fetal endocrine factors in the initiation of labor. However, the maternal-fetal interface, i.e. decidua, is a heterogeneous composite of multiple cell types. Although several studies have examined the human placental transcriptome of trophoblasts and stromal cells from later stages at the single-cell level, the subtypes of decidual T cells that play key roles in maternal-fetal immune tolerance during pregnancy, and their distribution in term labor vs. preterm labor remain largely elusive.

**Methods:** In the present study, a total of 8 single-cell RNA-seq libraries were prepared from T cells enriched by CD3<sup>+</sup> magnetic beads in two decidual compartments: decidua basalis (DB) and decidua parietalis (DP) from four women either at term in labor (TIL) or in preterm labor (PTL). ScRNA-seq libraries were prepared with the 10X Chromium system and were processed using the 10X Cell Ranger software, resulting in 64,249 cells being captured and profiled across all samples.

**Results:** We used Seurat to normalize expression profiles and identified 12 distinct T cell clusters in both DB and DP. Using Logistics regression analysis, we found that 4 of the 12 clusters were correlated with preterm labor, either in DB or in DP samples. Using previously reported marker genes, we identified a TIGIT<sup>high</sup>CTLA4<sup>high</sup>IL10RA<sup>high</sup>Treg cell cluster, which manifested a much higher distribution in TIL T cells compared to

PTL T cells, both in DB and DP samples. *In vitro* experiments showed that ORM1, a glycoprotein we recently found to be elevated in the plasma of patients undergoing PTL, could significantly inhibit Treg cell differentiation and increase its apoptosis. Interestingly, we also defined two new subtypes of CD8<sup>+</sup> T cells characterized by NKG7<sup>high</sup>PRF<sup>high</sup>GZMK<sup>high</sup> and NKG7<sup>high</sup>GZMK<sup>high</sup>TIGIT<sup>high</sup>IL10RA<sup>high</sup> in DB T cells, which manifested predominant distribution in PTL samples and TIL samples, respectively. Although these two types of T cells both express active and cytotoxic T cell markers, the latter subtype also highly expresses inhibitory T cell markers such as TIGIT and IL10RA.

**Conclusion:** The decreased distribution of NKG7<sup>high</sup>GZMK<sup>high</sup>TIGIT<sup>high</sup>IL10RA<sup>high</sup> cells and the increased distribution of NKG7<sup>high</sup>PRF<sup>high</sup>GZMK<sup>high</sup> CD8<sup>+</sup> T cells in PTL decidua may contribute to the occurrence of preterm labor.

## W-067

**Maternal and Neonatal Pregnancy Outcomes Following Fluoride Supplementation: A Pilot Randomized Controlled Trial.** Anna Maya Powell, Ramya Reddy, Kevin DeLong, Kimberly Jones-Beatty, Laura Ensign, Irina Burd\*. *Johns Hopkins University School of Medicine, Baltimore, MD, United States.*

**Introduction:** Maternal periodontal disease increases the risk for preterm birth. Fluoride supplementation has been added to water supplies to improve dental health. In mouse models, low dose fluoride supplementation showed decreased rate of preterm birth in a mouse model of intrauterine inflammation following lipopolysaccharide (LPS) challenge. The effect of fluoride supplementation on human pregnancy outcomes, including vaginal microbiome, is unknown. Maternal outcomes of interest included differences in vaginal microbiome between groups as well as mode of delivery and preterm birth rate. Primary neonatal outcomes of interest included anomalies, NICU admission, infectious and non-infectious comorbidity during hospital admission, and antibiotic requirement.

**Methods:** In this pilot double-blinded randomized controlled trial, maternal and fetal outcomes were compared following 3mg fluoride containing prenatal vitamin (treatment) compared to prenatal vitamin (control). Maternal urine samples and vaginal swabs were collected during the second and third trimesters and at delivery. The V4 region of 16S rRNA gene was sequenced from vaginal swabs, and richness, diversity and Community state types (CST) assignments were compared longitudinally. **Results:** 46 maternal-fetal dyads were eligible and 31 were enrolled through delivery. Among the 31 participants randomized, those receiving fluoride supplement had higher 2nd trimester urine fluoride concentrations [median 0.79 ng/mL (range 0.46-0.97) vs 0.6 ng/mL (range 0.49-0.76),  $p < 0.05$ ] but similar 3rd trimester and delivery urine fluoride concentrations. There were no differences in maternal mode of delivery or preterm births. While baseline CSTs differed by maternal race, there were no significant differences in the vaginal microbiome richness or diversity between treatment groups at any time-point. Neonates born to mothers receiving fluoride supplement had significantly higher Apgars at 1 ( $p < 0.05$ ) and 5 minutes ( $p < 0.05$ ), though similar weights at delivery and discharge, delivery length and head circumferences. There were no significant differences between neonates born to mothers who received fluoride supplement or control prenatal vitamin in terms of NICU admission ( $p=0.09$ ), neonatal anomalies ( $p=0.18$ ), comorbidities ( $p=0.63$ ) or illness during hospitalization ( $p=0.11$ ).

**Conclusion:** In this pilot RCT, fluoride supplementation was not associated with vaginal microbiome differences, mode of delivery or preterm birth rate. Fluoride supplementation was associated with higher Apgar scores at 1 and 5 minutes, however, was not associated with significant differences in NICU admission, febrile or infectious comorbidity among neonates, though sample size may limit interpretation.

**W-068**

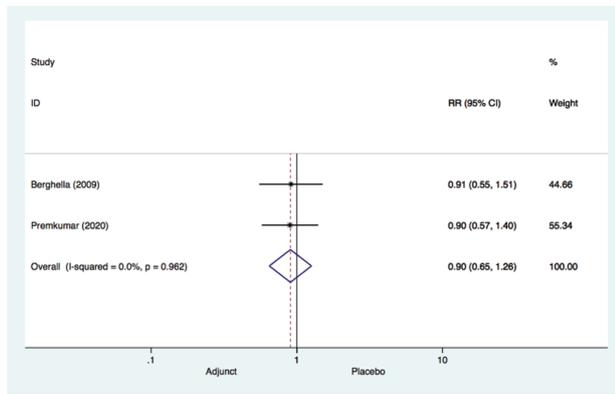
**Adjunct Therapy at Time of Exam-Indicated Cervical Cerclage in Singleton Pregnancies: A Systematic Review and Meta-Analysis.** Ann M Bruno<sup>†</sup>,<sup>1,2</sup> Ashley E Benson,<sup>1,2</sup> Torri D Metz,<sup>1,2</sup> Nathan R Blue\*.<sup>1,2</sup> <sup>1</sup>University of Utah Health, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Murray, UT, United States.

**Introduction:** The aim of this systematic review and meta-analysis was to compare gestational latency between those who did and did not receive adjunct antibiotic or tocolytic therapy at the time of exam-indicated cerclage.

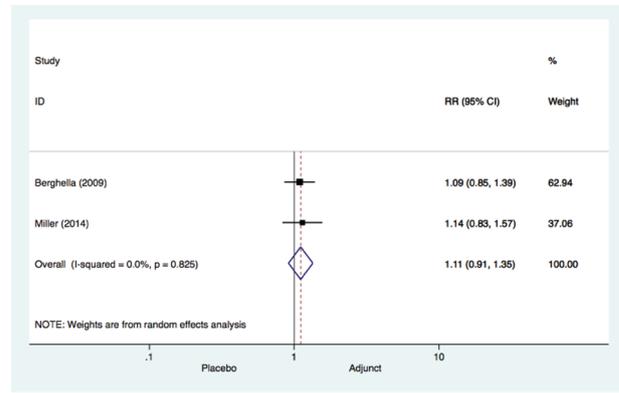
**Methods:** We searched electronic databases from 1966 to 2020 for randomized controlled trials (RCT) and cohort studies comparing adjunct antibiotic or tocolytic use versus non-use at time of exam-indicated cerclage. No language restrictions were applied. Exam-indicated cerclage was defined as placement for cervical dilation  $\geq 1$  cm between 16 0/7 and 24 0/7 weeks' gestation in a singleton pregnancy. Studies including individuals with intra-amniotic infection, cerclage already present, non-viable gestation, or ruptured membranes were excluded. The primary outcome was latency from cerclage placement to delivery. Secondary outcomes included preterm birth <28 weeks and neonatal survival. We used the Cochrane Risk of Bias tools to assess study quality. Heterogeneity was assessed using the  $\chi^2$  and  $I^2$  tests. Results were pooled and analyzed using a random-effects model.

**Results:** Of 923 unique records identified, 163 were reviewed in full. Three met inclusion criteria: one RCT and two retrospective cohort studies. The RCT was nested within one of the cohort studies, and therefore only one of these two studies was utilized for any given outcome to eliminate counting individuals twice. Risk of bias was "critical" for one cohort, "moderate" for one cohort, and "some concerns" for the RCT. Gestational age latency was reported by only one study and could not be pooled. Adjunct tocolytic-antibiotic therapy with exam-indicated cerclage was not associated with a decrease in risk of preterm delivery <28 weeks (RR 0.90, 95% CI 0.65-1.26;  $\chi^2=0$ ,  $I^2=0\%$ ) or neonatal survival (RR 1.11, 95% CI 0.91-1.35;  $\chi^2=0.05$ ,  $I^2=0\%$ ).

**Figure 1.** Forest plot of preterm birth at less than 28 weeks of gestation in selected studies comparing adjunctive use of tocolytics and antibiotics to non-use.



**Figure 2.** Forest plot of neonatal survival to hospital discharge in selected studies comparing adjunctive use of tocolytics and antibiotics to non-use.



**Conclusion:** Only three studies of adjunct antibiotic-tocolytic therapy at the time of exam-indicated cerclage were identified. The available studies were insufficient to draw any conclusions regarding the effect of these therapies on meaningful outcomes.

**W-069**

**Preterm Premature Rupture of the Membranes after Cerclage Placement.** Maria Andrikopoulou, Liping Lu, Chen Cheng, Samsiya Ona, Joy Vink, Cynthia Gyamfi-Bannerman\*. Columbia University Irving Medical Center, New York, NY, United States.

**Introduction:** The aim of the study is to determine the incidence of prelabor preterm rupture of membranes (PPROM) after cerclage placement and to determine the predictors of PPRM after cerclage.

**Methods:** This is a retrospective cohort study of women with singleton gestations who underwent history-, ultrasound- or examination-indicated cerclage over a 3-year period, excluding those without outcome data and pregnancies complicated with structural or genetic anomalies. Within this cohort we assessed the incidence of PPRM <37 weeks gestation. We then performed a nested case-control study to assess variables associated with PPRM. Cases included those with PPRM <37 weeks, and controls were those without preterm rupture at any time after cerclage placement. Participants had ultrasound prior to cerclage placement and sludge was noted if present. Sludge was defined as free-floating echogenic material at the internal os by review of transvaginal sonographic images during ultrasound. Demographics and clinical characteristic were compared between cases and controls using t-test, Kruskal-Wallis test, chi-square test, or Fisher's exact test as appropriate. We fit a logistic regression model to assess the contribution of each baseline variable to PPRM.

**Results:** Of 293 women receiving a cerclage in our study period, 243 were included. A total of 14 women (5.8%) delivered before 24 weeks. 60 women (24.6%) had PPRM after cerclage placement, of whom 12 (20%) had PPRM before 24 weeks of gestation. Specifically, among women with examination indicated, ultrasound indicated and history indicated cerclage, 35.7%, 26.4% and 18.8% had PPRM respectively. The mean gestational age of PPRM for examination indicated, ultrasound indicated and history indicated cerclage was 27 weeks, 26 weeks and 31 weeks respectively. Bivariable analyses showed that women who had PPRM after cerclage placement were more likely to be younger, have government insurance, have greater cervical dilation prior to cerclage placement, have an examination indicated cerclage, or have the cerclage placed at a later gestational age. However, after adjusting for confounders, no clinical variables, including maternal age, race, BMI, insurance, alcohol, tobacco, or drug use, cervical dilation prior to cerclage placement, gestational age of cerclage placement, indication for cerclage, the presence of sludge, or amniocentesis prior to cerclage placement were predictive of PPRM.

**Conclusion:** Approximately 1/4 of women who receive a cerclage will experience PPRM before 37 weeks, with 5.6% delivering prior to 24 weeks. Even though, relatively common, it can be challenging to predict the cohort of women who will PPRM after cerclage placement.

## W-070

**Survival of the Newborn with Diaphragmatic Hernia: EXIT versus Standard Procedure.** Daniele Francesco Lo Gerfo, Simona Lunardi, Marcello Bargione, Pietro Alimondi, Giulia Vellani, Antonio Vanella, Antonella Mercurio, Marzia Costanzo, Federica Cusimano, Roberta Vaccaro, Giorgia Ranieri, Domenico Incandela, Antonio Maiorana, Maria Chiara Di Liberto. *ARNAS Civico, Palermo, Italy.*

**Introduction:** Our study aims to compare the survival of infants with diaphragmatic hernia, born by caesarean section using the EXIT (Ex Utero Intrapartum Treatment) Procedures compared with those born by standard procedure.

**Methods:** This retrospective study was conducted on 18 women who underwent caesarean section and whose fetuses were affected by diaphragmatic hernia between 2015 and 2020. Out of these 18 patients, 13 gave birth between 2015 and 2018 and followed a standard procedure involving intubation of the fetus immediately after delivery. The remaining 5 patients followed the EXIT protocol, adopted in 2019, which consists in intubating the fetus after extraction of the head, neck and upper trunk, while keeping the rest of the fetal body inside the maternal uterus.

**Results:** 100% of fetuses born by EXIT procedure (n=5) survived and underwent hernia correction surgery. Of those born by standard procedure (n=13), only 25% survived (n=3) and underwent surgery.

**Conclusion:** The EXIT protocol has been adopted by the Obstetrics and Gynecology department of ARNAS Civico of Palermo since 2019. Despite the small number of cases, these results are very encouraging. Further investigations are required to demonstrate the effectiveness of the protocol.



## W-071

**Chaperon Mediated Autophagy and Macroautophagy in Placentas of Obese Women with or without Gestational Diabetes Mellitus: A Preliminary Study.** Chiara Mando, Cecilia Diceglie†, Gaia Maria Anelli†, Cristina Martelli†, Chiara Novielli, Fabrizia Lisso†, Alessia Lo Dico†, Anais Serati†, Irene Cetin, Luisa Ottobrini. *Università degli Studi di Milano, Milan, Italy.*

**Introduction:** Maternal obesity (OB) and gestational diabetes mellitus (GDM) are significant risk factors for both adverse pregnancy and long-term outcomes. We recently showed altered placental mitochondria in OB, suggesting increased oxidative stress. Chaperone Mediated Autophagy (CMA) is a form of autophagy activated by oxidative stress, interacting with macroautophagy in a mutual modulation, for the maintenance of cell homeostasis. Antioxidant, macroautophagy and CMA related gene expression has been evaluated herein in obese and GDM placentas.

**Methods:** 47 women with singleton pregnancies delivering by elective caesarean section were enrolled: 16 normal-weight [NW], 18 obese with no co-morbidities [OB GDM(-)], 13 obese with GDM [OB GDM(+)].

Placental gene expression was assessed by Real-time PCR. Parametric or non-parametric tests were applied for statistical analysis depending on data distribution.

**Results:** Placental efficiency resulted progressively lower from NW to OB GDM (-) and OB GDM (+). Antioxidant gene expression (*CAT*, *GPX1*, *GSS*) significantly decreased in OB GDM(-) compared to NW. In OB GDM(-) the pro-autophagic gene *ULK1* significantly increased its expression respect to NW, whereas the expression of the main chaperone involved in CMA (*HSC70*) decreased in both OB GDM(-) and OB GDM(+) compared to NW. The expression of the CMA regulator *PHLPP1* decreased in OB GDM (-) vs NW but its expression increased in OB GDM(+) vs OB GDM(-). When analyzing results in relation to fetal sex, we found sexual dimorphism for both antioxidant and CMA-related gene expression.

**Conclusion:** There are few reports correlating autophagy to obesity, suggesting autophagy deregulation in the obese omental and subcutaneous adipose tissues. However, the role of autophagy in human placentas remains controversial. In particular, only few contrasting studies in OB/GDM placentas reported alterations of macroautophagy, but to date placental CMA activity has never been specifically investigated. Moreover here we report for the first time an increase in *PHLPP1* expression in the OB GDM(+) group supporting a correlative hypothesis between *PHLPP1* activity and GDM. The sexual dimorphism might suggest different molecular mechanisms involved in GDM, with possible different resulting outcomes. These preliminary results can pave the way for further analyses aimed at elucidating the placental autophagy role in metabolic pregnancy disorders and its potential targetability for the treatment of diabetes outcomes.

## W-072

**Maternal Underweight and Obesity Are Associated with Placental Pathologies in Human Pregnancy.** Hailey Scott†,<sup>1</sup> David Grynspan,<sup>2</sup> Laura N Anderson,<sup>3</sup> Kristin L Connor\*.<sup>1</sup> *<sup>1</sup>Carleton University, Ottawa, ON, Canada; <sup>2</sup>University of British Columbia, Vancouver, BC, Canada; <sup>3</sup>McMaster University, Hamilton, ON, Canada.*

**Introduction:** Maternal underweight and obesity are prevalent conditions associated with chronic, low-grade inflammation, poor fetal development, and long-term adverse outcomes for the child. The placenta adapts in response to challenges in the pregnancy environment, such as suboptimal maternal body mass index (BMI), to support optimal fetal development. However, the mechanisms driving these adaptations, and the resulting placental phenotypes are poorly understood. We hypothesised that suboptimal maternal BMI would be associated with increased prevalence of placental pathologies related to inflammation and maturation in term (T) and preterm (PT) pregnancies.

**Methods:** A retrospective cohort study was conducted using data from 12,154 pregnancies with placental assessments from the Collaborative Perinatal Project. The associations between maternal BMI (primary exposure) and placental pathologies (primary outcomes) were assessed by unadjusted and adjusted linear or nominal logistic regression models (adjusted for fetal sex, maternal race, maternal age, maximum gestational weight gain, smoking history, maternal education, and diabetes status). We also explored the effect of fetal sex on placental pathologies. Data are adjusted  $\beta$  (95% CI).

**Results:** Among PT pregnancies, increased maternal BMI was associated with increased fetal inflammation (neutrophil infiltration of the umbilical vein, artery and cord substance;  $\beta=0.03$  [0.003, 0.05]). Among T pregnancies, increased maternal BMI was associated with increased fetal ( $\beta=0.01$  [0.007, 0.02]) and maternal (neutrophil infiltration of amnion and chorion of the placenta and amnion and chorion membranes;  $\beta=0.03$  [0.01, 0.04]) inflammation, increased odds of an appropriate mature placenta for gestational age ( $\beta=-0.06$  [-0.09, -0.03]), increased maternal vascular malperfusion (MVM) (placental infarcts and syncytial knots;  $\beta=0.007$  [0.004, 0.01]), and decreased placental efficiency ( $\beta=-0.02$  [-0.03, -0.02]). At PT, male placentae had lower fetal inflammation (unadjusted  $\beta=-0.06$  [-0.13, 0.02]) than female placentae, while at T, male placentae had both greater maternal (unadjusted  $\beta=0.07$  [0.03, 0.11]) and fetal (unadjusted  $\beta=0.05$  [0.03, 0.07]) inflammation. Male placentae also had

greater placental efficiency at PT (unadjusted  $\beta=0.12$  [0.05, 0.20]) and T (unadjusted  $\beta=0.08$  [0.06, 0.11]), and increased MVM at T (unadjusted  $\beta=0.02$  [0.01, 0.03]) compared to females.

**Conclusion:** Suboptimal maternal BMI is not an inert condition for the developing placenta. Characterising placental (mal)adaptations using clinically-relevant indicators can help to better understand the mechanisms through which these conditions affect the developing offspring, and may be useful predictors for future health trajectories.

#### W-073

**Gestational Diabetes Stratification According to Body Mass Index in a Multiracial Cohort.** Kevin Saiki†, Kelly Yamasato, Benny Beth Paula, Bartholomew Lisa Marguerite, Men-Jean Lee\*. *John A. Burns School of Medicine, University of Hawai'i, Honolulu, HI, United States.*

**Introduction:** Gestational diabetes (GDM) affects 2-10% of pregnancies in the United States (US), with health implications to both the pregnant woman and fetus. While several studies have investigated racial disparities in GDM in the mainland US, the unique multiracial populations of Hawaii remain under-represented.

**Methods:** We performed a retrospective study on 8,970 pregnant women from the Hawaiian Biorepository Database, of whom 987 had GDM. GDM was identified by ICD-9 codes. Pregnant women with gestational diabetes were stratified according to self-reported race and body-mass index (BMI). Multiracial women were categorized by their primary race.

**Results:** Native Hawaiian/Other Pacific Islander (NHOPI) and Filipino populations have significantly higher rates of GDM compared to White women and were less likely to be lean (BMI <25). When stratified by BMI, we observed higher rates of GDM in lean Filipino, Japanese and Chinese women. Although it is widely accepted that obesity is a driver of diabetes, there exists a large group of lean women who develop GDM with BMI between 18.5 and 25. The majority of NHOPI women with GDM were overweight/obese (BMI >25). Overall, an association between BMI and race was observed in women with GDM (chi-sq  $\chi^2$  test,  $p=6.27$  E-17).

**Conclusion:** Our data show that the spectrum of GDM is not limited to women who are overweight or obese. Concomitantly, race plays a role in the manifestation of GDM. Future work is needed to elucidate the molecular mechanisms underlying GDM in lean and overweight/obese pregnant women.

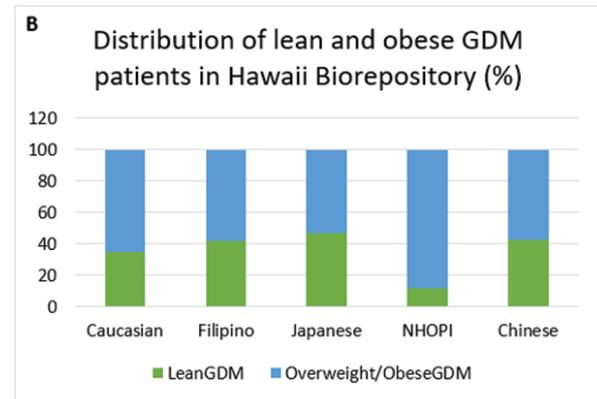
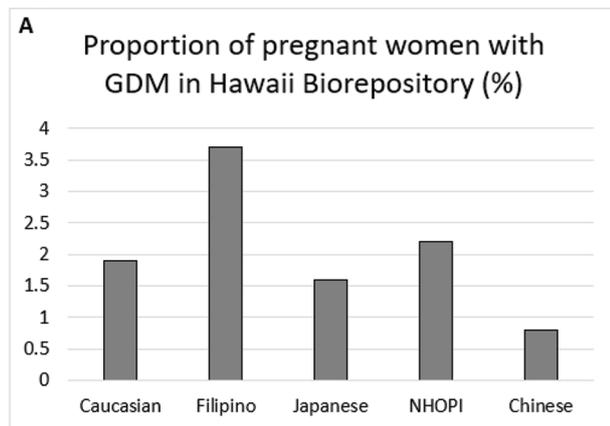


Figure A: Proportions of women with GDM in the different ethnicities in Hawaii  
Figure B: Stratification of women with GDM according to ethnicity and BMI.

#### W-074

**How Do Maternal BMI and Fetal Membrane Inflammation Influence Infant Outcomes at Birth?** Eleanor Duffley†, Marina White†, David Grynspan\*, Shannon Bainbridge\*, Kristin Connor\*. *1Carleton University, Ottawa, ON, Canada; 2Vernon Jubilee Hospital, Vernon, BC, Canada; 3University of Ottawa, Ottawa, ON, Canada.*

**Introduction:** Suboptimal maternal metabolic states associate with chronic, low-grade inflammation in pregnancy and poor offspring development. However, the effects of maternal BMI on inflammatory phenotypes in gestational tissues are poorly described. We hypothesised that maternal underweight (UW) and obesity (OB) associate with increased inflammation in fetal membranes (FM), and increased inflammation would associate with poor birth outcomes.

**Methods:** Clinical data and biospecimens were collected from Mount Sinai Hospital, Toronto. Mothers were classified based on pre-pregnancy BMI as UW (BMI <18.5 kg/m<sup>2</sup>; n=9), normal weight (BMI 18.5-24.9; n=22), overweight (OW; BMI 25-29.9; n=18) or OB (BMI >30; n=16). H&E stained FM sections were scored for histopathological characteristics with a clinical pathologist. Associations between maternal pre-pregnancy BMI, FM inflammation stage (S; defined as S0—no inflammation, S1—neutrophils in trophoblast layer, S2—diffuse neutrophils in fibrous chorion/amnion or S3—amnion/chorionic plate necrosis), and infant outcomes (birthweight z-scores, Apgar scores) were investigated for preterm + chorioamnionitis (PTC), preterm (PT) and term (T) pregnancies. Data were analysed using adjusted (for gestational weight gain, gestation length, infant sex) generalized linear ( $\beta$  [95% CI]) or standard least squares ( $\beta$  [95% CI], p value from Tukey's HSD *post hoc*) regression models. \*Sig= $p<0.05$ .

**Results:** Inclusive of BMI, PTC FM had higher inflammation (S1: n=7, 36.8%; S2: n=11, 57.9%) than PT (S1: n=8, 33.3%; S2: n=1, 4.2%) or T (S1: n=2, 9.1%; S2: n=1, 4.5%) FM ( $p<0.001$ ). Among T pregnancies maternal OW associated with increased FM inflammation (0.4 [0.02, 0.8],  $p=0.03$ ). Apgar scores at 5 min were lower for PT infants with S2 FM inflammation (vs. S1 and S0; -1.9 [-3.1, -0.7]  $p=0.01$ ) and T infants with S1 FM inflammation (vs. S0 and S2; -0.5 [-0.8, -0.2]  $p=0.005$ ).

**Conclusion:** Among T pregnancies, higher maternal BMI may be associated with increased FM inflammation and our data confirm PT + chorioamnionitis is most strongly linked with FM inflammation. Taken with our findings of increased inflammation in T and PT and lower Apgar scores, inflammation in utero, even if subtle, may have implications for fetal development and perinatal outcomes. Future studies in larger cohorts could determine if pregnancies with chronic subclinical inflammation, such as those with suboptimal metabolic status, increase risk for gestational tissue inflammation and adverse offspring outcomes.

## W-075

**Opiate Use Postpartum in Subjects with a History of Bariatric Surgery by Type of Surgery.** Samantha R Lauhon†, Meredith Cruz, Katherine Allen, Kia Semons-Booker, Rachel Harrison. *Medical College of Wisconsin, Wauwatosa, WI, United States.*

**Introduction:** Bariatric surgery is most commonly performed in women of reproductive age and remains a fundamental treatment of morbid obesity. Opioid abuse has reached epidemic levels in the United States prompting further characterization of vulnerable populations. Use of opioids is common after bariatric surgery and greater risk of chronic opioid use may be associated with this type of surgery. The objective of our study is to evaluate postpartum opiate use by type of bariatric surgery.

**Methods:** This is a retrospective cohort study in subjects with a history of bariatric surgery who delivered from 2009 to 2019 in a single academic center. Subjects were stratified by surgery type into those with a history of a primarily restrictive surgery type (gastric band or gastric sleeve) or a primarily malabsorptive type (Roux-en-Y gastric bypass or duodenal switch). Univariable and multivariable analyses were performed. Opiate use postpartum was quantified by total morphine milliequivalents (MME) and top quartile opiate use during inpatient postpartum stay. Linear regression was performed with possible confounders included in the adjusted model.

**Results:** A total of 190 subjects met inclusion criteria. Of those, 45 had a history of primarily restrictive surgery type and 145 primarily malabsorptive. Women with prior malabsorptive surgery had lower body mass index (BMI), were further out from surgery in the current pregnancy, and more like to be anemic than women with primarily restrictive surgery type (Table 1). Total ibuprofen use and MME use during admission were higher in women with malabsorptive procedure type (2400 mg vs 200 mg and 61.6 MME vs 22.5 MME, respectively). MME use was not statistically significantly different between groups after controlling for confounders (aLRC 42.31, 95%CI -3.42 to 88.03) and both groups were similarly likely to be in the top quartile of opioid users (aOR 2.05, 95% CI 0.65 to 6.47).

**Conclusion:** Subjects with primarily malabsorptive surgery used less ibuprofen than those with prior restrictive surgery. The type of bariatric surgery did not impact amount of opioid use postpartum.

	OR/LRC	95% CI	aOR/aLRC	95% CI
MME	26.61	-17.39 to 70.62	42.31	-3.42 to 88.03
Top quartile of MME use	1.14	0.49-2.67	2.05	0.65 to 6.47

OR=odds ratio, aOR=adjusted odds ratio, LRC=linear regression coefficient, aLRC=adjusted linear regression coefficient, MME=morphine milliequivalents  
Controlled for early pregnancy BMI, maternal race/ethnicity, time from surgery to pregnancy, chronic opioid use, mood disorder, cesarean delivery and maternal length of stay

	Restrictive Procedure Type N=45	Malabsorptive Procedure Type N=145	p-value
Age (years)	34.0 ± 4.2	33.9 ± 4.4	0.919
Body mass index at delivery (kg/m <sup>2</sup> )	40.0 ± 8.3	34.1 ± 6.6	<0.001
Nulliparity	14 (31.1%)	47 (32.4%)	0.870
Maternal race/ethnicity			1.000
White	28 (65.1%)	93 (64.1%)	
Non-Hispanic Black	11 (25.6%)	38 (26.2%)	
Hispanic	2 (4.7%)	7 (4.8%)	
Other	2 (4.7%)	7 (4.8%)	
Marital status			0.527
Single	12 (27.3%)	49 (34.0%)	
Married	30 (68.2%)	84 (58.3%)	
Divorced	2 (4.6%)	11 (7.6%)	
<18 months since bariatric surgery	13 (28.9%)	19 (13.1%)	0.013
Chronic hypertension	10 (22.2%)	25 (17.2%)	0.451
Anemia	12 (27.9%)	79 (58.9%)	<0.001
Pre-gestational diabetes mellitus	3 (6.7%)	10 (7.1%)	1.000
Gestational diabetes mellitus	8 (18.6%)	14 (10.3%)	0.182
Tobacco use	7 (15.9%)	15 (10.6%)	0.422
Opioid dependence	3 (6.8%)	11 (7.6%)	0.856
<b>Delivery/postpartum characteristics</b>			
Cesarean delivery	22 (48.9%)	51 (35.2%)	0.098
Gestational weeks at delivery	37.7 ± 3.1	37.8 ± 3.3	0.823
Composite adverse maternal outcome*	3 (6.7%)	10 (6.9%)	1.000
Preterm delivery	8 (17.8%)	25 (17.2%)	0.934
Postpartum stay length (days)	3.62 ± 1.83	3.67 ± 5.0	0.951
Preeclampsia or gestational hypertension	10 (22.2%)	17 (11.7%)	0.078
<b>Postpartum medication use</b>			
MME mg	22.5 (0-135.5)	61.6 (0-142.5)	0.234
Acetaminophen mg	5000 (1000-8000)	4000 (90-6000)	0.700
Ibuprofen mg	2400 (0-4200)	200 (0-3000)	0.038
Ketorolac mg	0 (0-90)	0 (0-90)	0.411
Top quartile of MME use	9 (23.7%)	33 (26.2%)	0.835

MME = morphine milliequivalents  
All data presented as N (%), mean ± standard deviation, or median (IQR)  
\*Composite includes postpartum hemorrhage, blood transfusion, wound infection, third and fourth degree laceration

## W-076

**Psychological Therapies Used to Improve Lifestyle Behaviors in (Pre) Pregnant Women: A Systematic Review.** Melissa van der Windt†, Sofie van Zundert†, Sam Schoenmakers\*, Pauline Jansen\*, Lenie van Rossem\*, Régine Steegers-Theunissen\*. <sup>1</sup>Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Erasmus University Rotterdam, Rotterdam, Netherlands.

**Introduction:** Poor lifestyle behaviors of women who are contemplating pregnancy or already pregnant affect reproductive, pregnancy and neonatal outcomes and can have transgenerational health consequences. However, adopting healthy lifestyle behaviors is challenging and interventions often do not lead to satisfactory results and sustainable change. Recently, psychological therapies were suggested as mean to enhance changes in lifestyle behaviors. We assessed the evidence for the effectiveness of psychological therapies used to change lifestyle behaviors among (pre) pregnant women.

**Methods:** A comprehensive search in online scientific databases was conducted with a focus on (pre)pregnant women and their lifestyle behaviors, including dietary intake, alcohol use, smoking, drug use, body weight (gain during pregnancy), and physical activity. Key words in our search strategy related to psychological therapies were psychotherapy, behavior therapy, cognitive behavioral therapy (CBT), motivational interviewing (MI), motivational enhancement therapy, mindfulness, hypnotherapy and incentives. We included studies that used a control group receiving usual care or an alternative version of a psychological intervention. Methodological quality was scored using the ErasmusAGE tool. A protocol of this systematic review has been registered in PROSPERO International prospective register of systematic reviews (PROSPERO 2020: CRD42020201172).

**Results:** Of 5760 unique citations found, 40 studies were included. The mean quality score was 6.8 (range: 0-10). MI (n=21), CBT (n=8), incentive-based contingency management (IBCM) (n=9), mindfulness (n=1) and hypnosis (n=1) were investigated in combination with lifestyle behaviors. The findings revealed that MI was effective in reducing (self-reported) smoking and alcohol consumption and restricting gestational weight gain (GWG). CBT was only studied as an intervention to restrict GWG and results predominantly confirmed its effectiveness. IBCM was the most effective intervention for reducing smoking and substance use. The studies using hypnosis or mindfulness to reduce smoking or restrict GWG, respectively, showed no associations.

**Conclusion:** The use of psychological therapies to improve lifestyle behaviors among (pre)pregnant women is relatively new and the emerging scientific proof is promising. Before wide implementation is legitimated, more evidence should become available on specific lifestyle factors, and on the subsequent consequences of lifestyle change on pregnancy outcomes.

## W-077

**Numeracy Scores and Perinatal Outcomes among Women with Gestational and Pregestational Diabetes.** Jennifer Jacobson†, Amy Goedecker, Jennifer Janik, April Eddy, Jacquelyn Adams\*. <sup>1</sup>University of Wisconsin School of Medicine and Public Health, Madison, WI, United States; <sup>2</sup>Unity-Point Health Meriter Hospital, Madison, WI, United States.

**Introduction:** Numeracy is the ability to understand and use numbers in daily life. Low diabetes (DM) related numeracy in patients is associated with poor knowledge of DM, lower perceived efficacy in managing DM and worse glycemic control. Numeracy data is limited in the pregnant population despite high prevalence, with 9% affected by gestational diabetes (GDM) and 6% with pregestational diabetes (preDM). We hypothesize that low Numeracy Score (NS) in diabetic pregnancies is associated with higher HbA1c at 4-8 weeks pre-delivery and up to 12 weeks postpartum (PP).

**Methods:** A retrospective cohort study of 295 women with preDM (prediabetes, type 2 DM, type 1 DM; n=121) or GDM (n= 174) between 1/1/2018 and 7/31/2020. Patients were identified using the American Diabetes Association pregnancy database. Demographic and pregnancy-related information was obtained from PeriData, a statewide quality improvement database, and the electronic medical record.

**Results:** Demographics were similar for GDM and preDM groups except for race (p<0.001). For maternal outcomes, women with low vs. high

NS did not have significantly different 3<sup>rd</sup> trimester A1c in all DM types (6.0% vs. 5.7%, p=0.069), preDM (6.6% vs. 6.1%, p=0.057) or GDM (5.6% vs. 5.4%, p=0.056). There was no difference between low NS and high NS groups in gestational weight gain (26.0 lb vs. 27.3 lb, p=0.695), positive Edinburgh Postpartum Depression Scale (EPDS) screen at 28-32 weeks (31.8% vs. 17.6%, p=0.122) or positive EPDS screen at 6 weeks PP (23.5% vs. 12.6%, p=0.098). In neonatal outcomes, there was no difference between low NS and high NS groups in 5 minute APGAR <7 (2.4% vs. 3.6%, p=0.719), hypoglycemia (38.1% vs. 42.2%, p=0.623), respiratory distress syndrome (11.9% vs. 6.5%, p=0.225) or NICU admission (26.8% vs. 19.8%, p=0.315).

**Conclusion:** Low NS was not associated with higher A1c in 3<sup>rd</sup> trimester or PP, however all low NS groups had higher A1c values than high NS groups, with PreDM and GDM subgroups approaching significance. Non-significant association of NS and A1c may be attributable to small sample size as well as loss to follow up. There was no significant interaction with NS and EPDS or neonatal outcomes. Given the trends noted, further prospective studies on NS and glycemic control in pregnancy are indicated.

Maternal Characteristic	Low NS		High NS	
	n(%)	p-value	n(%)	p-value
Age at Inception, mean (SD)	31.7(5)	0.0001	31.7(5)	0.0001
Pre-eclampsia	6 (3.02%)	<0.001	14 (6.98%)	0.007
Gestational	5 (4.89%)	<0.001	14 (6.98%)	0.056
Age at term, mean (SD)	41.1(1)	<0.001	41.1(1)	<0.001
Pre-eclampsia	5 (4.89%)	<0.001	14 (6.98%)	0.056
Gestational	5 (4.89%)	<0.001	14 (6.98%)	0.056
Gestational weight gain, mean (SD)	28.2 (8.6)	0.0001	27.3 (8.6)	0.0001
Pre-eclampsia	29.0 (9.5)	0.0001	25.1 (7.8)	0.0001
Gestational	29.4 (9.5)	0.0001	25.4 (7.8)	0.0001
APGAR-5, n (%)	201 (81.4)	0.0001	180 (65.4)	0.0001
Low (8-9)	33 (84.4)	<0.001	33 (84.4)	<0.001
Pre-eclampsia	33 (84.4)	<0.001	33 (84.4)	<0.001
Gestational	33 (84.4)	<0.001	33 (84.4)	<0.001
Low (8-9)	33 (84.4)	<0.001	33 (84.4)	<0.001
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Low (8-9)	33 (84.4)	<0.001	33 (84.4)	<0.001
Pre-eclampsia	33 (84.4)	<0.001	33 (84.4)	<0.001
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Pre-eclampsia	33 (84.4)	<0.001	33 (84.4)	<0.001
Gestational	33 (84.4)	<0.001	33 (84.4)	<0.001
Low (8-9)	33 (84.4)	<0.001	33 (84.4)	<0.001
Pre-eclampsia	33 (84.4)	<0.001	33 (84.4)	<0.001
Gestational	33 (84.4)	<0.001	33 (84.4)	<0.001
Low (8-9)	33 (84.4)	<0.001	33 (84.4)	<0.001
Pre-eclampsia	33 (84.4)	<0.001	33 (	

## W-080

**Increased Maternal Morbidity in Women with Iron Deficiency Anemia Who Receive Intravenous Iron Infusion Therapy.** *Martina S Burn†, Lisbet Lundsberg, Jennifer Culhane, Caitlin Partridge, Moeun Son\*. Yale University, New Haven, CT, United States.*

**Introduction:** Anemia in pregnancy has been linked with significant maternal morbidity including maternal blood transfusion and cesarean delivery. We sought to investigate whether women with iron deficiency anemia who receive intravenous (IV) iron infusion therapy have decreased risk for maternal morbidity compared to those who do not.

**Methods:** This is a retrospective cohort study of women with a diagnosis of iron deficiency anemia who delivered at term at a tertiary hospital from October 2013 to August 2020. Data were extracted from the hospital electronic medical record data warehouse using standardized definitions and billing and diagnosis codes. Women with iron deficiency anemia who received IV iron infusion therapy during the prenatal period were compared to women who did not. The primary outcome was a composite maternal morbidity outcome that included receipt of blood transfusion, hysterectomy, and/or admission to the intensive care unit. Bivariate analyses and multivariable logistic regression modeling were performed.

**Results:** Of a total of 35,265 deliveries, 6351 (18%) had a diagnosis of iron deficiency anemia. Among this sample, 731 (11.5%) received IV iron during their pregnancy. Women who received IV iron were more likely to be of Hispanic or Black race, have Medicare/Medicaid insurance, be single/divorced/widowed, or multiparous compared to women who did not receive IV iron (Table 1). There was an increased risk of the composite maternal morbidity outcome in the group who received IV iron therapy (n=43, 5.9%) compared with the group who did not (n=186, 3.3%), (OR 1.83, 95% CI 1.30-2.57). After adjusting for potential confounders, including mode of delivery, race-ethnicity, parity, insurance type, and marital status, this association persisted (aOR 1.80, 95% CI 1.27-2.55).

**Conclusion:** In contrast to our study hypothesis, composite maternal morbidity was increased among women who received IV iron. These findings, however, are not felt to suggest a lack of efficacy of IV iron. Instead, findings suggest that women at higher risk of maternal morbidity are more likely to receive IV iron therapy. Further investigation is warranted to determine why this study population had worse maternal outcomes.

Table 1

	Receipt of IV iron infusion therapy (n=731)	No receipt of IV iron infusion therapy (n=5620)	p-value*
Maternal age $\geq 35$ y	173 (23.7)	1233 (21.9)	0.29
Race/ethnicity			<0.01
Hispanic	216 (29.5)	1376 (24.5)	
Black	227 (31.1)	1555 (27.7)	
Non-Hispanic White	225 (30.8)	2112 (37.6)	
Asian	28(3.8)	333 (5.9)	
Other	35 (4.8)	244 (4.3)	
Insurance type			0.04
Commercial	337 (46.1)	2861 (50.9)	
Government	387 (52.9)	2696 (48.0)	
Self-pay/Other/Unknown	7 (1.0)	63 (1.1)	
Marital status			0.03
Married or living as married	365 (49.9)	3092 (55.0)	
Single/divorced/widowed	358 (49.0)	2478 (44.1)	
Other/Unknown	8 (1.1)	50 (0.9)	
Pre-pregnancy BMI $\geq 30$ kg/m <sup>2</sup> (n=883, 5253)	1463 (27.9)	207 (30.3)	0.41
Multiparous	502 (68.7)	3381 (60.2)	<0.01
Singleton gestation	701 (95.9)	5424 (96.5)	0.40
Cesarean delivery	234 (32.1)	1782 (31.9)	0.89
EBL $\geq 1000$ mL at delivery	55 (7.5)	332 (5.9)	0.09

All data are presented in n (%)

\*Chi square tests

IV=intravenous

BMI=body mass index

EBL=estimated blood loss

## W-081

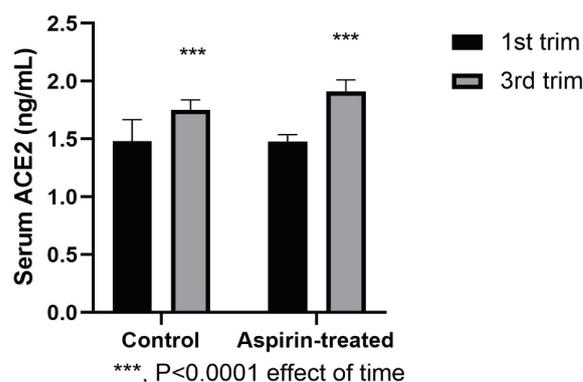
**Circulating ACE2 Increases during Pregnancy and Is Associated with Preterm Preeclampsia.** *Robin Shoemaker, Katherine Vignes, Hong Huang, Aarthi Srinivasan, Aric Schadler, Zachary Stanley, Cynthia Cockerham, Brittany McKinley, John Bauer, John O'Brien\*. University of Kentucky, Lexington, KY, United States.*

**Introduction:** The renin-angiotensin system (RAS) contributes to the physiological changes of pregnancy and angiotensin converting enzyme 2 (ACE2) is a key RAS regulator. Emerging evidence suggests circulating ACE2 is an indicator of cardiovascular stress outside of pregnancy. However, the significance of elevated ACE2 during pregnancy requires further study.

**Methods:** Data were derived from an observational cohort study of women at low risk for pre-eclampsia by USPSTF criteria and a trial of patients defined as high-risk for pre-eclampsia randomized to 81mg vs 162 mg of aspirin. Serum samples were obtained at two time points: 11-16 weeks (prior to aspirin) and 28-32 weeks gestation. Serum ACE2 activity was assessed by a natural substrate conversion assay where the product was quantified by liquid chromatography with tandem mass spectrometry (LC-MS/MS), considered to be the gold standard for quantification of angiotensin peptides. Serum concentrations of AngII and Ang-(1-7) (the substrate and product of ACE2, respectively), were also determined by LC-MS/MS. Analyses were completed with paired t-tests, RM 2-way ANOVA, and chi-square tests.

**Results:** 78 low- and high-risk women were included. 7 women developed preterm pre-eclampsia. Across all groups, serum ACE2 activity was increased in the third vs first trimester: in patients who did not develop pre-eclampsia (n=71; 1.81 +/- 0.07 vs 1.37 +/- 0.05 ng/mL, P<0.0001) and patients who did develop pre-eclampsia (n=7; 2.21 +/- 0.23 vs 1.25 +/- ng/mL, P<0.0001). Moreover, the change in serum ACE2 activity was significantly greater (1.2 fold) in patients who developed pre-eclampsia vs those who did not (P<0.01). Serum ACE2 activity was not different at either time point in between low-risk patients (n=35) and the high-risk cohort treated with aspirin (combined data 81 mg + 162 mg, n=36), see Figure. ACE2 activity was not correlated with serum AngII concentrations at either time point. Serum Ang-(1-7) concentrations were detectable in only 4/7 and 1/7 women who developed pre-eclampsia in the first and third trimester, respectively (vs 21/71 and 29/71 in those who did not, P=0.14 and P=0.17), and these peptides did not significantly change over time.

**Conclusion:** ACE2 increases during pregnancy and this change is significantly greater in women who developed preterm pre-eclampsia. However, the increased ACE2 concentrations do not appear to influence systemic angiotensin peptide levels.



W-082

**Maternal and Neonatal Morbidity Associated with TOLAC versus Elective Repeat Cesarean as a Function of VBAC Success Prediction.** Hayley Pierce†, Frank B. Williams†, Carole McBride†, Kelley McLean\*. *University of Vermont Medical Center, Burlington, VT, United States.*

**Introduction:** The Maternal Fetal Medicine Units Vaginal Birth After Cesarean (VBAC) Calculator is a validated resource for counseling women considering trial of labor after cesarean (TOLAC). We aim to correlate thresholds for composite maternal and/or neonatal morbidity with VBAC success prediction in TOLAC eligible women.

**Methods:** This is a retrospective cohort study of 810 women with prior cesarean delivery admitted for either TOLAC or elective repeat cesarean delivery (ERCD) from January 2016 through June 2019. All women with singletons in cephalic presentation without contraindications to TOLAC were included. Morbidity was defined by previously reported composites related to VBAC. Antepartum VBAC calculator scores were calculated and used to stratify patients by likelihood of VBAC success.

**Results:** TOLAC was attempted by 384 patients (47%), while 426 chose ERCD (53%). ERCD patients had a lower mean VBAC success prediction score (58% vs 66%,  $p < 0.001$ ). No differences were observed in maternal age, parity, race, ethnicity, hypertension or diabetes. The predicted VBAC success rate for the entire group was 66%, while the observed rate of vaginal delivery was 70%. Indications for repeat cesarean among patients undergoing TOLAC included intrapartum complications (53%) and arrest disorders (47%). Overall rate of MM was 3.8%, with higher rates in TOLAC patients compared to ERCD (Table 1). When stratified by VBAC calculator threshold of  $< 60\%$  predicted success, the rate of MM was 1.9% with ERCD versus 11.1% with TOLAC. Overall rate of NM was 5.4%, with again higher rates in the TOLAC group compared to ERCD (Table 1). When stratified by VBAC calculator threshold of  $< 60\%$  predicted success, the rate of NM was 3.3% with ERCD versus 12.8% with TOLAC.

**Conclusion:** In this population, TOLAC is associated with higher rates of composite maternal and neonatal morbidity compared to ERCD. Rates of MM and NM are highest among patients with likelihood of VBAC success  $< 60\%$ . MM was higher than has been previously reported with similar VBAC success prediction. The rate of NM was lower than in prior published reports. Our results provide a contemporary assessment of the correlation between VBAC prediction score and morbidity, and may help to better inform decision-making on mode of delivery for patients with prior cesarean.

	Rate of Maternal Morbidity (%)	p-value	Rate of Neonatal Morbidity (%)	p-value
All ERCS	1.18	$< 0.0001$	2.82	0.001
All TOLAC	6.75		8.31	
<b>VBAC score <math>&gt; 60\%</math></b>				
ERCS	0.47	0.01	2.35	0.047
TOLAC	4.85		6.35	
<b>VBAC score <math>&lt; 60\%</math></b>				
ERCS	1.89	0.001	3.30	0.001
TOLAC	11.1		12.82	

Maternal morbidity = blood transfusion, intensive care unit (ICU) admission, uterine rupture, hysterectomy, or need for concurrent surgical procedure  
 Neonatal morbidity = Apgar $< 4$  at 5 minutes of life, umbilical artery blood gas pH  $< 7.0$  and unanticipated neonatal ICU admission

W-083

**The Inhibitory Effect of Amniotic Fluid Contamination on Anticoagulation Pathway in the Maternal Plasma Measured by an Activated Protein C-Sensitivity Test.** Divyvanu Jain†, 1,2 Tomoaki Oda\*, 2 Naoki Tamura\*, 2 David M Olson\*, 1 Naohiro Kanayama\*, 2 Hiroaki Itoh\*, 2 *1University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada; 2Hamamatsu University School of Medicine, Hamamatsu, Japan.*

**Introduction:** It is known that amniotic fluid comes in contact with the maternal blood during childbirth and flows into the maternal circulation. In our previous study, we have reported that amniotic fluid promotes blood coagulation and platelet aggregation through activated tissue factor pathway. Although amniotic fluid contains some coagulation factors related to tissue factor- and contact activation pathways, it is not well-known which mechanisms work towards blood coagulation with amniotic

fluid contamination. Protein C is an anticoagulant, which inactivates factor Va and VIIIa with its cofactor protein S. Pregnancy is a hypercoagulable state due to increased coagulation factors and decreased potential of anticoagulation system, therefore; we hypothesized that on contamination of pregnant maternal plasma with amniotic fluid, the susceptibility to Activated protein C (APC) is decreased. The purpose of this study is to examine the effect on anticoagulation pathway when amniotic fluid is mixed with pregnant women's plasma.

**Methods:** Plasma collected from 51 healthy pregnant Japanese women in the 3rd trimester was mixed with increasing concentrations of amniotic fluid. Activated Protein C-sensitivity ratio (APC-sr) was calculated using an endogenous thrombin potential (ETP) based assay where thrombin production was measured by calibrated automated thrombography.

**Results:** APC-sr of maternal plasma significantly increased in an amniotic fluid volume-dependent manner indicating reduced sensitivity to APC. The difference in the ratio was approximately by 1 ( $p < 0.0001$ ) between without (0  $\mu$ L) and with 8  $\mu$ L of amniotic fluid addition. The ETP in the presence of APC was significantly elevated even with addition of 1  $\mu$ L of amniotic fluid ( $p < 0.05$ ).

**Conclusion:** Our results demonstrate that addition of amniotic fluid to pregnant women's plasma causes inhibition of anticoagulation pathway which may promote hypercoagulation and partly contribute to pregnancy disorders like pulmonary thromboembolism, which sometimes occurs during the early postpartum period. Further research is needed to identify the corresponding inhibitory mechanisms or factors in the amniotic fluid involved in coagulation in the maternal system.

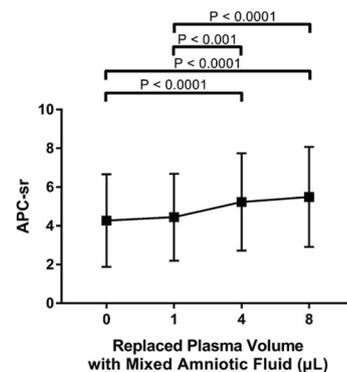


Fig. The activated protein C-sensitivity ratio (APC-sr) with the mixture of amniotic fluid. APC-sr was significantly increased by the contamination of amniotic fluid in a volume-dependent manner, indicating that increasing volumes of amniotic fluid resulted in further reductions in maternal sensitivity to APC.

W-084

**Longitudinal Metabolic Profiling in Pregnancy of Women with and without Pregestational Diabetes Who Develop Preeclampsia.** Kathryn J Gray, 1 Mengxi Yang†, 2 Liming Liang, 2 Richa Saxena\*, 3 *1Brigham and Women's Hospital, Boston, MA, United States; 2Harvard University, Boston, MA, United States; 3Massachusetts General Hospital, Boston, MA, United States.*

**Introduction:** Women with pregestational diabetes (DM) have an increased risk of pregnancy complications, including a 3-4-fold increased risk of preeclampsia (PE). In prior work, we found a signature of insulin resistance in the 2nd trimester in non-diabetic women who developed subsequent PE compared to controls. Given this, we hypothesized that shared metabolic alterations underlie the increased risk of PE in women with DM.

**Methods:** Using longitudinal global metabolic profiling data of the 1st and 2nd trimester maternal plasma and urine (T1, T2; mean 12, 26 weeks) previously generated on early-onset PE (EO-PE) cases (N = 68) with the Metabolon DiscoveryHD4™ platform, we performed linear regression with adjustment for clinical covariates (i.e., age, race, BMI) to compare

the metabolic profiles of PE cases with DM (N = 11) to those without (N=51). Six women who developed gestational diabetes were excluded from the analysis.

**Results:** In the plasma T2 samples, women with DM who later developed PE had elevated levels of branched-chain amino acids (BCAA), including derivatives of isoleucine, leucine, and valine, as well as elevated glutamate, methionine and long-chain fatty acids compared to non-diabetics who later developed PE (Table). Interestingly, these metabolic differences between cases with and without diabetes were not present in the T1 plasma and there were no significant differences in the urine at either T1 or T2.

**Conclusion:** These results reveal a unique metabolic signature in women with DM versus non-diabetics who later develop PE. As elevated BCAA and long-chain fatty acids are hallmark metabolic changes in non-pregnant diabetics associated with insulin resistance, future work aims to investigate the contributions of these metabolites to underlying PE pathophysiology.

### W-085

**TOLAC Morbidity as a Function of VBAC Predicted Success and Labor Onset.** Hayley Pierce†, Frank B. Williams†, Michael DeSarno\*, Carole McBride\*, Kelley McLean\*. *University of Vermont Medical Center, Burlington, VT, United States.*

**Introduction:** The Maternal Fetal Medicine Units Vaginal Birth After Cesarean (VBAC) Admission Calculator is a validated tool for counseling patients considering trial of labor after cesarean (TOLAC), which incorporates late pregnancy characteristics that build upon the Antepartum VBAC calculator. We aim to evaluate how Admission VBAC (AVBAC) scores relate to risk of maternal morbidity (MM) and/or neonatal morbidity (NM) among women electing a TOLAC on admission to our labor and delivery unit.

**Methods:** This is a retrospective cohort study of 368 patients who attempted TOLAC between January 2016 and June 2019. Singleton, cephalic deliveries with no contraindication to TOLAC were included. Morbidity was defined using previously reported composites. AVBAC scores were calculated through chart review. MM and/or NM was assessed in the overall group, and based on spontaneous (n=227) or induced (n=141) labor onset. Univariate and multivariate logistic regression analyses including AVBAC score and maternal characteristics associated with morbidity were generated for all patients attempting TOLAC. A receiver operate characteristic (ROC) curve was generated to determine the cutoff VBAC threshold for prediction of maternal, neonatal and combined morbidity.

**Results:** Mean predicted VBAC success of all patients attempting TOLAC was 66%, while observed rate of vaginal delivery was 70%. Lower mean AVBAC scores for patients undergoing induction compared to spontaneous labor (60% vs 81%, p<0.0001) were attributable to higher mean BMI, rates of hypertensive disease and diabetes, and induction status. No differences were observed in maternal age, gravidity, parity, EGA at delivery, race, history of vaginal delivery or prior VBAC. Induction was associated with higher rate of repeat cesarean compared to spontaneous labor (40% vs 25%, p=0.003). Repeat cesarean deliveries were due to intrapartum complications (n=58; 52%) and arrest disorders (n=53; 48%). MM was observed in 6.8%, NM in 8.3% and combined morbidity in 11.8% of all TOLAC attempts. Induced were more likely than spontaneous labors to have both MM (10%±0.3 vs 4.6%±0.2; p=.05), NM (12%±0.3 vs 5.9%±0.2; p=.04) and combined morbidity (18%±0.4 vs 9.7%±0.3; p=.02). In univariate and multivariate analyses, AVBAC score was predictive of both MM and NM in all patients who underwent TOLAC. The ROC curve for total MM by AVBAC score showed an AUC of 0.71 with a cutoff score of 65% (63% sensitive, 72% specific). The ROC curve for total NM by AVBAC score showed an AUC of 0.64 with a cutoff score of 46% (32% sensitive, 91% specific).

**Conclusion:** AVBAC score was predictive of both MM and NM. Low AVBAC scores were correlated with increased risk of adverse maternal and neonatal outcomes. We are better able to predict MM than NM. These findings can inform conversations between providers and women considering TOLAC at the time of admission, especially those considering induction.

### W-086

**Characteristics of Preventable Severe Maternal Morbidity.** Ashley Shea†, Michelle P Debbink, Susan Nourse†, Alexandra Kroes†, Sophie Janes†, Cara Heuser, Michael W Varner, Torri D Metz\*. <sup>1,2</sup> *University of Utah Health, Salt Lake City, UT, United States;* <sup>3</sup> *Intermountain Healthcare, Salt Lake City, UT, United States.*

**Introduction:** Many severe maternal morbidity (SMM) events are believed to be preventable through changes in patient, provider, and system factors. We aimed to assess the characteristics of preventable SMM (P-SMM) with a validated algorithm.

**Methods:** Retrospective cohort study of SMM events from 10/2015-9/2018 at a single tertiary care institution. SMM events were identified via CDC's ICD-10 algorithms and confirmed by medical record abstraction. Two independent reviewers used a published, validated algorithm by Koch et al to assess preventability. The algorithm identifies 18 non-mutually exclusive categories that could contribute to P-SMM. A descriptive analysis was performed using bivariate statistics and unadjusted logistic regression.

**Results:** 357 SMM events were identified out of 12,383 births (288.3/10,000); 88 (24.6%) were deemed preventable. The majority (n = 55, 62.5%) had ≥ 2 Koch preventability categories identified. Preventability differed by insurance, mode of delivery, gestational age, and timing of SMM (Table 1). In unadjusted logistic regression, P-SMM was associated with public compared to private insurance (OR 2.17, 95% 1.26 - 3.74) and preterm delivery <28 weeks compared to term delivery (OR 2.65, 95% CI 1.10 - 6.37). Cesarean birth was less likely to be associated with P-SMM (OR 0.58 95% CI 0.36 - 0.94). P-SMM was associated with postpartum readmissions compared to early postpartum events (referent) (OR 2.21, 95%CI 1.03 - 4.75). Among P-SMM, provider treatment choices, maternal stressors, and personal barriers were the most commonly identified contributors (Table 2).

**Conclusion:** An algorithm-driven review of P-SMM revealed differences in baseline characteristics between those with and without preventable events. This may help target intervention strategies to those at highest risk. More than 30% of P-SMM was associated with provider treatment choices. However, the majority of P-SMM had >1 category identified as an opportunity for improvement, suggesting that multifactorial approaches are needed.

**Table 1. Demographics of Severe Maternal Morbidity by Preventability<sup>1</sup>**

Demographic Factors	Preventable SMM (n=88)	Not-preventable SMM (n=261)	p <sup>2</sup>
Maternal Age	30.8 (25.5, 35.1)	31.7 (26.8, 350)	0.77
Maternal Race/Ethnicity			0.05
Non-Hispanic White	35 (40.7)	137 (53.1)	
Non-Hispanic Black	4 (4.7)	5 (1.9)	
Hispanic/Latina	36 (41.9)	69 (26.7)	
Asian	3 (3.5)	13 (5.0)	
Native Hawaiian/Other Pacific Islander	5 (5.8)	14 (5.4)	
Native American/Alaska Native	1 (1.2)	3 (1.2)	
Other or multiple, non-Hispanic	2 (2.3)	17 (6.6)	
Insurance status			0.02
Private/Commercial	41 (46.6)	157 (60.2)	
Medicaid/Other government	34 (38.6)	60 (23.0)	
Self-Pay/Emergency Medicaid	13 (14.8)	44 (16.9)	
Medical Comorbidities			
Body Mass Index	31.4 (30.0, 36.8)	31.0 (27.2, 36.1)	0.63
Pre-existing diabetes mellitus	24 (27.3)	41 (15.7)	0.02
Pre-existing hypertension	23 (26.1)	50 (19.2)	0.16
Obstetric Factors			
Gestational Age at Delivery (completed weeks)	38 (34, 40)	37 (34, 39)	0.32
PTB <37 weeks	31 (35.2)	111 (42.5)	0.22
PTB <34 weeks	19 (21.6)	49 (18.8)	0.56
PTB <28 weeks	10 (11.4)	12 (4.6)	0.03
Nulliparity	27 (30.7)	86 (33.0)	0.74
Multifetal gestation	7 (8.0)	19 (7.3)	0.86
Cesarean delivery	45 (51.1)	168 (64.4)	0.03
SMM Characteristics			
SMM by CDC criteria, excluding blood transfusion alone	54 (61.4)	34 (13.0)	0.02
Hospital-defined SMM: ICU or ≥4 unit blood transfusion	25 (28.4)	55 (21.1)	0.16
SMM Timing			<0.01
Antepartum <sup>3</sup>	20 (22.7)	50 (19.2)	
Intrapartum	6 (6.8)	41 (15.7)	
Early postpartum <sup>4</sup>	17 (19.3)	86 (33.0)	
Late postpartum <sup>5</sup>	22 (25.0)	57 (21.8)	
Postpartum readmission	23 (26.1)	26 (10.0)	

1) Data presented as n (%) or median (interquartile range).

2) Kruskal-Wallis for medians, Chi-square for categorical variables as indicated

3) Antepartum includes events that occur during admissions separate from and prior to the delivery admission, as well as events prior to labor during a delivery admission

4) Early postpartum is defined as up to 8 hours after delivery

5) Late postpartum is defined as more than 8 hours after delivery and prior to hospital discharge after delivery

Abbreviations: SMM – severe maternal morbidity; PTB – preterm birth; ICU – intensive care unit;

**Table 2. Koch Categories of Preventability<sup>1,2</sup>**

Category	Suboptimal or delayed care contributing to preventability
Barriers to healthcare entry	9 (10.2)
Referral difficulties	8 (9.1)
Inappropriate provider treatment choices	30 (34.1)
Mismanagement of medical hierarchy	3 (3.4)
Communication barrier	6 (6.8)
Hospital policy barriers	1 (1.1)
Documentation	9 (10.2)
Equipment unavailability or failure	2 (2.3)
Hospital barriers	7 (8.0)
Discharge barriers <sup>3</sup>	2 (2.3)
Patient transportation barriers	4 (4.5)
Unaddressed or mismanaged comorbidities	10 (11.4)
Unaddressed or mismanaged obstetric complications	11 (12.5)
Unaddressed previous obstetrical history	1 (1.1)
Other medical problems	3 (3.4)
Psychiatric or behavioral health complications	10 (11.4)
Significant maternal life stressors	12 (13.6)
Personal barrier <sup>4</sup>	13 (14.8)

- 1) Categories are not mutually exclusive
- 2) Data present as n (%) where the denominator is the 88 preventable SMM events
- 3) Inappropriate timing of follow up or patient discharged too early
- 4) Refusal of care or religious barriers

**W-087**

**Delivery Outside of a Transplant Center Is Associated with Emergent Cesarean and Increased Maternal and Neonatal Morbidity in Kidney and Liver Transplant Recipients.** *Ophelia Yin*<sup>†</sup>,<sup>1</sup> Kathleen Chung<sup>†</sup>,<sup>1</sup> Aneesh Kallapur<sup>†</sup>,<sup>1</sup> Lisa Coscia\*,<sup>2</sup> Serban Constantinescu\*,<sup>3</sup> Michael Moritz\*,<sup>4</sup> Yalda Afshar\*.<sup>1</sup> <sup>1</sup>University of California, Los Angeles, Los Angeles, CA, United States; <sup>2</sup>Gift of Life Institute, Philadelphia, PA, United States; <sup>3</sup>Temple University, Philadelphia, PA, United States; <sup>4</sup>Lehigh Valley Health Network, Morsani College of Medicine, Allentown, PA, United States.

**Introduction:** Pregnancies after liver and kidney transplantation are at a higher risk for antepartum admission and complications necessitating delivery. The objective of this study is to characterize the risk factors and outcomes of emergency prelabor cesarean delivery in kidney and liver transplant recipients.

**Methods:** Retrospective cohort study of all liver and kidney transplant recipients ≥20 weeks enrolled in Transplant Pregnancy Registry International. Women admitted antepartum who required an emergency cesarean were compared to those admitted antepartum who underwent non-emergent delivery. Multivariate logistic regression was conducted for neonatal composite morbidity, defined by NICHD criteria, and neonatal mortality.

**Results:** 1,979 births with known mode of delivery ≥20 weeks' gestation between 1976 and 2019 were screened. 181 pregnancies (188 neonates) with an antepartum admission were included. 53 neonates were delivered by emergent prelabor cesarean delivery (28%, 2 sets of twins). These were compared with the other 135 neonates (72%, 5 sets of twins) of mothers admitted to an antepartum service who subsequently did not require emergent delivery. Regarding risk factors, patients who had an emergent prelabor cesarean had increased rates of chronic hypertension (33% vs 17%, p=0.02) and Asian race (11.8% vs. 1.5%, p=0.03). Those who required an emergency cesarean were less likely to be delivered at a transplant center (37.3% vs 41.5%, p=0.04). For outcomes, these patients had increased rates of maternal surgical site infection (3.9% vs 0%, p=0.02), NICU admission (53% vs 36%, p=0.03), neonatal composite morbidity (43% vs 19%, p<0.001), and neonatal mortality (9.4% vs 0.7%, p=0.002). Mean gestational age for emergent prelabor cesarean deliveries was 33.4 vs 34.7 weeks for nonemergent deliveries (p = 0.02). After adjusting for year of conception, race, and hypertensive disorders, there was a persistent increased risk of neonatal morbidity (aOR 3.31, [1.53, 7.26], p = 0.002) with emergent prelabor cesarean delivery after transplantation.

**Conclusion:** Liver and kidney transplant recipients are at an increased risk for emergent prelabor cesarean delivery when they deliver outside of a transplant center. Pregnancies after transplantation should involve

multi-disciplinary transplant-obstetrics collaboration to attenuate the risk of severe maternal and neonatal morbidity and mortality in the setting of emergent prelabor cesarean delivery.

**W-088**

**Platelet Count on Admission to Labor and Delivery and Hemorrhage Risk.** *Megan Trostle*<sup>†</sup>, Iffath Hoskins, Ashley S Roman\*. *NYU Langone Health, New York, NY, United States.*

**Introduction:** Thrombocytopenia in pregnancy is defined as a platelet count less than 150,000/mL. Severe thrombocytopenia with platelet count <50,000/mL is a risk factor for procedural bleeding. However, the platelet count at which bleeding risk increases is unknown, especially relating to risk for postpartum hemorrhage (PPH). We hypothesized that women with any thrombocytopenia are at higher risk for PPH.

**Methods:** This was a retrospective cohort analysis of women ages 14-55 delivering at a single center from 7/2013 - 10/2018. Women at ≥ 28 weeks gestation with documented platelet count on admission to labor and delivery and quantitative blood loss (QBL) were included. The primary outcome was PPH, defined as QBL >1000cc. Secondary outcomes were median QBL, blood transfusion, administration of uterotonics (methylergonovine, carboprost, misoprostol, or tranexamic acid), and hemorrhagic shock. Subgroup analyses were then performed by platelet count in increments of 50,000/mL. Continuous variables were compared with Mann-Whitney or Kruskal-Wallis tests and categorical variables with chi-square or Fisher's exact tests with p<0.05 as significant.

**Results:** 29,348 women were included, 4402 of whom had thrombocytopenia. Women with thrombocytopenia were more likely to have PPH. They also had a higher median QBL and were more likely to require transfusion or uterotonics (Table 1). There was no difference in shock. When women with thrombocytopenia were stratified by platelet count in increments of 50,000/mL, women with platelet counts <50,000/mL were at the highest risk for PPH. As platelet count increased, risk for PPH, median QBL, and rates of transfusion and uterotonic use decreased, demonstrating a negative linear relationship (Table 2). Women with mild thrombocytopenia, platelet count from 100,000-149,000/mL, remained at higher risk for PPH than women with normal platelets (OR 1.57, 95% CI 1.42-1.74, p<0.001).

**Conclusion:** In our cohort, thrombocytopenia of any degree was associated with PPH. Women with the lowest platelet counts had the highest risk for PPH but even women with mild thrombocytopenia remained at increased risk compared to women with normal platelet counts.

	Platelets <150,000/ mL n=4402 n (%)	Platelets ≥ 150,000/mL n=24,946 n (%)	p-value
QBL >1000cc	593 (13.5)	2163 (8.7)	<0.001
QBL (cc) Median(IQR)	362 (500)	300 (400)	<0.001
Transfusion	106 (2.4)	234 (0.9)	<0.001
Uterotonic	1666 (37.8)	7346 (29.4)	<0.001
Shock	6 (0.1)	18 (0.1)	0.17

Table 2: PPH complications by platelet increments of 50,000/mL

	<50,000/ mL n=15 n (%)	50,000- 99,000/ mL n=365 n (%)	100,000- 149,000/ mL n=4021 n (%)	≥150,000/ mL n=24,946 n (%)	p-value
QBL >1000cc	6 (40)	66 (18.1)	521 (13)	2163 (8.7)	<0.001
QBL (cc) Median (IQR)	700 (800)	425 (600)	350 (500)	300 (400)	<0.001
Transfusion	3 (20)	17 (4.7)	86 (2.1)	234 (0.9)	<0.001
Uterotonic	10 (66.7)	165 (45.2)	1491 (37.1)	7346 (29.4)	<0.001
Shock	0 (0)	4 (1.1)	2 (0.04)	18 (0.1)	<0.001

**W-089**

**Perinatal Outcomes in Once versus Twice Weekly Antenatal Surveillance in A2 Gestational Diabetes Mellitus.** *Devon O'Brien†, Danielle Calvo†, Patrick Hilden\*, Jonathan O'Brien\*, Richard Miller\*, Kathy Matthews\*. Saint Barnabas Medical Center, Livingston, NJ, United States.*

**Introduction:** Although the recommendation for antenatal fetal testing (AFT) in pregnancies complicated by A2 gestational diabetes mellitus (A2GDM) begins at 32 weeks' gestation, there are no large clinical trials to evaluate optimal testing frequency. Our objective was to determine if there were differences in perinatal outcomes in pregnancies affected by A2GDM with once versus twice weekly AFT.

**Methods:** Retrospective cohort study of women with A2GDM who underwent antenatal surveillance at our institution between 9/1/2019-8/31/2020. During the peak of the COVID19 pandemic, AFT for women with A2GDM was reduced from twice weekly to once weekly, starting on 3/23/2020. Patients who received both testing frequencies were excluded. AFT consisted of a modified biophysical profile. We compared the incidence of pregnancy-induced hypertension (gestational hypertension, preeclampsia with or without severe features, or HELLP syndrome), primary cesarean delivery rate, macrosomia (neonatal weight >4000 grams), neonatal hypoglycemia (glucose ≤ 35 mg/dL in the first 48 hours of life), and NICU admission between women who had once weekly versus twice weekly AFT. Wilcoxon rank-sum test, chi-squared, and Fisher's exact test were used for statistical comparison with p<0.05 considered statistically significant. Continuous data are expressed as median [interquartile range].

**Results:** 156 women had one AFT frequency for A2GDM during the specified time periods; 62 underwent once weekly testing and 94 had twice weekly testing. There were no significant differences in baseline demographics between groups (Table 1). There were no significant differences in perinatal outcomes between women who had once versus twice weekly AFT (Table 2).

**Conclusion:** The frequency of AFT in pregnancies complicated by A2GDM was not related to differences in perinatal outcomes. There may be an opportunity to individualize antepartum surveillance in this population with consideration of other risk factors. This could have particular relevance during a pandemic when weighing risks and benefits of frequent outpatient visits.

Table 1. Maternal baseline demographics

Demographic	Frequency of Antenatal Surveillance		P-Value
	Once weekly (n=62)	Twice weekly (n=94)	
Maternal Age, years	35 (23, 47)	35 (24, 45)	0.893
Race	White	29 (46.8%)	44 (46.8%)
	Black	11 (17.7%)	18 (19.1%)
	Other	22 (35.5%)	32 (34%)
Nulliparity	25 (40.3%)	31 (33.0%)	0.444
BMI, kg/m <sup>2</sup>	34.1 (24.6, 52.3)	33.6 (21.7, 56.1)	0.361
Insurance Payor	Private	56 (90.3%)	84 (89.4%)
	Medicaid	6 (9.7%)	10 (10.6%)
Chronic Hypertension	9 (14.5%)	12 (12.8%)	0.941

Table 2. Perinatal outcomes in women with A2GDM, as stratified by frequency of antenatal surveillance

Perinatal Outcome	Frequency of Antenatal Surveillance		P-Value
	Once weekly (n= 62)	Twice weekly (n= 94)	
Pregnancy-induced hypertension	5 (8.1%)	14 (14.9%)	0.305
Primary cesarean delivery*	12/47 (25.5%)	18/65 (27.7%)	0.693
Macrosomia	4 (6.3%)	4 (4.3%)	0.714
Neonatal hypoglycemia	13 (21%)	19 (20.2%)	0.99
NICU admission	15 (24.2%)	25 (26.6%)	0.882

\*Repeat cesarean deliveries excluded.

**W-090**

**Uterine Conservation with Placenta Accreta Spectrum.** *Nicola C Perlman†, Michaela Farber, Jean Marie Carabuena, Daniela A Carusi\*. Brigham and Women's Hospital, Boston, MA, United States.*

**Introduction:** Though placenta accreta spectrum (PAS) is traditionally managed with hysterectomy, uterine conservation has been described. Placental retention is generally advocated to reduce the risk of immediate hemorrhage, though this places the patient at risk of delayed hemorrhage and infection. Here we describe a cohort of patients with planned uterine conservation utilizing intraoperative uterine artery embolization (UAE) with placental removal at time of delivery.

**Methods:** In this descriptive retrospective cohort study, patients with PAS suspected on antepartum imaging between 2004 and 2019 were identified. Those with intended conservative uterine management with intraoperative UAE were reviewed. Patient and delivery characteristics, intraoperative findings, and morbidities were abstracted from electronic charts. Categorical variables are given as rates while continuous variables are reported as median (range). Fisher exact and Wilcoxon tests were used to compare successful to unsuccessful conservation attempts.

**Results:** We identified 23 patients who met eligibility criteria between 2004 and 2019 at a single, tertiary hospital. Patient characteristics are given in Table 1. Of the 61% who had a previous cesarean delivery (CD) the majority had only one prior. Only two had concern for placental invasion (increta), and 83% had a previa at the time of delivery. Morbidly adherent placenta was confirmed for 78% at delivery, and outcomes for these patients are shown in Table 2. 89% of the patients had the placenta completely removed and 83% had successful uterine conservation. These patients had lower estimated blood loss than those with hysterectomy (p=0.04), and had fewer units of blood cells transfused (p=0.03). Of the three unsuccessful conservation cases, one had increta and one had percreta on final pathology. There were no postpartum readmissions in the cohort, and only two postpartum complications - one wound breakdown and one smooth muscle infarct on postpartum hysteroscopic biopsy.

**Conclusion:** Of our 18 confirmed cases of PAS at CD, 83% underwent successful uterine conservation with planned intraoperative UAE. While none had a delayed hemorrhage, infection, or readmission, the majority of patients had a significant postpartum hemorrhage. Patients should be selected for less invasive pathology to the degree possible, and should be delivered at a specialized center for PAS care.

**W-091**

**A Systematic Review of Prior Termination of Pregnancy as a Risk Factor for Cervical Health in Pregnant Women.** *Julia J Brittain†,1 Stacey E Wahl,2 John W Cyrus,2 Hope M Wolf,2 Jerome F Strauss III,2 Timothy P York\*.2,1 University of Richmond, Richmond, VA, United States; 2Virginia Commonwealth University, Richmond, VA, United States.*

**Introduction:** Current research reports a dose-response relationship between the number of prior terminations of pregnancy (TOP) and increasing preterm birth risk. While the mechanism for this relationship is unknown, uterine evacuation whereby the cervix is mechanically or osmotically dilated has been hypothesized to mediate risk for preterm birth due to the potential for cervical damage. Considering the known relationship between prior cervical surgery and an increased risk for preterm birth, an examination of procedures that result in cervical trauma can shed light on mechanistic reasons why cervical integrity in the mid-trimester is a strong predictor of preterm birth. The aim of this systematic review was to evaluate studies that examine the relationship between prior TOP and spontaneous abortion (SAB) on measures of cervical health in subsequent pregnancies.

**Methods:** This systematic review was conducted in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Research published over the last 60 years was assessed to capture the span of time whereby both surgical and medical TOP procedures were widely adopted. Studies that were included investigated the relationship between cervical health as the primary outcome and previous TOP/SAB status as the exposure. Research in which cervical health is a secondary focus was included if prior history of TOP/SAB was also reported. A systematic search for peer-reviewed literature was conducted in Medline (Ovid) and Embase (Ovid) in September, 2020 using a combination of keywords and controlled vocabulary for the concepts of TOP/SAB and cervical health. Risk of bias assessment was conducted using the Newcastle-Ottawa Scale.

**Results:** The search yielded 1,598 studies related to cervical health and TOP/SAB. After title-abstract and full text screening, four articles reporting 2,478,809 births from the United States, Netherlands and United Kingdom were included in this review. Three of the four studies report a significant relationship between at least one prior TOP on risk to cervical health at odds ratios of 2.80 (2.53-3.12), 2.99 (1.40-6.40) and 4.60 (3.33-6.36). No study reported association results separately for surgical versus medical TOP methods. A dose-response relationship between the number of previous TOPs and increasing risk for cervical insufficiency is found in two of these studies.

**Conclusion:** Prior TOP procedures were found to be consistently associated with risk of cervical insufficiency in subsequent pregnancies. The temporal ordering of the TOP and subsequent cervical health observations along with the identified dose-response relationships observed warrants further investigations on the mechanistic process whereby TOP can potentially cause cervical trauma and damage.

#### W-092

**Discrepancies between Ultrasound Based Composite Biometry for Estimated Fetal Weight and Abdominal Circumference Alone in Predicting Large for Gestational Age Fetuses and Newborns.** Lina Fouad, Bernard Gonik\*. *Wayne State University, Detroit, MI, United States.*

**Introduction:** In addition to estimated fetal weight (EFW) by ultrasound based composite biometry, abdominal circumference (AC) alone may be an important predictor of large for gestational age (LGA) newborns. We assessed if a fetus with an AC > 95th %tile in the setting of an EFW ≤ 90th %tile develops an EFW > 90th %tile on follow-up ultrasound imaging and are LGA at birth.

**Methods:** We identified singleton, non-anomalous fetuses at ≥ 24 weeks gestational age (GA) with an AC > 95th %tile and EFW ≤ 90th %tile over 5 years. Subsequent fetal growth scans were reviewed and categorized as 1) persistence of a dichotomy between AC (> 95th %tile) and EFW (≤ 90th %tile); 2) an EFW > 90th %tile; or 3) appropriate growth (AGA) (EFW ≤ 90th %tile and AC ≤ 95th %tile). Delivery records were extracted; birth weights (BW) were classified as LGA if they were > 90th %tile for GA.

**Results:** 280 subjects were identified as having dichotomous biometry at a mean (SD) GA of 34.3 (2.6) weeks. Of these, 61 had at least one subsequent scan ≥ 3 weeks later with 46% AGA, 18% LGA, and 36% remaining dichotomous. Of those who had more than one subsequent scan (n=9), 67% switched to another category. Based on last scan before delivery there were 43% AGA, 18% LGA, and 39% dichotomous fetuses. At birth, of these fetuses, 9%, 82%, and 10% were LGA newborns, respectively.

**Conclusion:** Approximately 1 in 5 fetuses with dichotomous biometry will have an LGA fetus on subsequent scanning. This supports the use of follow up scanning for suspected impending LGA. However, these ultrasound findings may again become discrepant later in pregnancy. Based on last scan before birth, ultrasound based LGA is a relatively accurate predictor of birth weight, with few cases in the other categories resulting in an LGA newborn.

#### W-093

**Persistence of Maternal Group B Streptococcal Colonization after Intravenous Ampicillin Administration.** Melissa L Kozakiewicz†, Sarah E White,<sup>1</sup> Mallory Alkis,<sup>2</sup> Rita Kaplon,<sup>1</sup> Brian C Brost.<sup>1</sup> <sup>1</sup>*Wake Forest University School of Medicine, Winston Salem, NC, United States;* <sup>2</sup>*Medical University of South Carolina, Charleston, SC, United States.*

**Introduction:** Group B streptococcal (GBS) disease is a leading infectious cause of neonatal morbidity and mortality. Intrapartum antibiotic administration is highly effective in preventing neonatal GBS infection. Bactericidal levels of ampicillin are detected in amniotic fluid and fetal blood within minutes of administration. The effect of ampicillin administration on maternal rectovaginal culture status is yet undetermined. Patients in preterm labor commonly receive antibiotics prior to transfer to tertiary care centers thus confounding results of subsequent GBS cultures. The primary objective of this study is to determine the time interval for continued detection of maternal GBS utilizing the standard rectovaginal culture after ampicillin administration.

**Methods:** Patients with a positive prenatal maternal GBS culture done within the prior 5 weeks and planned administration of ampicillin were approached for enrollment upon presentation to Labor and Delivery. Enrolled women had rectovaginal GBS cultures collected within one hour prior to the first dose of ampicillin followed by another rectovaginal culture collected at one, four and six hours after ampicillin initiation. Patient demographics, delivery information and culture results were obtained.

**Results:** 14 women were enrolled; 10 were maternal GBS positive by initial culture on Labor and Delivery. Of these, 60 percent (n=6) were culture positive at one hour, 60 percent (n=6) were culture positive at four hours and 60 percent (n=6) were culture positive at six hours after initiation of ampicillin. There were no significant differences in patient demographics.

**Conclusion:** GBS culture results within one and six hours after initiation of ampicillin may not accurately reflect initial maternal colonization status prior to antibiotic administration. Positive results should be considered true positives but negative results may be falsely negative. These data point toward treatment of fetal exposure over eradication of maternal colonization as a possible mechanism of action.

#### W-094

**COVID-19 Ethnic and Racial Disparity in the State of Maryland. A Report from the Maryland Study Group.** Liviu Cojocaru†, Irina Burd\*,<sup>2</sup> Ramya Reddy†,<sup>2</sup> Seung Hyunuk†,<sup>1</sup> Katelyn Uribe†,<sup>1</sup> Katherine Raja†,<sup>1</sup> Autusa Pahlavan†,<sup>1</sup> Sifa Turan\*. <sup>1</sup>*University of Maryland School of Medicine, Baltimore, MD, United States;* <sup>2</sup>*Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** COVID-19 had scrutinized the ethnic and racial (ER) health disparities (HD). We aimed to characterize COVID-19 infection and hospitalization rates in pregnant women in the State of Maryland. The demographic distribution in the State of Maryland is the following: white (W) (50%), black/ African-American (B/AA) (30%), Hispanic (H) (11%), and other races (O) (9%).

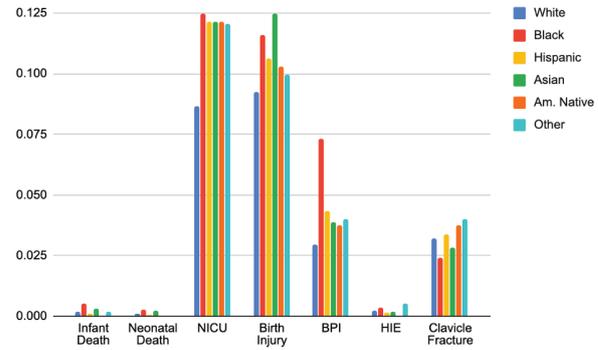
**Methods:** Maryland Study Group is a collaboration of major medical systems (University of Maryland Medical System and Johns Hopkins Health System) in the State of Maryland. We reviewed all patients with COVID-19 from March 2020 until January 2021 recorded in our registry. We collected demographic data, medical history (MH), and hospitalization rate. MH included comorbidities such as asthma, chronic hypertension, cardiac disease, chronic kidney disease, and hypertensive disorders of pregnancy (HDP). For bivariate analysis, we performed an analysis of variance. The  $\chi^2$  or Fisher's exact test was used to evaluate the associations. SAS version 9.4 was used for statistical analysis.

**Results:** We analyzed 347 cases. The mean age was 29.1 years. More than half (54%) of the patients had obesity and the same percentage had at least one comorbidity. The most predominant comorbidities were HDP (18%) and asthma (13%). The ER distribution was 39% H, 35% B/AA, 21% W and 5% (O). The hospitalization rate was the lowest in W (9.5%) as opposed to B/AA (20%), H (19%), and O (17.5%). The ER differences between age and obesity were not significant between the groups (0.42 and

0.09 respectively) (Table 1). The H patients were 3.5 times more likely to have no MH (OR=3.51, 95% CI =1.93- 6.39). The B/AA were 70% less likely to have no MH compared to the H (OR=0.3, 95% CI = 0.18, 0.51). **Conclusion:** After adjusting for age, obesity, and comorbidities, the Hispanic and Black/ African-American population were affected the most. Furthermore, the rate of hospitalization was double in H and B/AA compared to W. While the hospitalization rates in H and B/AA are comparable, the H had the lowest rate of comorbidities. Although we adjusted for common risk factors for COVID-19, other variables that could explain disparity warrant further research. For instance, recent data linked the angiotensin-converting enzyme deletion polymorphism with the susceptibility to COVID-19. Health care organizations should allocate more resources to investigate and eliminate ER HD.

Bivariate analysis						
		Age				
	Hispanic (n=134)	Black/ African-American (n=122)	White (n=74)	Asian (n=11)	Other (n=6)	p-value
Age	28.8(7.2)	28.8(6.3)	30.3(5.4)	27.2(4.4)	28.6(7.6)	0.42
	(n=35)	(n=34)	(n=7)	(n=2)	(n=1)	
Age (H)*	30.5(7.9)	30.1(6.7)	31.3(6.1)	25.4(8.1)	24.7	0.82
Obesity						
		Amongst all		Amongst hospitalized		
	BMI	BMI	p-value	BMI	p-value	
	Non-obese (n=156)	Obese (n=183)	0.09	Non-obese (n=22)	Obese (n=36)	0.22
Race						
Hispanic	5(635.9)	75(410)		9(40.9)	16(44.4)	
Black/ African-American	5(112.7)	67(36.6)		7(31.8)	16(44.4)	
White	3(623.3)	27(22.2)		2(12.6)	4(11.3)	
Asian	0	2(1.1)		2(9.1)	0	
Other	4(2.6)	2(1.1)		1(4.6)	0	
Comorbidities						
	Hispanic (n=134)	Black/ African-American (n=122)	White (n=74)	Asian (n=11)	Other (n=6)	p-value
No comorbidities	8(61.4)	4(315.3)	25(133.4)	5(45.5)	3(50)	<0.0001
Asthma	6(4.5)	27(22.3)	11(114.9)	1(9.1)	0	0.0008
CHTR	10(7.5)	16(13.1)	7(79.5)	1(9.1)	0	0.59
Cardiac disease	2(1.5)	5(4.1)	4(41.4)	0	1(16.7)	0.17
CKD	0	3(2.5)	1(1.1)	1(9.1)	0	0.08
DM	4(3.0)	6(4.9)	2(2.7)	0	1(16.7)	0.4
HDP	29(21.6)	23(18.9)	10(113.5)	0	0	0.3

Age (H)\*: age amongst hospitalized. BMI: body mass index; CHTR: chronic hypertension; CKD: chronic kidney disease; DM: diabetes mellitus; HDP: hypertensive disorders of pregnancy.



W-096

**Normalization of Circulating Adiponectin Levels in Obese Pregnant Mice Prevents Left Ventricle Mitochondrial Respiratory Dysfunction in Adult Offspring.** Jerad H Dumol†\*, Owen R Vaughan, Kathryn Erickson, Theresa L Powell, Thomas Jansson. *University of Colorado Anschutz Medical Campus, Aurora, CO, United States.*

**Introduction:** Maternal circulating levels of adiponectin (ADN) are lower in pregnancies of obese mothers and are associated with increased placental nutrient transport and fetal overgrowth. We have previously shown that normalizing maternal ADN in late gestation in obese mice reverses placental dysfunction and fetal overgrowth and prevents cardiac dysfunction in adult offspring. We hypothesized that maternal obesity decreases cardiac mitochondrial respiratory function in adult offspring and that normalization of circulating adiponectin levels in obese pregnant mice prevents these changes.

**Methods:** C57BL/6J female mice were fed either a control (CON) or obesogenic diet (OB). Pregnant females received a continuous infusion of sterile PBS or mouse recombinant full-length ADN from E14.5 until delivery resulting in the following groups CON-PBS, OB-PBS, and OB-ADN. At weaning, male and female offspring (n= 8-13/group) were fed a control diet until 7-9 months of age. Functional studies of high-resolution respiration on left ventricle samples used a lipid substrate-uncoupler-inhibitor titration (SUIT) protocol with the Oroboros Oxygraph-2K. Respiratory rates were measured following the stepwise addition of palmitoylcarnitine and malate (ADP independent), ADP (oxidative phosphorylation capacity), glutamate and succinate (Complex-I, II linked), oligomycin (leak state respiration following ATP synthase inhibition (L(Omy)), and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) until maximal uncoupling (electron transport system capacity). One-way ANOVA followed by Tukey’s multiple comparison post hoc tests was used to analyze results.

**Results:** ADP independent and oxidative phosphorylation capacity were similar in all groups. Offspring from OB-PBS dams had reduced Complex-I, II linked respiration (-32%, p<0.05) compared to CON-PBS and OB-ADN. Similarly, OB-PBS offspring had lower rates of L(Omy) (-30%, p<0.05) and maximal uncoupled electron transport system capacity (-27%, p<0.05) compared to CON-PBS offspring. Restoring maternal ADN in obese pregnancies prevented left ventricle mitochondrial dysfunction in adult offspring.

**Conclusion:** Exposure to maternal obesity led to decreased cardiac mitochondrial respiratory function in adult offspring and normalization of circulating ADN levels in obese pregnant mice prevented this cardiac mitochondrial dysfunction. We speculate that impaired mitochondrial respiratory function contributes to programming of cardiac dysfunction in offspring of obese mothers, and low maternal adiponectin in pregnancy may be an important programming factor.

W-095

**Influence of Maternal Race/Ethnicity on Adverse Neonatal Outcomes in the Setting of Shoulder Dystocia.** Sarina Rebecca Chaiken†, Claire H Packer†, Bharti Garg\*, Aaron B Caughey\*. *Oregon Health & Science University, Portland, OR, United States.*

**Introduction:** Here we examine the associations between maternal race and adverse neonatal outcomes in the setting of shoulder dystocia.

**Methods:** We conducted a retrospective cohort study of 1,514,924 non-anomalous, singleton pregnancies resulting in shoulder dystocia in California between 2007-2011 using linked vital statistics and discharge data. Race was categorized into White, Black, Hispanic, Asian, American Native and other. Outcomes of interest included infant death, NICU admission, birth injury, brachial plexus injury, HIE, and clavicle fracture. Chi-square tests were used to evaluate differences in shoulder dystocia complications by race and multivariable logistic regression used to control for maternal age, BMI, parity, education, number of prenatal visits, smoking status, mode of delivery, chronic hypertension and diabetes, and insurance status.

**Results:** The cohort consisted of 7,659 (30.49%) White, 1,163 (4.63%) Black, 13,004 (4.63%) Hispanic, 2,617 (10.42%) Asian, 107 (0.43%) American Native and 573 (2.28%) other race individuals. Black patients with shoulder dystocia had higher rates of infant death, NICU admission and brachial plexus injury than any other race. However in multivariate logistic regression, Hispanic (aOR 1.21, 95% CI 1.08-1.36), Asian (aOR 1.59, 95% CI 1.37-1.85) and other (aOR 1.35, 95% CI 1.02-1.77) race were associated with NICU admission. Asian race was significantly associated with birth injury (aOR 1.55, 95% CI 1.33-1.79). Black, Hispanic and Asian race/ethnicity were all associated with BPI after adjusting for confounders. Race was not significantly associated with HIE or skull fracture.

**Conclusion:** Black, Hispanic, Asian, and other races are associated with increased rates of adverse neonatal outcomes in the setting of shoulder dystocia. After adjusting for confounders, Black race was not significantly associated with most of the outcomes, indicating that confounding factors contribute to adverse outcomes in the population. This necessitates further understanding of the structural racism that exists in obstetric care.

## W-097

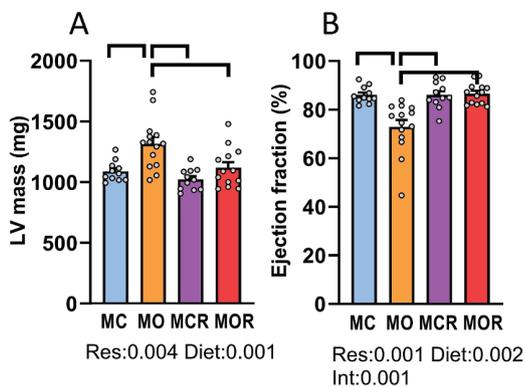
**Maternal Resveratrol Intervention Prevents Offspring Cardiac Dysfunction in a Rat Model of Obese Pregnancy.** Nozomi Itani<sup>†</sup>,<sup>1</sup> Guadalupe L Rodriguez-Gonzalez<sup>†</sup>,<sup>2</sup> Elena Zambrano,<sup>2</sup> Peter W Nathanielsz,<sup>3</sup> Paul D Taylor\*.<sup>1</sup> <sup>1</sup>King's College London, London, United Kingdom; <sup>2</sup>Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; <sup>3</sup>University of Wyoming, Laramie, WY, United States.

**Introduction:** Epidemiological and animal studies demonstrate that maternal obesity is a major risk factor for childhood obesity and related cardio-metabolic disease in adulthood (Godfrey KM, *et al. Lancet Diabetes Endocrinol.* 2017;5:53-64). Employing an established rodent model of maternal obesity, we hypothesised that maternal supplementation with resveratrol, a polyphenol with anti-inflammatory and antioxidant properties, would improve offspring cardiac structure and function.

**Methods:** Female rats (n=10/group) were fed control (C) or obesogenic (O) diet ad libitum from weaning with vehicle or resveratrol (R) treatment (20mg/kg/d) from 90d of age, through mating at 120d, to the end of lactation. Offspring litter size was standardised to 10 pups at 48 hours after birth and all pups were subsequently weaned on to normal chow. One male and one female from each litter (four groups; maternal (MC, MO, MCR and MOR) were subjected to micro-ultrasound echocardiography (VEVO770) under isoflurane anaesthesia at 20 weeks of age. Left ventricular (LV) mass, ejection fraction, and the aortic strain [(end-systolic - end-diastolic diameter)/ end-diastolic diameter (mm)] were determined. Data were calculated as mean±SEM and analysed by Two-way ANOVA with Tukey's post-hoc test.

**Results:** MO male offspring had significantly increased LV mass with reduced ejection fraction, compared to control offspring, which were both prevented by maternal R treatment (Figure 1A and B). Furthermore, MO male offspring had significantly reduced aortic strain, which was effectively normalised by maternal R (MC[n=7]:0.10±0.01; MO[n=9]:0.02±0.02\*; and MOR[n=12]:0.12±0.02. \*p<0.01). Similar, but less significant detrimental effects of maternal obesity were observed in female MO offspring.

**Conclusion:** Maternal supplementation with resveratrol throughout pregnancy and lactation prevented the developmental programming of cardiac dysfunction and aortic stiffening. This cardiac remodelling may be an indication of cardiomyopathy and risk of heart failure, established early in the life-course. Supported by the Newton Fund



**Figure 1: Male offspring cardiac function at 20 weeks of age** Mean ± S.E.M. for left ventricular mass (A) and ejection fraction (B). Two-way ANOVA with Tukey post hoc test, P<0.05

## W-098

**Long-Term Effects of a Placenta-Targeted Treatment during Hypoxic Pregnancies on Cardiac Capacity to Recover from Ischemia/Reperfusion Insult in Adult Offspring.** Nataliia Hula<sup>†</sup>,<sup>1</sup> Floor Spaans,<sup>1</sup> Jennie Vu,<sup>1</sup> Anita Quon,<sup>1</sup> Raven Kirschenman,<sup>1</sup> Christy-Lynn M. Cooke,<sup>1</sup> Tom J. Phillips,<sup>2</sup> C. Patrick Case,<sup>3</sup> Sandra T. Davidge.<sup>1</sup> <sup>1</sup>University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Cardiff University, Cardiff, United Kingdom; <sup>3</sup>University of Bristol, Bristol, United Kingdom.

**Introduction:** Fetal hypoxia is linked to fetal programming of cardiac dysfunction in the adult offspring. Notably, adult offspring born from hypoxic pregnancies are highly susceptible to cardiac ischemia/reperfusion injury (I/R). We previously showed that maternal treatment with the antioxidant MitoQ, encapsulated into placenta-targeted nanoparticles (nMitoQ), reduces placental oxidative stress and improves placental oxygenation. However, the long-term effects of prenatal nMitoQ treatment on cardiac function in the offspring are not known. We hypothesize that maternal nMitoQ treatment in hypoxic pregnancies increases cardiac tolerance to I/R in adult offspring by altering proteins involved in the regulation of calcium cycling.

**Methods:** Pregnant Sprague-Dawley rats were exposed to normoxia (21% O<sub>2</sub>) or hypoxia (11% O<sub>2</sub>) from gestational day (GD) 15 to GD21 and intravenously injected with saline or nMitoQ (100 µl of 125 µM) on GD15. Male and female offspring were aged to 4 months. Cardiac susceptibility to I/R (20 min. ischemia/40 min. reperfusion) was assessed ex vivo (isolated working heart). Proteins associated with calcium signaling (SERCA2α, PLN, pPLN, CaMK II, pCaMKII, PP2Ce) were assessed in post-I/R left ventricles by Western blotting. Data were analyzed with two-way ANOVA (n=4-6 dams/1 offspring per dam/group).

**Results:** Prenatal hypoxia decreased cardiac recovery from I/R in male (71.5±6.5 vs 102.2±4.2%, p=0.0002) and female (62.9±7 vs 82.7±4.3%, p=0.01) offspring compared to normoxia group, and nMitoQ treatment prevented this in male (96.5±3.5%, p=0.002) and female (84.8±2.3%, p=0.008) hypoxic offspring. SERCA2a levels were decreased in hypoxic females (104.6±11.2 vs 73.1±8.5%, p=0.04) only, with no effect of nMitoQ. Compared to saline, nMitoQ increased PLN levels (156.7±12.7 vs 117.3±12%, p=0.03) in male normoxic and hypoxic offspring, while increasing pPLN/PLN ratios (179.9±27.1 vs 100.7±11.6%, p=0.02) in normoxic and hypoxic females. pCaMKII/CaMK II ratios tended to be lower (97.8±5.9 vs 82.2±4.7%, p=0.051) in nMitoQ-treated normoxic and prenatally hypoxic male offspring only. nMitoQ treatment increased PP2Ce levels (79.5±5.9 vs 117.2±15.3%, p=0.02) in hypoxic males only, compared to saline controls.

**Conclusion:** Placenta-targeted treatment with nMitoQ during hypoxic pregnancies improved offspring cardiac tolerance to I/R. While resulting in a similar functional improvement of cardiac recovery from I/R, there were sex-specific differences in the effects of maternal nMitoQ treatment on the levels and phosphorylation of cardiac proteins involved in calcium cycling in offspring born from hypoxic pregnancies.

## W-099

**The Role of HPA-Axis Genetics in the Relationship between Birthweight and Adult Disease.** Carol A Wang,<sup>1,2</sup> Wriwu N Martin<sup>†</sup>,<sup>1</sup> Stephen J Lye,<sup>3</sup> Rebecca M Reynolds,<sup>4</sup> Stephen G Matthews,<sup>3,5</sup> Carly E McLaughlin,<sup>6</sup> Roger Smith,<sup>1,2</sup> Craig E Pennell\*.<sup>1,2</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>4</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>5</sup>University of Toronto, Toronto, ON, Canada; <sup>6</sup>Curtin University, Western Australia, Australia.

**Introduction:** Animal and human data suggest an important role of the HPA-axis (HPA-A) in the developmental origins of health and disease. Previous work suggests HPA-A function moderates rather than mediates the relationship between birthweight and adult cardiometabolic outcomes. Despite multivariable modelling with established risk factors, significant residual confounding still exists in these analyses. Increasing evidence suggest genetics could potentially play a role in explaining these significant residual confounding these relationships.

**Aim:** To evaluate the role of HPA-A genetics in the relationship between birthweight and adult cardiometabolic disease.

**Methods:** Detailed obstetric and neonatal biometric data were collected in the Raine Study. A total of 1137 participants aged 18 underwent the Trier Social Stress Test (TSST). Plasma and salivary measures of cortisol and ACTH were measured before and after the TSST. GWAS were performed for basal cortisol, TSST cortisol range and AUCg cortisol on the 598 participants with genome wide SNP data to develop polygenic risk scores (26, 17 and 25 SNPs respectively) for each measure. The relationship between polygenic scores, birthweight and cardiometabolic outcomes (at ages 20 and 22) were evaluated using multivariable models.

**Results:** No associations were demonstrated between the three polygenic scores and birthweight. Multiple associations were demonstrated between HPA-A polygenic scores and adult cardiometabolic outcomes (see table). Significant associations were limited to obesity, hypertension and lipid profile; none were evident with fasting glucose or fasting insulin.

**Conclusion:** Genetic variants associated with HPA-A function (TSST) were associated with adult cardiometabolic outcomes independent of birthweight. These associations may explain, in part, the residual confounding seen in analyses evaluating the role of HPA-A function in moderating the relationship between birthweight and markers of adult disease.

### W-100

**Associations of Gestational Hypertensive Disorders and Maternal Blood Pressure with Offspring Blood Pressure, and Early Markers of Atherosclerosis at the Age of 10.** Clarissa J. Wiertsema<sup>†</sup>, Vincent W.V. Jaddoe, Annemarie G.M.G.J. Mulders, Romy Gaillard. *Erasmus MC, Rotterdam, Netherlands.*

**Introduction:** Gestational hypertensive disorders and a higher maternal blood pressure across the full range during pregnancy are associated with higher offspring blood pressure. Animal studies suggest that early gestational exposure to an adverse in utero environment is related to vascular remodeling in the offspring of affected pregnancies. Therefore, we hypothesized that gestational hypertensive disorders and maternal blood pressure across the full range are not only associated with higher blood pressure, but also with early atherosclerotic changes in the offspring.

**Methods:** Among 4777 mother-offspring pairs, we examined the associations of gestational hypertensive disorders and maternal blood pressure with offspring blood pressure, carotid intima media thickness and distensibility at the age of 10. We examined the effect of mediation by gestational age and weight at birth, breastfeeding status and child adiposity. We also explored critical periods for fetal exposure to a higher maternal blood pressure, as we expected the strongest effect in early pregnancy.

**Results:** Compared to normotensive pregnancies, offspring of mothers with gestational hypertension had higher systolic and diastolic blood pressure (difference: 0.17 (95% CI 0.02,0.31) and 0.23 (95% CI 0.08, 0.38) SDS in offspring systolic and diastolic blood pressure, respectively), which was not explained by birth and child factors. Offspring of mothers with preeclampsia had higher systolic blood pressure, but this association attenuated after correction for birth and child factors. No associations were found for offspring carotid IMT and distensibility with any gestational hypertensive disorder. Higher maternal systolic and diastolic blood pressure in early, mid and late pregnancy were associated with higher offspring systolic and diastolic blood pressure and lower carotid distensibility (all p-values <0.05), but not with IMT. These associations persisted after correction for birth and child factors. We found the strongest effects for higher maternal blood pressure in early pregnancy.

**Conclusion:** Gestational hypertension and already a higher maternal blood pressure across the full range during pregnancy are associated with higher blood pressure in the offspring. Higher maternal blood pressure is also associated with decreased offspring carotid distensibility. These findings suggest that higher maternal blood pressure influences blood pressure and arterial stiffness in the offspring. Further studies need to assess whether these associations reflect intrauterine mechanisms, or family-based shared lifestyle and genetic factors.

### W-101

**Sex-Specific Upregulation of Estrogen Receptor 2 and Insulin-Like Growth Factor 1 Receptor in the Left Ventricle of Fetal Sheep by Prenatal Testosterone Excess.** Adel Ghnenis<sup>†</sup>, Vasantha Padmanabhan,<sup>1</sup> Arpita Vyas\*,<sup>2</sup> <sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>California Northstate University, Elk Grove, CA, United States.

**Introduction:** Gestational hyperandrogenism predisposes offspring to intrauterine growth retardation (IUGR) and cardiometabolic dysfunction in postnatal life. IUGR is associated with left ventricular (LV) hypertrophy and increases the risk for cardiovascular disease. In this regard, prenatal testosterone (T) excess induces IUGR and programs LV myocardial disarray and hypertension in adult female offspring. However, the early impact of prenatal T excess in programming cardiovascular dysfunctions and if it is female-specific is not known. We have previously shown prenatal T excess 1) increases fetal T levels in female fetuses to control male fetal levels while also increasing estradiol and 2) induces a transient increase in insulin-like growth factor 1 (IGF1). Because IGF-1 plays a key role in cardiac hypertrophy, metabolism and function, steroids are major programming agents, and males are exposed to higher levels of T during fetal life we hypothesized that the impact of prenatal T excess on LV remodeling involved early changes in expression of AR, ESR1, ESR2, and IGF-1R in the female fetuses but not in male fetuses. We further hypothesize early molecular changes adversely alter myocardial morphology (fibrosis and increased left ventricular mass).

**Methods:** Pregnant ewes were injected with testosterone propionate (T), or oil-vehicle (CON), 100 mg i.m. twice weekly from gestational day 30 to 90; term: 147 days. At 90 days of gestation, fetal and heart weights were recorded, and LV tissues (n=6/sex/group) were collected, and snap-frozen or fixed in formaldehyde in PBS pH 7.4 and paraffin-embedded for molecular and histological analyses. Steroid and IGF-1R expression were measured by RT-PCR and collagen content using Masson trichrome staining. Data were analyzed by one-way ANOVA.

**Results:** Heart/body weight ratios did not differ between the groups. While LV AR mRNA gene expression did not differ between T and CON fetuses, LV ESR2 was upregulated (P=0.027) in T treated females but not in males compared to their CON fetuses (11.1±3.3 vs. 1.8±0.7 and 6.2±2.5 vs. 1.6±0.7 respectively). No significant differences were observed in LV ESR1 in both female and male T fetuses. IGF1R was significantly increased (P=0.007) in T treated females but not T males compared to their CON fetuses (4.1±0.99 vs. 1.23±0.3 and 2.1±0.3 vs. 1.14±0.25 respectively). Histological analysis of LV showed no difference in collagen content among the groups.

**Conclusion:** These data suggest that maternal exposure to excess T during pregnancy alters myocardial signaling pathways involved in programming cardiomyocyte structure and metabolism. Furthermore, the influence of gestational T excess on cardiac reprogramming may be mediated via estrogenic rather than androgenic programming. Supported by NIH R01HL139639.

### W-102

**Chronobiological Effects of Gestational Hypoxia on the Placental-Cardiac Clock Axis.** Rachael C Crew<sup>†</sup>, Kimberley C. W Wang,<sup>1</sup> Peter B Noble,<sup>1</sup> Peter J Mark,<sup>1</sup> Caitlin S Wyrwoll,<sup>1</sup> Dino A Giussani\*,<sup>2</sup> <sup>1</sup>The University of Western Australia, Perth, Australia; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.

**Introduction:** Circadian rhythms, controlled by clock genes at a molecular level, regulate cardiovascular function under both healthy and pathological conditions (Crnko et al. *Nat. Rev. Cardiol* 16(7): 437-447, 2019). The circadian system is also vital during fetal development to prepare the offspring for external cues, and recent evidence shows crosstalk between clock genes and hypoxia signalling via HIF1 $\alpha$  (Wu et al. *Cell Metab* 25(1): 73-85, 2017). Therefore, chronobiological processes may be linked to the programming of cardiovascular dysfunction by developmental hypoxia, which is one of the most common complications of human pregnancy, however this idea has never been tested. Here, in a study using mice, we provide the first evidence that gestational hypoxia influences clock

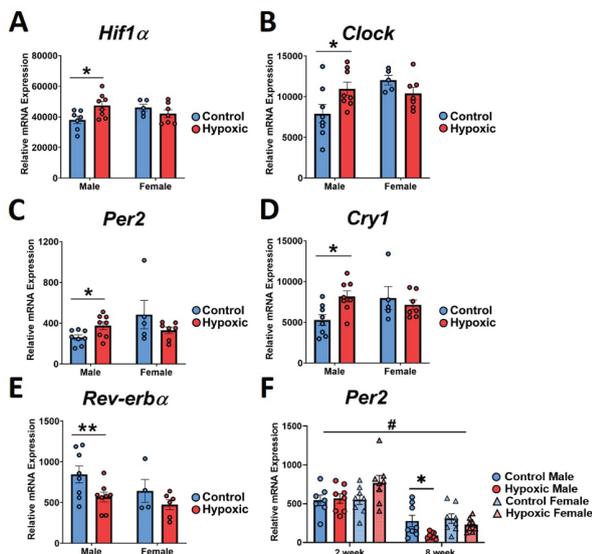
gene expression in the placenta, the key interface between the maternal 24h rhythm and the fetus, and induces long-lasting effects on the cardiac clock of the adult offspring.

**Methods:** Pregnant BALB/c mice were exposed to control (21% O<sub>2</sub>) or hypoxic (10.5% O<sub>2</sub>) conditions from embryonic day (E)11-E17.5 (term is E21). Placental tissue was sampled at 10 am on E17.5 in a cohort of animals. Another cohort delivered, and offspring were raised under normoxic conditions. Offspring hearts were then collected at the juvenile (2 week) and adult (8 week) stages. Clock gene expression was measured in tissues via RT-qPCR.

**Results:** While there was no effect in females, hypoxic male placentas had greater expression of *Hif1α* (25%,  $P=0.02$ ) and the core clock genes: *Clock* (39%,  $P=0.046$ ), *Per2* (44%,  $P=0.027$ ) and *Cry1* (55%,  $P=0.009$ ), but reduced expression of *Rev-erba* (-32%,  $P<0.05$ ) compared to controls (Fig. 1 A-E). Disruptions induced by gestational hypoxia also extended to postnatal life; cardiac clock gene expression was unaffected in juveniles, however adult male offspring of hypoxic pregnancy showed a substantial reduction in cardiac *Per2* expression (-67%,  $P<0.05$ ; Fig. 1 F).

**Conclusion:** We show a molecular link between clock genes in the placenta and the hearts of adult offspring, whereby they are impacted by gestational hypoxia in a sex-dependent manner. We propose that molecular clocks should be exploited therapeutically to protect against developmental programming of cardiovascular disease by adverse intrauterine conditions.

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**Figure 1.** Expression of *Hif1α* (A) and clock genes (B-E) in the placenta at E17.5, and *Per2* expression in juvenile (2 week) and adult (8 week) offspring heart tissue (F) in mice exposed to hypoxic or control conditions during gestation. Values are the mean  $\pm$  SEM, n=5-8 per group. \* $P<0.05$  in control vs. hypoxic males; Two-Way ANOVA and *post hoc* Student's t-test following treatment-sex interaction. \*\* $P<0.05$  hypoxic vs. control males; Two-Way ANOVA and *post hoc* LSD comparison following treatment effect. # $P<0.001$  Two-Way ANOVA showing significant age effect.

## W-103

**Transcriptomic Responses Are Sex-Dependent in the Skeletal Muscle and Liver in Offspring of Obese Mice.** Amy C Kelly<sup>†</sup>,<sup>1</sup> Jeannie Chan,<sup>2</sup> Theresa L Powell,<sup>1</sup> Laura A Cox,<sup>2</sup> Thomas Jansson\*.<sup>1</sup> <sup>1</sup>University of Colorado Anschutz, Aurora, CO, United States; <sup>2</sup>Wake Forest, Winston-Salem, NC, United States.

**Introduction:** Infants born to obese mothers are at risk to develop obesity and metabolic disease in childhood and adult life, including glucose intolerance and hepatic steatosis. Emerging data indicates metabolic programming responses to maternal obesity are sexually dimorphic. In a mouse model of diet-induced obesity with metabolic features of obese women delivering large babies, we used RNAseq to identify pathways involved in programming of liver and skeletal muscle in male and female offspring of obese dams.

**Methods:** Female C57BL/6J mice were fed chow (11% fat) or an obesogenic high-calorie diet (41% fat + 20% sucrose) before mating and throughout pregnancy and lactation. The liver and gastrocnemius muscle were snap frozen following collection from fetuses on E18.5 from obese (OB-fetus) and control dams (CON-fetus) and from 3 month old offspring from obese (OB-offspring) and control dams (CON-offspring). Each group (n=10) consisted of 5 males and 5 females. RNAseq determined differentially expressed transcripts in the liver and muscle. Significant genes (DEG;  $P<0.01$ ) were generated for each sex independently using edgeR. DEG were queried for enrichment and modeled to functional pathways.

**Results:** OB-male and female E18.5 fetuses were heavier ( $P<0.05$ ) than controls. There was minimal overlap (<3%) between male and female DEG. In liver, male OB-fetuses had 399 DEG, female OB-fetuses had 173 DEG. Male DEG were significantly enriched for broad metabolic and protein processing pathways; however, female DEG were enriched for fatty acid metabolism and PPAR signaling. In muscle, male OB-fetuses had 211 DEG and female OB-fetuses had 289 DEG compared to controls. Female DEG were enriched for RNA transport yet mTOR signaling was enriched in both sexes. Only male OB-offspring gained more weight ( $P<0.05$ ) than CON-offspring. In liver, male OB-offspring had 571 DEG and female OB-offspring had 273 DEG compared to controls. DEG seen in both sexes were enriched for steatosis including increased apolipoproteins and perilipins. The muscle of female offspring showed greater response than that of males (females 580 DEG and males 275) and female muscle DEG support greater glucose uptake and metabolism in response to maternal obesity. In both tissues, less than 3% of fetal DEG were also identified as DEG in the offspring.

**Conclusion:** Transcriptomic data support that fetuses of obese mothers modulate metabolism in both muscle and liver and that adult metabolic processes continue to be impacted as overt disease develops. These changes were strikingly sexually dimorphic in agreement with published findings that male offspring of obese dams exhibit more pronounced metabolic disease. In both males and females, the transcriptomic responses in the fetus were different than those at 3 months, implicating adaptive mechanisms throughout adulthood.

## W-104

**In Utero Exposure to  $\Delta^9$ -tetrahydrocannabinol Leads to Rapid Postnatal Catch-Up Growth and Hepatic Dysmetabolism.** Shelby Louise Oke<sup>†</sup>,<sup>1,2</sup> Kendrick Lee,<sup>1</sup> Rosie Papp,<sup>1</sup> Patti Kiser,<sup>1</sup> Steven Laviolette,<sup>1</sup> Daniel B Hardy\*.<sup>1,2</sup> <sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>Children's Health Foundation, London, ON, Canada.

**Introduction:** Postnatal catch-up growth exacerbates the inverse relationship between birth weight and long-term metabolic disease. We have previously demonstrated that catch-up growth following perinatal protein deficiency leads to impaired mitochondrial function and hepatic dysmetabolism in postnatal life. Given that this occurs exclusively following catch-up growth, we have implemented an *in utero* model of  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC, the psychoactive component of *cannabis*) exposure to determine if catch-up growth promotes impaired mitochondrial and liver function in an alternate model of fetal growth restriction (FGR). We hypothesized that  $\Delta^9$ -THC-exposed offspring would undergo catch-up growth, leading to compromised mitochondrial and hepatic function.

**Methods:** Pregnant rat dams received daily dose of vehicle or  $\Delta^9$ -THC (3 mg/kg *i.p.*) from embryonic day (E) 6.5 through E22. At birth, body weight (BW) and organ to BW ratios were recorded. Hepatic and mitochondrial outcomes were assessed via immunohistochemistry (IHC), lipid analysis, and western immunoblotting at three weeks and six months. IHC images were subject to blinded pathological analysis, while immunoblot data were analyzed via an unpaired Student's t-test.

**Results:**  $\Delta^9$ -THC offspring had reduced BW and liver to BW ratio, followed by whole body and hepatic catch-up growth by three weeks. At six months,  $\Delta^9$ -THC offspring exhibited increased adipose to BW ratio, suggestive of dyslipidemia. Male  $\Delta^9$ -THC offspring displayed increased hepatic triglycerides ( $p<0.05$ ) at six months, concomitant with increased hepatic diglyceride acyltransferase 1 (DGAT1) and DGAT2

protein ( $p < 0.05$ ). Furthermore, male and female  $\Delta 9$ -THC offspring demonstrated decreased hepatic PAS staining at six months, indicating reduced glycogen stores. Male  $\Delta 9$ -THC offspring also exhibited increased protein abundance of p66Shc ( $p < 0.05$ ) and mitochondrial complexes I, III, and V ( $p < 0.05$ ), along with decreased SOD1 ( $p < 0.05$ ), suggesting mitochondrial dysfunction. At three weeks, male  $\Delta 9$ -THC offspring had increased DGAT2 ( $p < 0.001$ ) and fatty acid binding protein 1 (FABP1;  $p < 0.01$ ) protein, while there were no differences in markers of oxidative stress or mitochondrial dysfunction.

**Conclusion:** Overall, our data indicate that catch-up growth may disrupt hepatic metabolism and mitochondrial function independent of gestational insult. It also suggests that  $\Delta 9$ -THC-exposed offspring may be vulnerable to development of the metabolic syndrome later in life, and that early elevation of FABP1 and DGAT may serve as a potential mechanism to explain mitochondrial dysfunction in adulthood.

### W-105

**Fetal Hyperuricemia in Pregnancy Induces Severe Postnatal Sex-Specific Development Deficits in an Animal Model.** Benjamin P. Lüscher†,<sup>1,2</sup> Andreina Schoeberlein,<sup>1,2</sup> Daniel V Surbek\*,<sup>1,2</sup> Marc U Baumann\*,<sup>1,2</sup> <sup>1</sup>Department of Obstetrics and Gynaecology, University Hospital of Bern, Bern, Switzerland; <sup>2</sup>Department of Clinical Research, University of Bern, Bern, Switzerland.

**Introduction:** Elevated uric acid (UA) levels in maternal serum are frequent in pre-eclampsia (PE). Maternal and fetal outcome in PE has been shown to correlate with the degree of hyperuricemia: higher maternal UA plasma have worse maternal / fetal outcome. However, to date, it is unknown if hyperuricemia is just a “bystander” phenomenon in more severe forms of PE, or if increased uric acid itself fosters severity or fetal complications of PE. To gain insight into the effect of fetal hyperuricemia on postnatal development, we used a systemic GLUT9 KO mouse model. GLUT9 belongs to the glucose transporter family and is the major regulator of the placental UA transport system. In this model, GLUT9 KO fetuses have reduced clearance of UA through the placenta, leading to fetal hyperuricemia, allowing to study the effect of fetal exposure to high UA levels on postnatal development.

**Methods:** GLUT9 (+/-) mice were crossed to obtain litters with wild type (WT) and knock out (KO). During pregnancy one group received inosine 1mg/g BW in order to increase fetal UA serum levels, the other group got regular food. The body weight was assessed daily until day 70 after birth. With the age of 70 days, animals were euthanized and perfused with 4% formaldehyde. The kidneys were isolated for histological analysis.

**Results:** The body weight development after birth of the KO pups did not differ from the WT pups under normal feeding conditions. However, we observed a significant difference in male KO mice development if their mothers had been fed with inosine supplement during gestation. Specifically, they show reduced weight development over time. Furthermore, kidneys isolated from the KO offspring at 70 days of age show massive morphologic developmental deficits, severe inflammation with macrophage invasion, and early-onset nephropathy. These changes could not be observed in fetuses on day 18.5 of gestation.

**Conclusion:** Our data show for the first time that hyperuricemia during gestation may lead to severe developmental deficits in the offspring, appearing only during postnatal development. This study suggests that fetal exposure to hyperuricemic environment during gestation affected by PE may play a crucial role in the pathophysiology of fetal programming of long-term developmental deficits.

### W-106

**Are There Sex-Specific Effects of Placental Gross Morphology on Early Childhood Growth of Term Newborns in a Low Risk Community Based Setting?** Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,3</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongsachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,4</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,4</sup> Adwoa Nantwi,<sup>5</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*,<sup>1,3,2</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>Institute for Basic Research, Staten Island, NY, United States; <sup>4</sup>CUNY Hunter College, New York, NY, United States; <sup>5</sup>New York University College of Global Public Health, New York, NY, United States.

**Introduction:** We have previously shown sex-specific effects of placental gross features on birth weight in the Collaborative Perinatal Project, a cohort of university-based births >50 years ago. We search to confirm these findings in a modern community-based cohort of low-risk term births.

**Methods:** A community hospital based sample with universal placental examination was searched for those births followed to at least age 2 years at our institution. Gross placental examination was performed according to a protocol that recorded trimmed placental weight (PW), major and minor disk axes, minimum and maximum disk thickness and cord eccentricity. Infant sex, and centiles for weight and length/height at birth and ages 1 and 2 years were extracted from medical records. Gross measures and centiles of growth were both analyzed with nonparametric tests due to non-normal distributions.

**Results:** 1631 infants met inclusion criteria. Placental measures of disk thickness (minimum and variance), chorionic disk ellipsivity, and eccentricity of cord insertion relative to the mean chorionic surface radius were significantly reduced in males ( $p=0.02$ ,  $p=0.003$ ,  $p=0.01$ ,  $p=0.02$ , respectively) compared to females. While PW and chorionic plate area (CPA) were significantly related to birth weight and length centiles in both sexes, disk thickness was related to birth centiles in females only (e.g.,  $p=0.01$  v.  $p=0.10$ ). PW and CPA showed effects on growth centiles at years 1 and 2 in both sexes (each  $p < 0.01$ ), while the effects of disk thickness in females did not persist to years 1 and 2.

**Conclusion:** Placental measures show significant relationships to weight and length centiles at birth, with disk thickness associated with growth only in female infants. The unique effect of disk thickness on female birth centiles was lost by age 1. Disk thickness represents the placental villous arborization and parallels the nutrient exchange surface area available to the fetus. The different impacts of such villous branching on male and female fetal growth may contribute to differential fetal programming and account for divergent lifelong health risks.

### W-107

**Differential DNA Methylation Signatures Following Antenatal Corticosteroids in Human Neonatal Blood.** Bona Kim†, Aya Sasaki, Kellie Murphy, Stephen G Matthews\*. University of Toronto, Toronto, ON, Canada.

**Introduction:** Antenatal corticosteroids (ACS) are a critical treatment for the survival of neonates born preterm. However, increased risk of behavioural disorders in children have been associated with ACS. Animal studies have reported significant modification to brain DNA methylation patterns following ACS, but the changes in humans have yet to be investigated. Here, we have examined the neonatal blood as a surrogate tissue since human brain samples are not accessible. *We hypothesized that a differential methylation signature will be identified in peripheral blood following ACS treatment in neonates born at term.*

**Methods:** ACS-treated women and their neonates (born at term) were retrospectively identified through the Ontario Birth Study at the Sinai Health System and matched (for maternal age, BMI, parity, and fetal sex) to untreated control women ( $n=14$ /gp). Genomic DNA was extracted from neonatal blood spot cards and differentially methylated CpG sites (DMS) were identified using bioinformatic approaches (*MethPipe*) following reduced representation bisulfite sequencing (RRBS). Reads with >30x coverage were evaluated, and significance was defined as  $FDR < 0.05$ .

Comparison of DNA methylation between ACS-treated and untreated subjects was performed through regression analysis. Gene set enrichment analysis was performed through the STRING database.

**Results:** A total of 1815 DMS were identified, of which 843 were hypermethylated and 972 were hypomethylated. Applying a filter to highlight DMS that were >10% different in effect size, 98 DMS were hypermethylated and mapped to 64 genes, while 140 DMS were hypomethylated and mapped to 46 genes. Gene set enrichment analysis of all hypermethylated genes identified pathways of cell proliferation and the hypomethylated gene set identified the ubiquitin-proteasome system (UPS) and intracellular protein trafficking pathways following ACS exposure.

**Conclusion:** Through this study, we have identified genome-wide changes in DNA methylation patterns and their associated gene networks in term-born neonates following ACS treatment. The UPS regulates antigen degradation and presentation in immune cells, while regulating synaptic function and neurotransmitter transport from cell body to synapse in neurons. We are currently conducting animal studies to investigate methylation changes in the neonatal brain. These studies will elucidate the direct impact of ACS on target brain methylation patterns and identify correlations in blood-brain signatures. Determining a brain-specific signature of ACS and their surrogate biomarkers in peripheral blood will create a powerful clinical tool to improve quality of care for the early identification of neonates who may develop later adversities.

### W-108

**Enrichment and Absolute Quantification of Cell-Free Placenta DNA in Maternal Blood.** Samantha L Wilson<sup>†</sup>,<sup>1</sup> Shu Yi Shen,<sup>1</sup> Tim Triche Jr.,<sup>2</sup> Daniel D De Carvalho,<sup>1</sup> Michael M Hoffman\*.<sup>1</sup> <sup>1</sup>Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada; <sup>2</sup>Van Andel Institute, Grand Rapids, MI, United States.

**Introduction:** Cell-free methylated DNA immunoprecipitation with high-throughput sequencing (cfMeDIP-seq) was originally used to enrich for tumour DNA in patients' blood. As cancer and placenta have similar DNA methylation profiles, we hypothesized that cfMeDIP-seq would also enrich for cell-free placental DNA in maternal blood. Additionally, we designed spike-in controls to account for biological and technical bias and absolutely quantify placental DNA.

**Methods:** We designed DNA spike-in controls with fragment lengths [80bp, 160bp, 320bp], G+C contents [35%, 50%, 65%], and CpG content within a fragment [1/80bp, 1/40bp, 1/20bp] using Markov models. We generated two distinct sequences for each parameter combination, one *in-vitro* methylated using CpG methyltransferase (M.SssI) and one left unmethylated to assess non-specific antibody binding. As picomoles of spike-in control put into the experiment is known, we used generalized linear models to quantify methylated DNA captured. We performed cfMeDIP-seq on maternal plasma drawn at 17wks (N=3). Samples were sequenced on Illumina Nova-seq (paired-end 100bp). We calculated picomoles of DNA present in the original sample. We used publicly available DNA methylation microarray data from placenta and cfDNA from non-pregnant females to assess placental DNA enrichment.

**Results:** cfMeDIP has >>99.9% specificity for methylated DNA. Spike-in controls adjust for uninformative biological and technical variance. CfMeDIP results correlate with placenta ( $r=0.67$ ,  $p<2\times 10^{-16}$ ). Principal component (PC) analysis shows PC2 separates cfMeDIP and placenta from the nonpregnant cfDNA. The top genes contributing to PC2 are placenta methylome regulators. *RASSF1*, a tumor suppressor uniquely methylated in placenta, contributes dramatically to PC2. Linear dimension-reduced space of PC1 and PC2 shows >5-fold greater separation of cfMeDIP results from nonpregnant cfDNA than from placental DNA, giving clear evidence that cfMeDIP enriches for placental DNA.

**Conclusion:** Spike-in controls improve cfMeDIP-seq, adjusting for biological and technical variance and absolute quantification of cell-free DNA. We show that cfMeDIP-seq can enrich for placental DNA in maternal plasma opening a new avenue for non-invasive prenatal screening.

### W-109

**Noninvasive Prenatal Genetic Testing after Uterus Transplant.** Jessica Ruth Walter<sup>†</sup>, Nawar Latif, Eileen Wang, Kathleen O'Neill\*. *University of Pennsylvania, Philadelphia, PA, United States.*

**Introduction:** Uterus transplant (UTx) has increased fertility options for patients with absolute uterine factor infertility. As experience grows, reporting challenges encountered in reproductive care is paramount. We report for the first time inconsistencies of noninvasive prenatal testing (NIPT) after UTx. Erroneous NIPT has been observed after other organ transplants, suspected in the setting of DNA shedding from the grafted organ. The American College of Obstetricians and Gynecologists and Society for Maternal Fetal Medicine recently affirmed cell free DNA (cfDNA) is the most sensitive and specific screening option, regardless of maternal age or baseline risk. Circumstances precluding use of first line technologies are essential to report to improve antenatal counseling.

**Methods:** Three patients have received UTx in University of Pennsylvania's Uterus Transplantation for Uterine Factor Infertility (UNTIL) trial. Functionality of the grafted uterus was established with return of menses and the endometrium was prepared with either a programmed (exogenous estrogen and progesterone) or natural cycle. Ultrasound-guided frozen single embryo transfer was performed. Sequential screening and cfDNA were performed during antenatal care.

**Results:** NIPT testing of the first two patients resulted with low or borderline fetal fraction (Table 1). Although recipient 1 had previously undergone preimplantation genetic testing with transfer of a euploid embryo, she was categorized as high risk. Notably, she was on therapeutic enoxaparin—a known risk factor for low fetal fraction. Both patients with low/borderline fetal fraction, had subsequent negative sequential screening, normal nuchal translucency measurements, and healthy live births. The third recipient (currently pregnant) was low risk by cfDNA, but had an elevated maternal serum alpha-fetoprotein (MSAFP).

**Conclusion:** NIPT may be less reliable in UTx patients given potential interference of DNA shedding by the grafted uterus, further complicated by the concurrent use of prophylactic or therapeutic anticoagulation. Inconsistencies and limitations in prenatal genetic testing after UTx will require broader reporting of efficiency and accuracy to ensure appropriate patient counseling.

Prenatal Genetic Testing after Uterus Transplant							
Patient	Donor Type	Blasto-cyst Grade	Gestational Age at Testing	Maternal Age (yrs)	Maternal Weight (lbs)	cfDNA Result (Fetal Fraction)	Sequential Screen (MoM)
Recipient 1	Deceased	Day 5 3AA (euploid)	16 weeks 1 day	32	151	High risk Low fetal fraction (1.8%)	Negative NT 0.73 PAPP-A 1.46 HCG 1.32 UE3 1.51 Inhibin 1.16 MSAFP 1.79
Recipient 2	Deceased	Day 5 3AB (untested)	16 weeks 0 days	28	190	No call Borderline low fetal fraction (2.8%)	Negative NT 0.84 PAPP-A 0.65 HCG 0.83 UE3 1.28 Inhibin 1.13 MSAFP 1.18
Recipient 3	Living	Day 6 3AB (untested)	17 weeks 0 days	33	141	Low risk (20.2%)	Positive NT 1.2 PAPP-A 1.76 HCG 4.02 UE3 0.98 Inhibin 3.04 MSAFP 3.5

**W-110**

**Maternal Diabetes Alters Gene Expression in the Developing Mouse Embryonic Heart.** Rolanda Lister, Chisom Ezenekwe, Etoi Garrison, Scott Baldwin. *Vanderbilt University Medical Center, Nashville, TN, United States.*

**Introduction:** Women with pre-gestational diabetes are five times more likely to deliver an infant with a heart defect yet the underlying mechanisms of hyperglycemia-induced embryopathy are incompletely understood. Genome-wide mRNA expression is an unbiased way to investigate how maternal hyperglycemia affects gene expression of the developing heart. Our hypothesis is that maternal diabetes will cause changes in gene expression in the developing embryonic heart throughout development.

**Methods:** Hyperglycemia, defined as  $\geq 250$  mg/dL was induced in eight-week-old female CD1 mice by injecting a one-time intraperitoneal dose of 150mg/kg streptozotocin. Control mice received an equal volume of normal saline. Hyperglycemic and control females were mated with CD-1 males. At Embryonic Day(E) 14, 17, and Post-natal day (P) 0 pregnant mice were euthanized, and the embryonic and neonatal hearts were harvested. RNA was extracted from homogenized pooled cardiac tissue (N=2 litters/group/timepoint). Total RNA was prepared using the Illumina Tru-Seq RNA sample prep kit (Illumina, San Diego, USA), and sequencing was conducted on the Illumina HiSeq 2500. RNA was extracted from the pooled pup hearts in both groups at the timepoints described above. RNA sequencing was then used to compare genome-wide differential expression throughout development within control mice, diabetic mice, and within diabetic and control pup hearts. This differential expression of genes was assessed within each group and across disease (diabetic) and normal (control) maternal conditions across various stages of development. Two-way ANOVA and Post Hoc Tukey statistical tests were employed to examine the effect of stage of development and disease state on number of DEG's. P-values of  $\leq 0.05$  were considered significant.

**Results:** The average maternal blood glucose concentration between control and diabetic dams was 112+/-24 and 473+/- 47 respectively ( $p \leq 0.0001$ ). Control embryonic hearts exhibited differential expression in 2450 genes from E14- P0. Diabetic hearts exhibited differential expression of 2104 from E14-P0. However, this contrasts with the number of DEG ranged from 65, 95, and 210 with each timepoint E14, E17 and P0 respectively between diabetes and control.

**Conclusion:** Results from this study suggest that the number of differentially expressed genes within the developing embryonic heart are more greatly impacted by advancing stages of development over maternal diabetes. In a mouse model of pregestational type 1 diabetes, whole transcriptome sequencing can illuminate mechanisms diabetes induced cardiac embryopathy. These genetic insights can identify potential genetic pathway targets for therapy or prevention.

**W-111**

**Vaginal Delivery Is Feasible and Safe in Giant Omphaloceles.** Nicole R Gavin†, Amanda C Mahle†, Eric B Jelin, Clark T Johnson, Angie C Jelin\*. *The Johns Hopkins Hospital, Baltimore, MD, United States.*

**Introduction:** The appropriate delivery mode for abdominal wall defects, particularly giant omphaloceles, is unclear. This study was undertaken to examine delivery methods in cases of omphalocele and gastroschisis with respect to size of defect, presence of associated anomalies, and presence of extracorporeal organs other than bowel.

**Methods:** This is a retrospective cohort study of all cases with an abdominal wall defect visualized by ultrasound who underwent delivery at Johns Hopkins Hospital between 2008 and 2019. Elective terminations and fetal demises in utero were excluded.

**Results:** We identified 80 cases of abdominal wall defects (57 cases of gastroschisis, 23 cases of omphalocele). Cesarean deliveries were performed in 31.6% of gastroschisis cases and 52.2% of omphalocele cases ( $P = 0.085$ ). Vaginal delivery was attempted in 93% of cases of gastroschisis and 82.6% of cases of omphalocele. The majority of cesarean deliveries performed were for standard obstetric indications, rather than scheduled due to an abdominal wall defect. There were 9 cases of giant omphalocele (measuring greater than 5cm), including 1

case with sonographically identified prenatal sac rupture. Six cases of giant omphalocele underwent trial of labor with a successful vaginal delivery rate of 66%. There were no cases of sac rupture or damage to intra-abdominal organs during delivery in any of these cases.

**Conclusion:** In pregnancies complicated by fetal abdominal wall defects, including giant omphalocele, vaginal delivery can be performed without damage to intra-abdominal organs or sac rupture. This suggests that more consideration should be given to delivering fetuses with giant omphaloceles vaginally, reducing the maternal morbidity that is associated with cesarean delivery.

Trial of Vaginal Delivery in Giant Omphaloceles				
Largest Measured Dimension (cm)	Gestational Age at Delivery	Attempted Vaginal Delivery	Successful Vaginal Delivery	
6.9	35w4d	Yes	Yes	
5.3	38w4d	Yes	No	Non-reassuring FHR
5.4	40w2d	Yes	Yes	
7.1	36w4d	Yes	Yes	
6.5	37w1d	Yes	Yes	
9.4	38w2d	Yes	No	Failed Induction

**W-112**

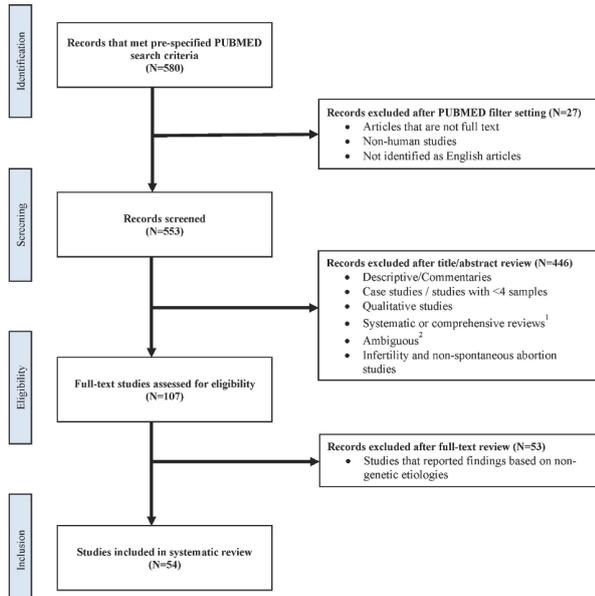
**A Systematic Review to Guide Future Efforts in the Determination of Genetic Causes of Pregnancy Loss.** Andrew Zaricor Carey†, Nathan R Blue†, Michael W Varner, Jessica M Page, Aaron R Quinlan, D Ware Branch, Robert M Silver, Tsegaselassie Workalemahu\*. *University of Utah Health, Salt Lake City, UT, United States.*

**Introduction:** Pregnancy loss, the most common obstetric complication, occurs in almost 30% of conceptions and 12-14% of clinically recognized pregnancies, yet knowledge of the genetic causes is limited. We conducted a systematic review to highlight genetic studies of pregnancy loss and identify key strategies to guide future research.

**Methods:** PUBMED was searched for full-text articles published in English between 01/01/00-01/01/20 and examined factors involved in pregnancy loss in humans. We excluded review articles, case studies, studies with sample sizes  $< 4$ , descriptive studies, commentaries, infertility/non-spontaneous abortion studies and studies with non-genetic etiologies. Studies were classified based on developmental periods in gestation to synthesize data across developmental epochs.

**Results:** Our search yielded 580 titles, of which 553 (95%) were eligible after title/abstract screening. Of these, 107 (18%) matched eligibility after full-text review (Figure 1). Fifty-four (50%) studies reporting findings with genetic predictors of pregnancy loss were subsequently included in the review. These studies examined early pregnancy loss (n=9 [17%]), stillbirth (n=10 [18%]), recurrent pregnancy loss (n=32 [59%]), unclassified fetal death (n=2 [4%]), or self-reported miscarriage or stillbirth (n=1 [2%]) as their primary outcomes. Multiple genetic pathways known to be critical for life and novel pathways found to be necessary for *in utero* survival are implicated to play a role in pregnancy loss (Table 1).

**Conclusion:** Pregnancy loss is multi-factorial, but recent studies utilizing various genetic modalities identified genetic pathways thought to be essential for embryonic and fetal survival. These pathways may provide novel biomarkers for risk stratification and therapeutic targets for improvement of pregnancy outcomes. Further research systematically evaluating pregnancy loss by utilizing whole genomic sequencing may further elucidate causal genetic mechanisms and identify other pathways critical for embryonic/fetal survival.



<sup>1</sup> Studies in systematic reviews were extracted and included in the current systematic review if they met the PUBMED search criteria  
<sup>2</sup> Studies were ambiguous if the outcome or the predictor studied are unknown or unclear

Pregnancy Loss Phenotype	Genes, microRNAs, mRNAs, or chromosomes	Functional Pathway	Number of Studies
Early Pregnancy Loss (<20 weeks gestation)	SGK1, miR-575, miRNA-17, miRNA-19b, VEGF	Placental function	7
	TET family, 5-hmC	Epigenetic reprogramming	
	miR-125a, miR-3663-3p	Mitosis, meiosis, cell cycle progression	
	miR-3663-3p, miR-135a, miR-122, let-7, miR-378a-3p	Apoptosis	
	miR-125a, miR-135a	Hematopoiesis	
Stillbirth (≥20 weeks gestation)	HOX family	Implantation	6
		Endometrial function	
	F5, PAI-1, eNOS	Coagulation	
	AOX-1, GPER	Oxidation and cellular aging	
	LPA	Lipoprotein synthesis	
Recurrent Pregnancy Loss (≥2 failed pregnancies)	Ch 1q31.3, NOS3, RCAS1	Inflammation and immunity	32
	eNOS	Mitosis, meiosis, cell cycle progression	
	eNOS	Vascular tone	
	NOD1, ITI-H4, KLKB1, IL-22, HLAG, CD16, CD68, CD56, S100A8, S100A9, KISS1, IL1B, CD46, FOXP3, NLRP2, NLRP5, NLRP7, IDO2	Inflammation and immunity	
	CREB5, DYNC2H1, PLCD4, OSBP15, STIL	Mitosis, meiosis, cell cycle progression	
	CREB5, BAX, CASP9	Apoptosis	
	NUP98, IFT122, APAF1, CASP9, CSPP1, NLRP5, PADI6	Embryonic development	
	MTRR, VDR	Folate and other vitamin metabolism	
	Cx43, VEGF, ALOX15	Placental function	
	Cx43, VEGF, VEGFA, FLT1, EPAS1	Angiogenesis	
	ANXA5, TAFI, THBD, FGA, FGB, PROCRA	Coagulation	
	KISS1, CHRNA1, RYR1, MUSK	Cell signaling	
CGB5	Implantation		
KIF14, IFT122, DYNC2H1	Ciliogenesis		
MMP10	Extracellular matrix organization		
CAPS	Ion transport		
Unclassified Fetal Death	PROCRA, F5, F2	Coagulation	3
	MTHFR	Folate and other vitamin metabolism	

W-113

**Late Gestation Fetal Hyperglucagonemia Results in Lower Fetal Weight, Fetal Hypoaminoacidemia, and Decreased Uteroplacental Nutrient Uptake.** Sarah Cilvik,<sup>1</sup> Stephanie R. Wesolowski,<sup>2</sup> Russ V. Anthony,<sup>3</sup> Laura D. Brown,<sup>2</sup> Paul Rozance\*.<sup>2</sup> <sup>1</sup>Wake Forest University Health Sciences, Winston-Salem, NC, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>Colorado State University College of Veterinary Medicine, Fort Collins, CO, United States.

**Introduction:** Fetal glucagon concentrations are elevated in intrauterine growth restriction. Glucagon receptors are expressed in the placenta, with prior data suggesting that glucagon may inhibit umbilical uptake of certain amino acids. We hypothesized that chronic fetal hyperglucagonemia would result in decreased amino acid transfer to the fetus and decreased growth. **Methods:** Late gestation fetal sheep received a direct intravenous infusion of glucagon (5 or 50ng/kg/min; n=7 and 5, respectively) or a vehicle control (n=10). After 8-10 days, uterine and umbilical blood flows were measured using the <sup>3</sup>H<sub>2</sub>O transplacental diffusion technique. Uteroplacental and umbilical (fetal) uptake rates of oxygen, glucose, lactate, and amino acids were calculated using the Fick principle. Total RNA was isolated from cotyledons, and gene expression analysis was performed using qPCR. All glucagon-exposed fetuses were grouped and compared to controls using the unpaired Student's t-test to evaluate global trends related to experimental hyperglucagonemia.

**Results:** Fetuses receiving glucagon had 13% lower fetal weight compared to controls ( $p<0.05$ ). Fetal hyperglucagonemia resulted in 30% higher fetal oxygen concentrations ( $p<0.05$ ), 42% lower lactate ( $p<0.01$ ), and significantly lower concentrations of nearly all amino acids. Glucose concentrations were not different. Uterine blood flow was 33% lower in hyperglucagonemic fetuses ( $p<0.05$ ), while umbilical blood flow was similar between groups. Fetal hyperglucagonemia resulted in lower uteroplacental uptake of oxygen by 32% ( $p<0.001$ ), glucose by 34% ( $p<0.01$ ), and several amino acids by 53-79% (valine, isoleucine, leucine, arginine, serine, proline, methionine, tryptophan;  $p<0.05$ ). Placental lactate production was 33% lower than controls ( $p<0.05$ ). Fetal uptake rates of valine, isoleucine, leucine, phenylalanine, proline, and tyrosine were 36-51% lower than controls ( $p<0.05$ ), while fetal glucose and oxygen uptake were not different. Cotyledons from hyperglucagonemic fetuses had 40% lower mRNA expression of both placental lactogen and vascular endothelial growth factor A compared to controls ( $p<0.01$ ).

**Conclusion:** These results demonstrate that fetal hyperglucagonemia significantly lowers uteroplacental blood flow, as well as uteroplacental oxygen and nutrient uptake. As glucagon does not cross the placenta, we speculate this effect is mediated by glucagon-activated signaling through the placenta via receptors localized on the fetal-facing basal membrane. This leads to reduction in fetal nutrient supply, particularly of the branched chain amino acids, and lower fetal weight.

#### W-114

**Gestational Diabetes Mellitus-Associated Changes in the Proteomic Profile of Extracellular Vesicles in the Fetal Circulating Reveal a Potential Role in Cell Metabolism.** Ormazabal Valeska\*,<sup>1</sup> Soumyalekshmi Nair†,<sup>2</sup> Andrew Lai†,<sup>2</sup> Katherin Scholz-Romero†,<sup>2</sup> Emilio Diaz\*,<sup>1</sup> Felipe Zuñiga\*,<sup>1</sup> McIntyre H. David\*,<sup>2</sup> Martha Lappas\*,<sup>3</sup> Salomon Carlos\*,<sup>2,1</sup> <sup>1</sup>University of Concepcion, Concepcion, Chile; <sup>2</sup>The University of Queensland, Brisbane, Australia; <sup>3</sup>University of Melbourne, Melbourne, Australia.

**Introduction:** The link between metabolic disorders during pregnancy and an increased risk of developing chronic non-communicable diseases in later life has been well established. However, the mechanisms underlying this link, whereby fetuses exposed to gestational diabetes mellitus (GDM) have a higher risk of abnormal glucose homeostasis in later life, is poorly understood. Extracellular vesicles (EVs) have been well characterized in the maternal circulation in GDM and normal and pregnancies; however, little is known about EVs' profile in the fetal circulation.

**Methods:** A retrospective, case-control study design was used to identify EV-associated proteins that vary in fetal circulation from normal ( $n=35$ ) and GDM ( $n=40$ ) pregnancies. EVs were isolated from plasma by differential centrifugation and size exclusion chromatography and characterised by enrichment of protein associated with EVs, morphology, and size distribution using Western blot, electron microscopy, and nanoparticle tracking analysis, respectively. Quantitative proteomic (Sequential Windowed Acquisition of All Theoretical Mass Spectra [SWATH]) and bioinformatic analysis by Ingenuity Pathway Analysis (IPA) was performed.

**Results:** A total of 1431 proteins in circulating EVs in fetal plasma were identified. A total of 117 proteins (56 upregulated and 61 downregulated in GDM compared to normal) were significantly different ( $p<0.05$ , False discovery rate 1%) between normal and GDM EVs. Interestingly, Fructose-1,6-bisphosphatase isozyme 2, Pyruvate kinase PKM, Hexokinase-1, Phosphoglycerate kinase 1, Superoxide dismutase were the proteins with the more significant changes in GDM compared to controls. Bioinformatics analysis showed that the proteins identified in EVs regulate insulin secretion genes in response to a glucose stimulus, insulin receptor signaling, and glucose homeostasis.

**Conclusion:** Overall, our data proposed the modified protein cargo of EVs present in the fetal circulation in GDM might be associated with the changes in cell metabolism and fetal growth observed in GDM. We suggest that EVs may alter the metabolic function and the gene expression, and ultimately the cell phenotype, by transferring specific proteins to target cells.

#### W-115

**Metformin Has Direct Signaling and Metabolic Effects in Fetal Hepatocytes.** Amanda Jones, Michael Nash, Dong Wang, Paul Rozance, Laura Brown, Stephanie Wesolowski\*, University of Colorado Anschutz Medical Campus, Aurora, CO, United States.

**Introduction:** Metformin reduces hepatic glucose production in adult humans with diabetes and is used by some women during pregnancy. Importantly, metformin crosses the placenta, yet little is known about its tissue specific effects in the fetus. We hypothesized that metformin would have direct signaling and metabolic effects in the fetal hepatocyte. This has important implications for understanding the potential direct effects that may develop in the liver of fetuses exposed to metformin.

**Methods:** Primary hepatocytes from late gestation fetal sheep were isolated ( $n=4-6$ ), incubated overnight, and treated with 0, 250, or 1000  $\mu\text{M}$  metformin for 24 h. Phosphorylated (P-) and total protein expression for AMPK, ACC, mTOR, and S6 was measured by western blotting. Gene expression was measured by RNA-sequencing (Illumina NovaSEQ6000). Paired end reads were mapped (Oar\_v3.1 genome) and filtered data were analyzed by paired t-test. Genes with an FDR adjusted q-value  $<0.05$  and absolute fold change  $>1.5$  were used for KEGG pathway enrichment (DAVID 6.8) and discovery of putative upstream regulators (Ingenuity Pathway Analysis). Hepatocytes also were stimulated (500 nM dexamethasone + 100  $\mu\text{M}$  cAMP) in glucose-free media with lactate plus pyruvate and glucose production was measured. Expression of *OCT1*, metformin transporter, was measured by qPCR in fetal tissues.

**Results:** In fetal hepatocytes, metformin increased P-AMPK and P-ACC and decreased P-mTOR and P-S6, classic metformin targets. We identified 954 upregulated and 929 downregulated genes in response to metformin. These genes were enriched in pathways for glucose and lipid metabolism, DNA replication, and signaling via MAPK, FOXO, ERBB2, and glucagon. The stress inducible protein, *NUPRI*, was the top predicted upstream activator. Expression of *HNF4A*, a transcription factor for hepatocyte differentiation and metabolism, was decreased 4.7 fold and top predicted upstream regulator inhibited by metformin. Metformin decreased stimulated glucose production by 75%. Of the genes specific to glucose production, metformin decreased *G6PC* and *FBP1*, yet *PCK1*, *PCK2*, and *PC* were unchanged. Expression of *OCT1* was greatest in fetal liver compared to other tissues (muscle, pancreas, adipose, heart, lung).

**Conclusion:** We show that the fetal liver has abundant *OCT1* expression, the major metformin transporter, and that metformin has signaling and metabolic effects in fetal hepatocytes. While metformin decreased glucose production and expression of genes in the latter steps of gluconeogenesis, this was not the most impacted pathway in the fetal hepatocyte, as it is in the adult. Rather, we identified novel effects of metformin on activation of stress pathways via *NUPRI* and impaired metabolic genes via decreased *HNF4A*. Thus, fetuses exposed to metformin may have previously unrecognized effects that disrupt normal hepatic metabolism.

#### W-116

**Association between Maternal and Cord Serum Lipid Profile Markers with Anthropometrical Newborn Outcomes.** Catherine Everest†, Jessica L Puranda†, Danilo F da Silva†, Sara C.S Souza†, Alexandra D Goudreau†, Velislava Tzaneva, Kristi B Adamo\*. University of Ottawa, Ottawa, ON, Canada.

**Introduction:** A balanced maternal blood lipid profile is important for reducing the risk of pregnancy-related complications (i.e., excessive gestational weight gain (GWG), gestational diabetes, risk of preterm birth). High maternal cholesterol and triglyceride (TG) levels have been associated with increased fetal birthweight and body fat percentage. Physical activity (PA) during pregnancy has beneficial effects by lowering blood lipids levels such as cholesterol and TG, while promoting appropriate GWG. It is currently unknown if late gestation maternal serum and venous umbilical cord serum (UCS) lipid profile is associated with newborn anthropometrics. This study aims to investigate the association between late gestation maternal and UCS lipid profile on newborn outcomes.

**Methods:** Pregnant women ( $n=40$ ) from the Ottawa, ON region were recruited in mid-pregnancy (24-28 weeks' gestation) to participate in

the PLACENTA study. Participants wore accelerometers to objectively measure moderate-to-vigorous PA status in mid and late (34-38 weeks') pregnancy. Maternal sugar and unsaturated fat intake were measured by dietary records at late pregnancy. Serum from late pregnancy and UCS were used to analyse lipid profile markers (e.g., total cholesterol [TC], HDL, LDL, remnant cholesterol, TG and glucose) using Cholestech LDX Lipid Profile-Glu Cassette and biochemical assays. UCS was taken at birth, and 24-48 hours later, fetal weight, length, and body fat percentage were measured using Ultrascale MBSC-55 and Harpenden Skinfold Caliper respectively. Anthropometric measures were used to determine weight-for-age (W/A) percentile and weight-for-length (W/L) z-score based on WHO standards. Associations between maternal and UCS lipid levels, and newborn outcomes were examined using three linear regression models, controlled for maternal age, pre-pregnancy BMI, unsaturated fat and sugar intake at late pregnancy. Model 1 additionally controlled for the average moderate-to-vigorous PA over gestation only, Model 2 GWG only, and Model 3 combined PA and GWG.

**Results:** Maternal TC concentration was significantly associated with fetal W/L z-score in Model 1 ( $\beta$ : 0.32; 95% Confidence Interval (CI): 0.09-0.56;  $p=0.009$ ), Model 2 ( $\beta$ : 0.33; CI: 0.09-0.57;  $p=0.009$ ), and Model 3 ( $\beta$ : 0.33; CI: 0.09-0.57;  $p=0.010$ ). Maternal TG was significantly associated with fetal W/A percentile in Model 1 ( $\beta$ : 0.38; CI: 0.03-0.73;  $p=0.033$ ). Maternal LDL was significantly associated with fetal W/L z-score in Model 1 ( $\beta$ : 0.34; CI: 0.03-0.65;  $p=0.031$ ), 2 ( $\beta$ : 0.34; CI: 0.03-0.65;  $p=0.031$ ), and 3 ( $\beta$ : 0.35; CI: 0.03-0.66;  $p=0.034$ ).

**Conclusion:** No associations were found between UCS lipid levels and fetal anthropometrics. This study highlights that maternal TC, LDL, and TG may have a more powerful influence on fetal anthropometrics than glucose.

#### W-117

**Long Noncoding RNA-XIST Augments miR-424 to Regulate MEK1-FGFR1 Pathway in Intrauterine Growth Restriction.** Lu Huang<sup>†,1</sup>, Nanbert Zhong<sup>\*,2,1</sup> *Wuxi Maternity and Child Healthcare Hospital, Wuxi, China; <sup>2</sup>New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States.*

**Introduction:** Intrauterine growth restriction (IUGR) may result from gene-environmental interaction is hypothesized. We aim to investigate the epigenetic involvement of long non-coding RNA (lncRNA) in IUGR. **Methods:** Quantitative measurement was applied for the gene expression of lncRNA-XIST (lncXIST), and lncXIST-targeted molecules including miRNA, mRNA, and proteins. A transgenic trophoblast culture model and an over-expression mouse model were developed to demonstrate the epigenetic regulatory function of lncXIST-miRNA-targets.

**Results:** We firstly documented that lncXIST is significantly up-regulated. The lncXIST expression is increased ( $p=0.01$ ) in the placentas of IUGR. Through bioinformatic prediction with programs *mv22v2* and *bibiserv*, we identified a microRNA, the miR-424, as a candidate target of lncXIST and determined that the gene expression of miR-424 in IUGR was increased ( $p=0.002$ ). Further prediction with programs *Target Scan*, *PicTar*, and *DIANA LAB*, genes FGFR1 and MEK1 were identified as the potential downstream targets of miR-424. In vivo measurement of FGRF1 and MEK1 in IUGR placentas showed that the mRNAs of FGRF1 and MEK1 were down-regulated significantly ( $p=0.021$  and  $p=0.001$ , respectively), and both of proteins were decreased ( $p<0.05$ ). To assess the association among the up-regulated lncXIST and miR-424 with the down-regulated FGRF1 and MEK1, a statistical toll *Spearman* was applied with the CRISPR-cas9 gene editing system. A statistically positive correlation was documented between lncXIST and miR-424 ( $p<0.001$ ), and between MEK1 and FGRF1 ( $p<0.05$ ). However, a negative correlation was shown between lncXIST and FGRF1 ( $R^2=0.0035$ ), lncXIST and MEK1 ( $R^2=0.0433$ ), miR-424 and FGRF1 ( $R^2=0.0084$ ), and miR-424 and MEK1 ( $R^2=0.0593$ ). In phase II, a plasmid containing miR-424 was transfected into cultured human trophoblast HTR8/SVneo cells, in which the endogenous mRNAs of FGRF1 and MEK1 were documented to be down-regulated ( $p<0.05$  in both). A retroviral CRISPR-Cas9 system was used to over-express exogenous lncRNA-XIST in a stabilized human trophoblast HTR8/SVneo cell line, in which miR-4244 was co-transfected.

Our results showed that there was no significant change on cell apoptosis and cell cycles, however, lncXIST may significantly inhibit cell mobility through miR-424.

**Conclusion:** Identification of a single gene mutation that may associate with IUGR has not been success. However, many epigenetic factors such as methylation and lncRNA have been identified and characterized. In this study, we reported lncXIST and miR-424 as the epigenetic regulators involved in MEK1-FGFR1 pathway in IUGR, which has opened a novel avenue for further exploration of pathogenic mechanism and designing a rational intervention strategy.

#### W-118

**Chronic Intrauterine Hypoxia Alters Mitochondrial Dynamics in the Fetal Guinea Pig Heart in a Sex Dependent Manner.** Loren P. Thompson, Hong Song. *University of Maryland SOM, Baltimore, MD, United States.*

**Introduction:** Chronic gestational hypoxia imposes a metabolic stress to fetal organ growth and contributes to developmental programming of organ dysfunction in the offspring. The fetal heart responds to hypoxic stress by undergoing concomitant changes in cell proliferation/differentiation and mitochondrial biogenesis, which impacts respiratory function Mitochondrial number or content is regulated by formation of networks via fusion of outer (OMM) and inner (IMM) mitochondrial membranes and removing defective mitochondria via fission. We hypothesize that chronic hypoxia alters mitochondrial function of fetal hearts by altering its mitochondrial dynamics (i.e. the balance between fusion and fission). This may contribute as an underlying mechanism in contractile dysfunction, as well as, in developmental programming in offspring hearts.

**Methods:** Pregnant guinea pigs were exposed to either normoxia (NMX, N=7) or hypoxia (HPX, 10.5%O<sub>2</sub>) starting at 25d (early-onset, N=7) and 50d gestation (late-onset, N=7) until term (~65d) to test the effects of HPX on gestational onset. Male and female fetuses (N=7 each group, 2 per litter) were obtained by cesarean section of anesthetized sows and fetal body, placenta, and organ weights measured and fetal heart left ventricles (LV) excised. Mitochondrial (mito) proteins were extracted from frozen LV and expression of fusion [(MFN-2 (OMM), OPA-1 (IMM)] and fission [(DRP1 (OMM), FIS1 (IMM)] proteins were measured by Western blot, normalized to VDAC as a loading control. Mito content was measured by the mitoDNA/nuclearDNA ratio using qPCR.

**Results:** Both early- and late-onset HPX decreased FBW (10-15%) and increased relative placental wt (20-35%) in both sexes compared to NMX. For fusion, neither early- or late-onset HPX had any effect on MFN2 expression in either sex, although, both early- and late-onset HPX increased ( $P<0.05$ ) OPA1 expression in both sexes. For fission, early-onset HPX increased ( $P<0.05$ ) both DRP1 and FIS1 in males but only DRP1 in females. Late-onset HPX increased ( $P<0.05$ ) both DRP1 and FIS1 in males but only FIS1 in females. Both early- and late-onset HPX decreased ( $P<0.05$ ) mitoDNA content in males only compared to their NMX controls.

**Conclusion:** In a growth-restricted HPX model, the fetal cardiac mitochondria shifted their balance towards fission and reduced mitoDNA content, regardless of gestational age of exposure but in a sex dependent manner favoring females. Thus, a reduced mitochondrial population identifies how fetal heart function may be compromised with chronic intrauterine exposure. Further, this may be an underlying mechanism by which HPX reduces the metabolic and/or respiratory capacity in both fetal, and subsequently, offspring hearts via developmental programming. (NIH HL 126859).

#### W-119

**Mitochondrial Respiration and Citrate Synthase Activity Are Lower in the IUGR Fetal Sheep Heart.** Eileen I. Chang<sup>†</sup>, Jane E. Stremming, Leslie A. Knaub, Jane E. Reusch, Laura D. Brown\*. *University of Colorado School of Medicine, Aurora, CO, United States.*

**Introduction:** Intrauterine growth restriction (IUGR) causes greater risks for ischemic heart diseases and heart failure for the fetus in adulthood. Previously, we reported that the late gestation IUGR sheep fetus has lower percentages of binucleated cardiomyocytes and a less mature

heart compared to control fetuses. The mitochondria are the main supply of ATP in the heart, and a delay in cardiomyocyte maturation may alter cardiac mitochondrial function in the IUGR myocardium. We hypothesize that the late gestation IUGR fetal heart has less capacity for oxidative phosphorylation and reduced mitochondrial function.

**Methods:** Left (LV) and right (RV) ventricles were collected from IUGR (n=6) and control (CON, n=7) fetal sheep at 133±1 day gestation (term=147 days). Mitochondrial respiration was measured from permeabilized muscle fibers using Oroboros Oxygraph-2k with either carbohydrate [state 3 (Carb-S3): pyruvate/malate/glutamate/succinate + ADP; state 4 (S4): oligomycin; and respiratory control ratio (RCR): S3S/S4] or lipid [state 3 (Lipid-S3): palmitoylcarnitine/malate + ADP] substrates. Maximal uncoupled respiration (MaxR) was measured using carbonilcyanide p-trifluoromethoxyphenylhydrazone (FCCP). Citrate synthase (CS) activity was measured to estimate mitochondrial content. Protein expressions of five mitochondrial oxidative phosphorylation complexes (CI-CV) were measured using western blot and normalized to total protein. Paired two-way ANOVA was used to determine the effects of treatment (CON, IUGR), ventricle (LV, RV), and interaction with Fisher's LSD *post hoc* test.

**Results:** Lipid-S3 and MaxR were 38% and 35% lower in IUGR vs. CON, respectively ( $P<0.05$ , group), and CS activity was 18% lower in IUGR LV vs. CON LV ( $P<0.05$ , *post hoc*). However, relative CII and CV protein amounts were 11-12% higher in IUGR vs. CON ( $P<0.05$ , group); CV was 20% higher in IUGR LV vs. CON LV ( $P<0.05$ , *post hoc*). Interestingly, Carb-S3, S4, and MaxR were 44-88% higher in RV vs. LV ( $P<0.0005$ , ventricle). RCR was 30% higher in RV vs. LV ( $P<0.005$ , ventricle). Lipid-S3 and MaxR were 47% and 42% higher in RV vs. LV, respectively ( $P<0.005$ , ventricle). CS activity was 7% lower in CON RV vs. CON LV ( $P<0.05$ , *post hoc*). Protein expression of CII-CIV were higher in RV vs. LV (16-36%,  $P<0.05$ , ventricle); CII was 21% higher in CON RV vs. LV, and CIII was 19% higher in IUGR RV vs. LV ( $P<0.05$ , *post hoc*).

**Conclusion:** Lower mitochondrial respiration in response to lipid substrate in the IUGR heart compared to CON is consistent with delayed cardiomyocyte maturation. We speculate that the IUGR heart compensates for lower CS activity by increasing CII and CV expressions to maintain glycolysis for ATP production. Interestingly, the differences observed between RV and LV reveal that more research is needed to elucidate how the immature IUGR heart responds to the challenges of parturition as LV becomes the main pump.

## W-120

**Novel Gasotransmitter Cardio-Protection in the Developing Heart: Comparative Roles of Hydrogen Sulphide and Carbon Monoxide.** Y Niu<sup>†</sup>, Q. Lyu<sup>†</sup>,<sup>1,2</sup> R. M. Hess<sup>†</sup>,<sup>1</sup> S. G. Ford<sup>†</sup>,<sup>1</sup> T. A. Garrud<sup>†</sup>,<sup>1</sup> A. Iqbal<sup>†</sup>,<sup>1</sup> J. O. Louca<sup>†</sup>,<sup>1</sup> W. Tong<sup>†</sup>,<sup>1</sup> K. J. Botting<sup>†</sup>,<sup>1</sup> D. A. Giussani<sup>\*</sup>.<sup>1</sup> *University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>The Fourth Military Medical University, Xi'an, China.*

**Introduction:** Hydrogen sulphide (H<sub>2</sub>S) and carbon monoxide (CO) protect the heart against episodes of ischemia/reperfusion (I/R) in adult individuals (1,2). However, whether H<sub>2</sub>S or CO protect the fetal heart is completely unknown, when tissue I/R injury is just as relevant, for instance as a result of birth asphyxia. Using the chicken embryo model, we isolate direct preconditioning effects of H<sub>2</sub>S and CO, which confer significant protection against I/R in the developing heart. Further, we show that the molecular basis underlying fetal cardiac protection by H<sub>2</sub>S and CO is different.

**Methods:** Fertilized Bovans Brown eggs were incubated under normoxia (21% O<sub>2</sub>). On day 19 of incubation (term is 21 days), the heart was excised following cervical transection and mounted on a Langendorff preparation. Following measurement of basal cardiac function, hearts were randomly treated with a bolus of water vehicle (Control), a H<sub>2</sub>S donor (NaHS) or a CO donor (CORM-3), with or without the non-selective K<sub>ATP</sub> channel inhibitor Glibenclamide (Glib) or the mitochondrial K<sub>ATP</sub> channel inhibitor 5-hydroxydecanoate (5-HD). An I/R challenge was induced 10 min after treatment for 30 min, followed by 2h of reperfusion. Myocardial infarct size was determined by tetrazolium staining.

**Results:** Treatment with either H<sub>2</sub>S and CO donors improved cardiac function and reduced cardiac infarct size after I/R in the chicken embryo. However, H<sub>2</sub>S improved cardiac function earlier while CO did so later after I/R. While the cardioprotective effects of H<sub>2</sub>S were blocked by Glib but not 5-HD, the cardioprotective effects of CO were blocked by both Glib and 5-HD (Fig. 1).

**Conclusion:** We introduce new interventional gasotransmitter therapy against I/R injury in the developing heart. While H<sub>2</sub>S confers cardiac protection by opening of myocardial sarcolemmal K<sub>ATP</sub> channels, CO does so via myocardial mitochondrial K<sub>ATP</sub> channels.

References: 1. Papapetropoulos et al. PNAS 106:21972, 2009 2. Otterbein et al. *Circ Res* 118:1940, 2016

Supported by The British Heart Foundation

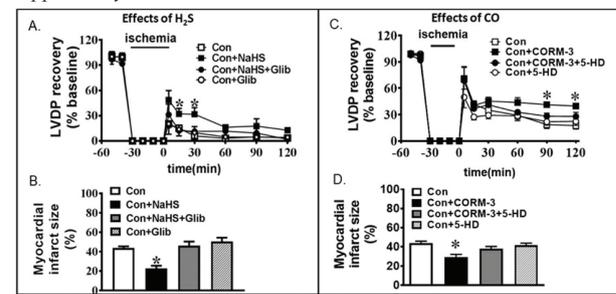


Figure 1. Cardiac recovery and myocardial infarction following I/R. Values are mean ± S.E.M. for left ventricular developed pressure (LVDP) recovery (A and C) and myocardial infarct size (B and D). N=7-10 for each group. Significant differences ( $P<0.05$ ): \*, vs. Con. Two way ANOVA+Tukey test, with repeated measures if appropriate.

## W-121

**Pyrroloquinoline Quinone (PQQ) Supplementation Negatively Alters Skeletal Muscle Gene Expression in Addition to Oxidative Stress/IUGR's Effects.** Allyson Wood<sup>†</sup>,<sup>1</sup> Lin Zhao,<sup>1</sup> Timothy Regnault<sup>\*</sup>.<sup>1,2,3</sup> *<sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>Lawson Research Institute, London, ON, Canada; <sup>3</sup>Children's Health Research Institute, London, ON, Canada.*

**Introduction:** Placental insufficiency results in an adverse in-utero hypoxic environment for the developing fetus, resulting in intrauterine growth restriction (IUGR) associated with oxidative stress and muscle mitochondrial dysfunction. Oxidative stress, the imbalance between free radicals and antioxidants, is believed to be a significant driving force underlying IUGR skeletal muscle mitochondrial dysfunction. PQQ, a novel antioxidant-like compound, shows promise in rescuing postnatal oxidative stress situations, but the mechanisms underlying its antioxidant and positive mitochondrial effects have not been examined in an in-utero environment setting. It is postulated that PQQ will prevent oxidative stress and restore muscle mitochondrial function.

**Methods:** Model 1: Immortalized mouse myoblasts (C2C12) were differentiated for 7 days to form myotubes. PQQ (1uM and 10uM) was administered either every other day for 7 days, or for the last 48 hours, or for the last 5 hours of differentiation. 750uM H<sub>2</sub>O<sub>2</sub> was administered for 24 hours on day 6 of differentiation to induce oxidative stress. qPCR was performed to examine relative mRNA expression of genes of interest. Data were analyzed by one-way ANOVA and Dunnett's multiple comparisons test. Model 2: PQQ was administered to guinea pig dams in drinking water (1mg/L) from mid-gestation until 65d (term ~69d). Fetuses with brain-to-liver ratio >0.65 and body weight <80g were classified as spontaneous IUGR and those outside both thresholds classified as normal intrauterine growth (NG). Male fetal gastrocnemius muscle was collected and qPCR was performed to examine relative mRNA expression of genes of interest. Data were analyzed by one-way ANOVA and Dunnett's multiple comparisons test.

**Results:** H<sub>2</sub>O<sub>2</sub> insult, and unexpectedly PQQ exposure alone, both significantly ( $p<0.05$ ) decreased relative mRNA expression of Cox7a1, Myod1, Ndufb6, Pax7, and Tfam in vitro. Similarly, the relative mRNA expression of Atp5a1, Cox7a1, Cpt1b, CS, Myh1, Myh4, Myod1, Myog, Ndufb6, Pgc1a, Sirt1, Sirt3, Tfam, and Ucp2 was significantly ( $p<0.05$ )

decreased in IUGR gastrocnemius and was not rescued by PQQ. PQQ alone significantly ( $p < 0.05$ ) decreased relative gastrocnemius mRNA expression of CS, Myh4, Myod1, Myog, Pax7, Pgcl1a, Sirt1, and Sirt3.

**Conclusion:** Our results demonstrate that PQQ does not attenuate oxidative stress-induced damage nor promote muscle mitochondrial function in both an in vitro and in vivo model of oxidative stress/IUGR. PQQ alone appears to instead negatively alter key muscle mitochondrial genes and augment the negative effects of oxidative stress/IUGR. These changes have unknown long-term consequences and warrant further investigation.

## W-122

**High Altitude Fetal Genetics and the Influence on Birthweight.** Sara L Hillman\*,<sup>1</sup> Sushil Bhandari,<sup>2</sup> Padma Dolma,<sup>3</sup> Mitali Mukerji,<sup>4</sup> Bhavana Prasher,<sup>4</sup> Hugh Montgomery,<sup>5</sup> David J Williams,<sup>6</sup> Aniket Bhattacharyya,<sup>7</sup> Gianpiero Cavalleri.<sup>2</sup> <sup>1</sup>Univ. College London Institute for Women's Health, London, United Kingdom; <sup>2</sup>Royal College Surgeons Ireland, Dublin, Ireland; <sup>3</sup>Sonam Norboo Memorial Hospital, Leh, Ladakh, India; <sup>4</sup>Institute for Genomics and Integrative Biology, Delhi, India; <sup>5</sup>Univ. College London Institute for Human Health, London, United Kingdom; <sup>6</sup>David Williams, London, United Kingdom; <sup>7</sup>Institute for Genomics and Integrative Biology, Delhi, United Kingdom.

**Introduction:** Birth weight is a complex trait. Genome wide association studies have robustly identified approximately 70 single nucleotide polymorphisms (SNPs) that have a recognised effect. Fetal growth restriction is an important influencer of adverse outcomes. Understanding its genetic aetiology may pave the way for new treatments. Studies suggest that pregnancies at high altitude hypoxic environments experience higher rates of growth restriction. However, populations who have resided for many generations at high altitude, seem to be relatively protected. Genetic adaptation in long ancestry populations may explain the relative birth weight sparing effect seen. Leh in India is located at 3,500m within the Himalayan plateau. Many families have resided at this altitude for thousands of years, making the population a unique group to study potential genetic influences on birth weight.

**Methods:** Over 2 years, 300 pregnant women were recruited from Sonam Norboo Memorial Hospital, Leh Ladakh to the HAPS (hypoxia and pregnancy) study. Collection of umbilical cord blood and placenta was done after birth. Birth details (gestation- with accurate ultrasound dating confirmed, fetal sex, weight and length) were recorded. Samples were processed at the Institute for Genomics, Delhi using the Illumina Global Screening Array. Genetic associations were tested through principal component analysis based on fine structure analysis data and an admixture test and selection analysis testing to investigate birth weight associations.

**Results:** Admixture analysis suggests a considerable component of the contemporary Ladakhi genome descends from ancestral highlander populations residing on the Tibetan plateau for < 35,000 years, with subsequent admixture with neighbouring Indo-European populations. Although no gene variants reached genome wide significance in relation to birth weight analysis this was to be somewhat expected with  $n=300$ . However, the top 60 variants reached  $p$  value of ( $1 \times 10^{-4}$  to  $1 \times 10^{-7}$ ) with enrichment of SNPs previously associated with birth weight (HMG2), along with LING02 (SNP associated with body mass index) and PPP2R2C (body weight gene).

**Conclusion:** This study supports evidence of enrichment in high altitude populations of SNPs in key metabolic genes involved in body mass/ weight and height. Strengthening our findings, a key replication was found in the gene HMG2 that has been previously reported by the EGG birth weight consortium.

## W-123

**The Cardiac Adenosinergic System Is Developmentally Regulated.** Lowell Davis, Samantha Louey, Sonnet S Jonker\*. Oregon Health & Science University, Portland, OR, United States.

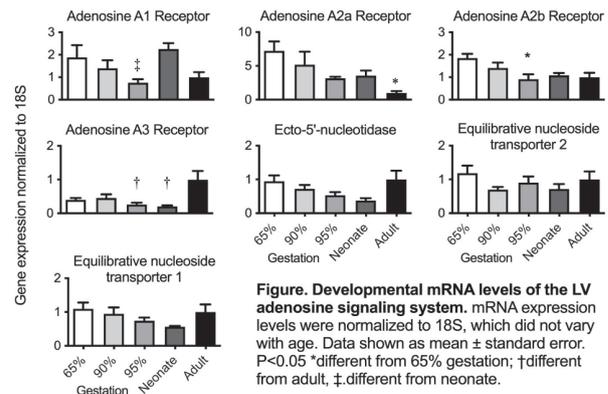
**Introduction:** Adenosine is a metabolic by-product and paracrine factor released by cardiomyocytes. Adenosine acutely improves local oxygen delivery by increasing blood flow, while longer-term effects on myocardial function are mediated by changed mRNA and protein levels.

In addition to acute production of adenosine, the adenosinergic system is transcriptionally regulated by hypoxia. The purpose of this study was to determine how the cardiac adenosinergic system is regulated developmentally, including the transition from relatively hypoxic fetal environment to postnatal life.

**Methods:** PCR was performed on left ventricular myocardium from 65% (94 days) and 93% (136 days) gestation, 4 days postnatal, and adult sheep. Expression of genes involved in adenosine signaling were normalized to 18s ribosomal RNA, and analyzed by ANOVA followed by Tukey's test with significance at  $P < 0.05$ .

**Results:** Neonatal levels of ecto-5'-nucleotidase (NT5E) are lower than adult levels. Levels of equilibrative nucleoside transporters (ENT) 1 and 2 were not significantly different. Adenosine receptor (AR) levels were different by developmental age. A1 levels were lower in the late-term fetus and adult compared to the neonate. A2a levels were higher in the mid-gestation fetus compared to the adult. A3 levels were lower in fetuses and neonates compared to the adult.

**Conclusion:** Together, these data suggest increasing capacity for adenosine production after the neonatal period by the extracellular NT5E, decreasing reliance on the A2a receptor and increasing reliance on the A3 receptor. The importance of the A1 receptor appears to increase transiently in the neonate. These findings indicate developmental differences may be important for acute coronary vasoregulation and for the chronic preconditioning, contractile and growth effects of the cardiac adenosinergic system.



**Figure 7. Developmental mRNA levels of the LV adenosine signaling system.** mRNA expression levels were normalized to 18S, which did not vary with age. Data shown as mean  $\pm$  standard error.  $P < 0.05$  \*different from 65% gestation; †different from adult; ‡different from neonate.

## W-124

**Antenatal Synthetic Glucocorticoid Exposure Modifies Blood-Brain Barrier Function after Birth.** Margaret E Eng†,<sup>1</sup> Alice Kostaki,<sup>1</sup> Stephen G Matthews\*,<sup>1,1,2</sup> <sup>1</sup>The University of Toronto, Toronto, ON, Canada; <sup>2</sup>Sinai Health System, Toronto, ON, Canada.

**Introduction:** Antenatal synthetic glucocorticoids (sGCs) are a life-saving treatment in managing pre-term birth. However, the off-target effects of sGCs can lead to acute changes in P-glycoprotein (P-gp; encoded by *Abcb1*) levels in the fetal blood-brain barrier (BBB). Breast cancer resistance protein (BCRP; *Abcg2*) is another key BBB transporter that is modified by sGCs. sGCs regulate P-gp and BCRP in part via the glucocorticoid receptor (GR). There are known sex differences in GR isoform expression, and therefore signaling. Additionally, studies in young animals exposed to multiple courses of prenatal sGCs found increased HPA axis sensitivity in males compared with females. Drug transporters are essential for fetal brain protection. Whether there are longer-term effects of antenatal sGCs on BBB drug transporters after birth, and whether these are sex-specific are not known. We hypothesized that antenatal sGC treatment will increase P-gp and BCRP expression and function at the BBB of juvenile offspring in a sex-dependent manner.

**Methods:** Pregnant guinea pigs were treated with a single course (gestation day (GD) 50) or 3 courses (GD 40, 50 & 60) of betamethasone (1mg/kg) or vehicle ( $n=10$ ). Cerebral microvessels were collected from post-natal day (PND) 14 male and female offspring to measure gene and protein expression of drug transporters (*Abcb1*, *Abcg2*). Brain endothelial

cells were further isolated from microvessels and cultured to identify differences in P-gp and BCRP function, through accumulation of the fluorescent substrates calcein-AM, and chlorin-e6, respectively.

**Results:** Data were analyzed by student's t-test unless indicated otherwise. Multiple courses of sGC treatment resulted in a significant decrease in P-gp function in endothelial cells derived from male ( $P<0.05$ ), but not female offspring. P-gp protein expression was also decreased ( $P<0.05$ ) in microvessels derived from male offspring that had been exposed to sGC, *in utero*. There was no effect of a single course of sGC on function or expression of P-gp. BCRP was not affected by antenatal sGC. When comparing drug transporter function between sexes, males had significantly higher P-gp function compared to females ( $P<0.05$ , 2-way ANOVA).

**Conclusion:** Our discovery of sex differences in drug transporter function in the BBB is novel, and may underlie sex differences in drug sensitivity. Further, reduced P-gp function at the BBB of young male offspring following prenatal sGC exposure, could result in increased transfer of P-gp substrates into the brain. This is clinically relevant as many of the drugs given in the NICU are actively transported by P-gp. Importantly, single course exposure, the current gold-standard treatment in humans, had no effect on drug transport function after birth.

### W-125

**Targeting Reactive Astrocyte Polarity as a Strategy for Neuroprotection in Acute Perinatal White Matter Injury.** Amanda Brosius Lutz†,<sup>1</sup> Patricia Renz†,<sup>2</sup> Vera Tscherrig†,<sup>2</sup> Marialuigia Giovannini-Spinelli†,<sup>1</sup> Valerie Haesler,<sup>2</sup> Shane Liddelow\*,<sup>3</sup> Andreina Schoeberlein\*,<sup>2</sup> Daniel Surbek\*.<sup>1</sup> <sup>1</sup>University Hospital Insel, University of Bern, Bern, Switzerland; <sup>2</sup>University of Bern, Bern, Switzerland; <sup>3</sup>New York University, New York, NY, United States.

**Introduction:** Acute perinatal white matter injury (WMI) is the most common form of perinatal brain injury in preterm infants resulting from insults to the developing brain during a peak period of oligodendrocyte vulnerability. WMI is characterized by reactive microgliosis, reactive astrocytosis and failed myelination. Recent studies in the injured mature brain show the formation of diverse reactive astrocyte subtypes, some supporting brain repair and other «inflammatory» reactive astrocytes driving neurodegeneration. The specific nature of astrocyte reactivity after WMI remains obscure. We report progress on a basic research project aimed to investigate the formation, function and therapeutic modulation of inflammatory astrocytes in WMI.

**Methods:** We tested the formation of inflammatory astrocytes across multiple rodent WMI models. Inflammatory astrocytes were identified using *in situ* Hybridization (ISH). Microfluidic qRT-PCR of mRNA isolated from immunopanned primary astrocytes evaluated the expression of a panel of reactive astrocyte subtype-specific genes. Purified astrocytes were also analyzed through scRNA seq. C1q/III-a/TNF mutant mice were used to investigate the necessity of inflammatory astrocytes for WMI outcomes. Finally, exosomes purified from mesenchymal stem cells were added to inflammatory astrocytes *in vitro* to evaluate influence on astrocyte polarization.

**Results:** Experiments demonstrate a significant increase in the prevalence of inflammatory astrocytes after WMI. qRT-PCR supports this finding, revealing an upregulation of a panel of inflammatory astrocyte-specific transcripts. scRNAseq provides a deeper characterization of reactive astrocyte subtypes. Ongoing experiments in mutant mice test whether inflammatory astrocytes are central drivers of WMI pathogenesis. *In vitro* experiments demonstrate cell-autonomous changes in astrocyte polarization in the presence of exosomes.

**Conclusion:** Our experiments demonstrate the formation of inflammatory reactive astrocytes in WMI, test the ability of these cells to drive WMI outcomes and work towards a deep characterization of astrocyte polarity in this disease. Further studies will investigate therapeutic approaches to modifying astrocyte polarity, aiming to prevent WMI and improve longterm outcome of neonates.

### W-127

**Targeted Blockade of STAT-3 Signaling Inhibits Cell Proliferation of Uterine Leiomyosarcoma Cells.** Collin Sittler†, Minnie Malik, Joy Britten, Paul Driggers, William H Catherino\*. *Uniformed Services University of Health Sciences, Bethesda, MD, United States.*

**Introduction:** Uterine leiomyosarcoma (uLMS) is a rare and aggressive tumor. Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that plays a key role in cell proliferation and survival. It has been previously reported that the JAK/STAT pathway is constitutively active in many cancers and diseases including uterine leiomyosarcoma. We investigated the hypothesis that direct inhibition of STAT3 decreases cell proliferation of uLMS.

**Methods:** Leiomyosarcoma cells (SK-UT-1) were exposed to Stattic, a small molecule inhibitor of STAT3 activation and nuclear translocation. Cell proliferation was measured using a 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Western blotting was used to analyze expression of cyclin D1, beta-catenin, active Y705-phosphorylated STAT3 (p-STAT3), Bcl-2, and Caspase-3. Immunohistochemistry was used to detect p-STAT3 in 3D cell cultures of SK-UT-1 and in normal myometrium and leiomyoma tissue specimens.

**Results:** SK-UT-1 cells exhibited a 2.52-fold higher expression of p-STAT3 compared to a normal uterine myometrial cell line, as assessed by western blotting. IHC results also demonstrated significantly higher p-STAT3 immunoreactivity in 3D cultures of SK-UT-1 compared to normal myometrial tissue. uLMS cells treated with Stattic showed a concentration-dependent decrease in cell proliferation with 54% growth inhibition observed at 1uM concentration after 48 hours ( $p<0.01$ ). Initiation of apoptosis was indicated by an increase in expression of Caspase-3 following treatment with 2.5uM or 5uM of Stattic for 2 hours ( $1.91\pm 0.34$ ,  $p=0.080$  and  $2.21\pm 0.50$ ,  $p=0.073$ ). Exposure to Stattic resulted in a concentration dependent decrease in p-STAT3 after 2 hours of treatment. 2.5uM Stattic produced a 2.9-fold ( $0.34\pm 0.04$ ,  $p<0.001$ ) decrease in p-STAT3. Cyclin D1 expression levels were 3.6-fold higher in uLMS cells compared to myometrial cells. At 2 hours of exposure a concentration dependent increase was observed in cyclin D1 (3.6-fold) and beta-catenin (3.2-fold) with 2.5uM Stattic. After 6 hours of exposure to 2.5uM Stattic, SK-UT-1 cells demonstrated a 1.7-fold ( $0.58\pm 0.04$ ,  $p<0.001$ ) decrease in cyclin D1. By 24hrs decreases in p-STAT3 and Cyclin D1 were observed at lower concentrations of Stattic. To determine if uLMS cells responded to upstream effector of JAK/STAT pathway, we exposed cells to 10ng/ml of IL-6 for 3 hours and observed a  $3.64\pm 0.33$  ( $p<0.001$ ) increase in expression of p-STAT3.

**Conclusion:** Targeted inhibition of STAT3 with the small molecule inhibitor Stattic induced a decrease in cell proliferation. This suggested that the JAK/STAT pathway may play a key role in cell proliferation of uterine leiomyosarcoma and serve as a possible target for development of new treatment therapies.

### W-128

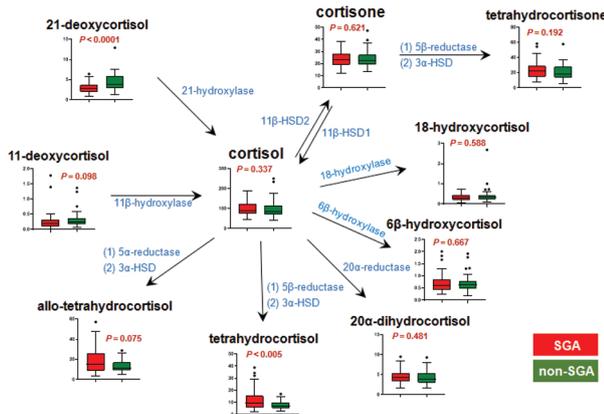
**First Trimester Maternal Cortisol Signatures in Small-for-Gestational Age Infants.** Chaelin Lee,<sup>1</sup> Seung Mi Lee,<sup>2</sup> Dong Jun Byun,<sup>1</sup> So Yeon Kim,<sup>2</sup> Hugh I Kim,<sup>3</sup> Do Yup Lee,<sup>4</sup> Young Mi Jung,<sup>2</sup> Chan-Wook Park,<sup>2</sup> Joong Shin Park,<sup>2</sup> Man-Ho Choi.<sup>1</sup> <sup>1</sup>KIST, Seoul, Korea, Republic of; <sup>2</sup>Seoul National University College of Medicine, Seoul, Korea, Republic of; <sup>3</sup>Korea University, Seoul, Korea, Republic of; <sup>4</sup>Seoul National University, Seoul, Korea, Republic of.

**Introduction:** Abnormal maternal hypothalamus-pituitary-adrenal axis is associated with fetal growth, and we hypothesized that the alteration in metabolic signatures of cortisol might be detectable during early pregnancy. The objective of this study was to identify predictable maternal serum signatures in cortisol metabolism during the first trimester of women who are expected to deliver small-for-gestational age (SGA) neonates.

**Methods:** In this prospective cohort study, maternal serum samples were obtained from 112 singleton pregnant women (with and without SGA, n = 56 each) at 10~14 gestational weeks and the levels of cortisol and its precursors and metabolites were evaluated by liquid chromatography-mass spectrometry.

**Results:** Increased maternal serum levels of tetrahydrocortisol (THF,  $11.82 \pm 8.16$  ng/mL vs.  $7.51 \pm 2.90$  ng/mL,  $P < 0.005$ ) and decreased 21-deoxycortisol (21-deoxyF,  $2.98 \pm 1.36$  ng/mL vs.  $4.33 \pm 2.06$  ng/mL,  $P < 0.0001$ ) were observed in pregnant women carrying SGA infant. In conjunction with individual steroid levels, metabolic ratios corresponding to the activity of related enzymes were calculated. In addition to increased THF/cortisol ratio ( $P < 0.006$ ), the SGA group showed a significant increase in the two metabolic ratios including cortisol/11-deoxycortisol (F/11-deoxyF;  $P < 0.03$ ) and cortisol/21-deoxycortisol (F/21-deoxyF;  $P < 0.0003$ ) indicating cortisol biosynthesis. The ROC curve generated in combination with three variables of 21-deoxyF concentration and two metabolic ratios of F/21-deoxyF and THF/F resulted in AUC = 0.824 (95% confidence interval, 0.713 ~ 0.918).

**Conclusion:** A significant decrease in maternal serum levels of 21-deoxyF and an increase in two metabolic ratios of F/21-deoxyF and THF/F, indicating cortisol biosynthetic rate, represent a reliable biomarker for the prediction of SGA in the first trimester.



## W-129

**Differential Expression Changes in Human Decidua at Labor, Term versus Preterm - Role for Upstream Targets in the PG Pathway.** Kylie Hornaday<sup>†</sup>, Moss Bruton Joe<sup>†</sup>, Stephen Wood<sup>\*</sup>, David Anderson<sup>\*</sup>, Donna Slater<sup>\*</sup>. University of Calgary, Calgary, AB, Canada.

**Introduction:** The decidua, situated at the maternal fetal interface, is hypothesized to play a role in the onset of labor, and this process may be mediated by the prostaglandin (PG) synthesis pathway (an important clinical target for labor induction and augmentation). Most studies, however, rely solely on tissue drawn from fetal membranes and adherent scrapes of decidua, which is likely not reflective of the entire decidua interface. PG synthesis is highly complex with at least three rate-limiting steps, i) the release of arachidonic acid (AA) by phospholipase A2 (PLA2) ii) conversion of AA to PG intermediates by PTGS1/2, and iii) isomerization by select PG synthases. We hypothesize that the decidua 1) exhibits a distinct expression profile of genes in the PG pathway with the onset of labor, and 2) these genes exhibit differential expression in preterm labor (PTL) compared to term labor (TL). The aim of this study is to conduct a complete expression profile of the PG pathway in a unique set of decidua biopsy samples.

**Methods:** Decidual samples dissected from the myometrium at cesarean section were used to assess PG gene expression by RNAseq and qRT-PCR (n=7 preterm non-labor (PTNL), n=11 preterm labor (PTL), n=31 term non-labor (TNL), n=31 term labor patients (TL)). Expression analyses were conducted using student's t-test, one-way ANOVA, and adjusted for multiple testing using the Benjamini-Hochberg method.

**Results:** RNAseq analysis identified 30 of the 53 unique PG genes to be highly expressed in decidua. We observe increased expression in PTGS2 ( $p=0.011$ ) and PTGES ( $p=0.003$ ) and decreased expression of PG-D synthases ( $p<0.05$ ) with labor (term or preterm). In contrast, the expression of upstream enzymes is differentially expressed with pregnancy

outcome. At term, we observe a decrease in PLA2 subtypes 2D, 4B and 6 while preterm labor is associated with lower expression of PLA2G16 and PLAA ( $p<0.05$ ).

**Conclusion:** We observe that downstream PG synthases are differentially expressed with labor irrespective of gestational age at delivery. However, cases of preterm labor exhibit differential expression profiles when compared to term births at the level of upstream PG enzymes (PLA2). The presence of multiple PLA2 subtypes within the decidua suggests the potential for fine tuning the release of AA during PG synthesis during labor. Further investigation of upstream activity of PG synthase may provide insight on the mechanism of PTL.

## W-130

**Attenuated Piezo1-Mediated Vasodilation in the Reduced Uteroplacental Perfusion Model of Preeclampsia in Rat.** Danielle Marasat<sup>†</sup>, Susannah Chilton, Annie Glessner-Fischer, Nga Ling Ko<sup>\*</sup>. The University of Vermont, Burlington, VT, United States.

**Introduction:** Women with preeclampsia (PE) and intrauterine growth restriction frequently demonstrate impaired uteroplacental blood flow (UPBF) and inadequate uterine vascular adaptation. Endothelial nitric oxide (NO) mediates arterial vasodilation and expansive remodeling of the maternal uterine circulation to ensure normal UPBF during gestation. We, and others, have found that shear stress secondary to hemochorial placentation, which reduces distal resistance, is the principal physiological stimulus for both NO and remodeling. Knockdown of Piezo1, a shear-stress sensitive cation channel, was shown to inhibit increases in endothelial NOS (eNOS) activity. Using the reduced uteroplacental perfusion pressure (RUPP) model of PE in rat, we tested the hypothesis that Piezo1 functionality is impaired in PE.

**Methods:** The RUPP surgery was performed on time-pregnant Sprague-Dawley rats (12-14 week old) at Day 10/22 of gestation (RUPP, n=6). Briefly, the abdominal aorta (below the renal arteries and above the aortic bifurcation) and the uterine arteries of both uterine horns were partially occluded with silver clips to reduce blood flow into the uterine circulation. Pregnant rats with sham surgery (without clips) were served as controls (Sham, n=4). To determine the effect of NOS on Piezo1 functionality in pregnancy, rats (n=6) were given L-NAME in drinking water (0.5 g/L) to inhibit NOS from Day 10 of gestation until euthanasia, with age-matched, normal pregnant rats as control (LP, n=4). Animals were euthanized at late pregnancy (day 20). Main uterine arteries (MUAs) were isolated, pressurized and assessed in physiological conditions by pressure myography. The functionality of Piezo1 channels was evaluated by Yoda1 (10 and 20  $\mu$ M), a chemical Piezo1 activator that mimics the effect of fluid shear stress on endothelial cells and induces vasodilation.

**Results:** The RUPP surgery was performed in early-pregnancy before prominent uterine vascular remodeling. In RUPP, unstressed cervical-end MUAs tended to have smaller lumen diameter ( $p=0.12$ ) while the pressurized MUA diameter and distensibility were significantly increased. Surprisingly, there was no significant difference in pup or placental weights. Yoda1-induced vasodilation (20  $\mu$ M) was inhibited by ~45% in RUPP vs. Sham MUAs ( $27 \pm 4\%$  vs.  $49 \pm 8\%$ ,  $p=0.016$ ). With *in vivo* NOS inhibition, MUA diameter and distensibility were significantly decreased as expected. At 20  $\mu$ M of Yoda1, vasodilation was reduced ~68% in the L-NAME group ( $20 \pm 5\%$  vs.  $62 \pm 7\%$ ,  $p<0.0001$ ).

**Conclusion:** Yoda1-induced MUA vasodilation was reduced in the RUPP and L-NAME treated groups, indicating that Piezo1 functionality and/or its signaling were impaired by reduced UPBF in PE. These findings suggest that therapeutic interventions targeting Piezo1 signaling may be beneficial for treating pregnancy complications such as PE.

**W-131**

**Pregnant Women Release Greater Levels of Small Extracellular Vesicles after an Acute Bout of Moderate-Intensity Physical Activity Compared to Non-Pregnant Women.** Shuhiba Mohammad<sup>†</sup>,<sup>1</sup> Kelly Ann Hutchinson<sup>†</sup>,<sup>1</sup> Danilo Fernandes da Silva<sup>†</sup>,<sup>1</sup> Dylan Burger\*,<sup>1,2</sup> Kristi B Adamo\*.<sup>1</sup> <sup>1</sup>University of Ottawa, Ottawa, ON, Canada; <sup>2</sup>Ottawa Hospital Research Institute, Ottawa, ON, Canada.

**Introduction:** Engagement in physical activity (PA) during pregnancy mediates numerous health benefits for both the mother and fetus. The physiological mechanisms contributing to these benefits are unclear but are thought to act upon the placenta. In men, acute PA results in the release of small extracellular vesicles (EVs) into circulation which may be important for tissue cross-talk during exercise. It is unknown whether small EVs are released in women after an acute bout of PA, and whether pregnancy impacts this response. The objective of this study was to define the circulating small EV profile after a single bout of moderate-intensity PA by size and concentration in healthy pregnant and non-pregnant women. **Methods:** Pregnant (13-28 weeks gestation, N=10) women and non-pregnant controls (N=9) performed a single session of moderate-intensity treadmill walking for 30min. Plasma was collected immediately pre- and post-PA, and small EVs (<200 nm) were isolated by differential ultracentrifugation. The presence of EVs was confirmed by western blotting for the small EV markers TSG-101 and Flotillin-1. EVs were quantified by size and concentration using nanoparticle tracking analysis. Percent change post-PA was compared between groups for mean vesicle size with an unpaired *t*-test and ANCOVA was used to compare the percent change between groups post-PA for concentration adjusting for baseline as a covariate. Significance was set at  $p < 0.05$ .

**Results:** At baseline, pregnant women had higher levels of small EVs compared to non-pregnant women ( $1.83 \times 10^{10} \pm 1.25 \times 10^{10}$  particles/mL and  $8.11 \times 10^9 \pm 4.04 \times 10^9$  particles/mL,  $p = 0.03$ ). Mean vesicle size was not different between groups. After an acute bout of moderate-intensity PA, the mean difference for the percent change of small EVs was 88 between pregnant versus non-pregnant women (CI 95% = 7-169,  $p = 0.04$ ,  $\eta_p^2 = 0.25$ ). Percent change of mean vesicle size showed no differences pre- versus post-PA. All isolated EV fractions were positive for TSG-101 and Flotillin-1.

**Conclusion:** On average, pregnant women had a greater release of small EVs after a single bout of moderate-intensity PA when compared to non-pregnant women.

**W-132**

**Suppression of Maternal Iron-Regulatory Hormone Heparin Is Essential for Healthy Pregnancy and Is Mediated by Secreted Placental Proteins.** Veena Sangkhae,<sup>1</sup> Yoel Sadovsky,<sup>2</sup> Tomas Ganz,<sup>1</sup> Elizabeta Nemeth\*,<sup>1</sup> <sup>1</sup>UCLA, Los Angeles, CA, United States; <sup>2</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States.

**Introduction:** Iron is essential for maternal and fetal health but the molecular mechanisms ensuring increased iron availability during pregnancy are not well understood. Heparin is the key iron-regulatory hormone and functions by blocking iron absorption, recycling and mobilization from stores, effectively decreasing plasma iron levels. In healthy human and rodent pregnancies, maternal hepcidin decreases starting in the second trimester and is nearly undetectable by late pregnancy, and it is hypothesized that this allows for greater iron availability for transfer to the fetus. However, fetal hepcidin may also regulate iron transfer across the placenta. We explored the regulation of maternal and fetal hepcidin during pregnancy, and their effect on iron homeostasis.

**Methods:** 1) To determine if maternal or embryo hepcidin regulates iron homeostasis, we used mouse models and generated combinations of dams and embryos lacking hepcidin or not and quantified embryo iron endowment. 2) To determine consequences of elevated maternal hepcidin on pregnancy, pregnant mice received daily injections of a hepcidin mimetic starting in the second trimester and we assessed pregnancy outcomes and iron parameters. 3) We also developed a bioassay to identify pregnancy-associated hepcidin regulators.

**Results:** 1) Maternal but not fetal hepcidin determines embryo iron endowment in a healthy pregnancy. Maternal hepcidin was inversely related to embryo iron stores: embryos from hepcidin-deficient dams had higher hepatic iron stores ( $P < 0.001$  Two-way ANOVA), regardless of embryo hepcidin genotype. 2) Augmented maternal hepcidin during the second half of pregnancy in mice led to dose-dependent embryo iron deficiency, anemia, and even death. Even with mild maternal hepcidin elevation, where maternal hematological parameters were unaffected, embryos developed systemic iron deficiency which even led to decreased brain iron content ( $0.7 \pm 0.2$  to  $0.3 \pm 0.1$   $\mu\text{g/g}$ ,  $P = 0.002$ ), indicating that embryos are remarkably sensitive to maternal iron restriction. 3) Despite the critical role of maternal hepcidin suppression for healthy pregnancy, the physiological mechanism of suppression remains unknown. Using our *in vitro* bioassay with primary mouse hepatocytes or human hepatocytes, we determined that the placental trophoblast is the source of a hepcidin-suppressing factor. Treatment with trophoblast supernatants suppressed hepcidin mRNA more than 10-fold ( $P < 0.001$ ) for up to 48hr. Studies to determine the identity of the placenta-derived hepcidin suppressor(s) are ongoing.

**Conclusion:** Suppression of maternal hepcidin is essential to ensure adequate iron supply for transfer to the fetus and for the increase in maternal red blood cell mass. A placenta-derived hepcidin suppressor likely plays an important role in this adaptation.

**W-133**

**Women Who Develop Placental Maternal Vascular Malperfusion Show Evidence of a Procoagulant Phenotype in Early Pregnancy.**

Carole A McBride, Maria C Bravo, Kelley C McLean, Thomas Orfeo, Ira M Bernstein\*. *University of Vermont Larner College of Medicine, Burlington, VT, United States.*

**Introduction:** Placental maternal vascular malperfusion (MVM) is associated with adverse pregnancy outcomes, including preeclampsia and gestational hypertension (PAH). We sought to examine whether women who develop MVM have increased coagulation potential, which may influence early placentation.

**Methods:** Platelet-poor plasma from 29 women was collected pre-pregnancy (PP) in the follicular phase, and early pregnancy (EP), 12-13 weeks). Outcome data and placentas were collected at delivery, with placental histopathologic features of MVM characterized, including decidual arteriopathy (DA) and infarcts or agglutination (INAG). Plasma was analyzed for levels of factors II, V, VII, VIII, IX, X, antithrombin (AT), and Protein C, reported as % of mean physiological. Thrombin generation (TG) parameters (lagtime, peak level, rate, and endogenous thrombin potential [ETP]) were assessed at each study visit, using tissue factor (TF) initiation +/- soluble thrombomodulin (TM). Comparisons were made between PP, EP and changes from PP to EP within each parameter in the context of MVM. Mean  $\pm$  standard deviation are presented.

**Results:** Women were  $30 \pm 4$  years of age, body mass index of  $25.9 \pm 5.6$   $\text{kg/m}^2$ , 90% white race, and 69% nulliparous. DA was identified in 13 placentas, 7 from pregnancies complicated by PAH. INAG was identified in 13 placentas, 9 with PAH. DA and INAG were identified in 7 placentas, 5 from pregnancies with PAH. While there were no PP differences between women +/- placental DA, those who developed DA showed a greater increase in TG parameters between PP and EP ( $\Delta$ lagtime DA =  $-0.11 \pm 0.15$  vs.  $0.11 \pm 0.33$  min.;  $p = .046$ ;  $\Delta$ peak DA =  $143.7 \pm 43.2$  vs  $101.5 \pm 39.7$  nM,  $p = .02$ ). The same held true for TG +TM for women with DA ( $\Delta$ rate DA =  $77.1 \pm 26.4$  vs  $49.2 \pm 49.2$  nM/min,  $p = .03$ ;  $\Delta$ peak DA =  $169 \pm 56$  vs  $115 \pm 69$  nM,  $p = .047$ ).

PP, women with placental INAG had higher factor VIII compared to those without INAG (INAG =  $109.3 \pm 9.0$  vs  $80.7 \pm 23.7\%$ ,  $p = .03$ ) and lower AT (INAG =  $90.6 \pm 4.9$  vs  $98.3 \pm 8.3\%$ ,  $p = .02$ ). In EP, AT remained lower (INAG =  $82.5 \pm 6.8$  vs  $92.4 \pm 8.3\%$ ,  $p = .01$ ). TF-initiated TG was greater in EP for INAG (peak INAG =  $400 \pm 67$  vs  $357 \pm 42$  nM,  $p = .07$ ; rate INAG =  $160.6 \pm 44.4$  vs  $128.6 \pm 24.5$  nM/min,  $p = .04$ ). TF+TM TG was also higher in EP (peak INAG =  $312 \pm 60$  vs  $237 \pm 75$  nM,  $p = .01$ ; rate  $135.7 \pm 34.4$  vs  $102.9 \pm 37.6$  nM $\cdot$ min,  $p = .04$ ; ETP INAG =  $1627 \pm 315$  vs  $1246 \pm 423$  nM $\cdot$ min,  $p = .02$ ). Comparisons from PP to EP resulted in a larger decrease in TF-initiated TG ( $\Delta$ lagtime INAG =  $-0.11 \pm 0.11$

vs  $0.12 \pm 0.34$  min,  $p=.04$ ) and greater increase of TF+TM TG ( $\Delta$ peak INAG=  $180 \pm 67$  vs  $106 \pm 48$  nM,  $p=.005$ ;  $\Delta$ ETP INAG= $1001 \pm 381$  vs  $607 \pm 284$ ,  $p=.008$ ).

**Conclusion:** Enhanced TG potential from PP to EP in women who develop features of MVM, characterized by DA and INAG, suggests a more hypercoagulable phenotype in these women, which may result in abnormalities at the fetal/maternal interface and ultimately influence pregnancy-associated morbidity.

**W-134**

**Paternal Deficiency of Complement Component C1q Leads to Vascular Dysfunction in Apolipoprotein-E Female Knockouts: A Novel High-Risk Mouse Model of Preeclampsia.** Mary Gemmel†, Elizabeth Sutton, Marcia Gallaher, Robert W. Powers\*. *University of Pittsburgh, Pittsburgh, PA, United States.*

**Introduction:** Preeclampsia is clinically defined by new onset hypertension and proteinuria, and affects 3-5% of pregnancies worldwide. Preeclampsia is associated with an increased risk of later-life cardiovascular disease. However, it remains difficult to interpret whether adverse outcomes and mechanisms of preeclampsia differ between healthy women and women predisposed to poor vascular health. To address these questions, relevant animal models are needed. Previous work demonstrates that breeding complement component C1q knockout (C1q<sup>-/-</sup>) males to wildtype female mice results in preeclampsia-like phenotypes. The current aim was to determine whether C1q<sup>-/-</sup> male mice induce preeclampsia-like vascular dysfunction in apolipoprotein-E knockout (ApoE<sup>-/-</sup>) females who are predisposed to poor vascular health.

**Methods:** ApoE<sup>-/-</sup> female mice were time mated either to male C1q<sup>-/-</sup> (ApoE<sup>-/-</sup> x C1q<sup>-/-</sup>) or wildtype C57BL/6 mice (ApoE<sup>-/-</sup> x C57). Blood pressure was measured at the end of pregnancy from gestation day (GD) 14.5-17.5. Body weight, heart weight, heart/body weight ratio and ex-vivo vascular function data were collected at GD 17.5.

**Results:** Preliminary data indicate that high-risk ApoE female mice bred to C1q<sup>-/-</sup> male mice (ApoE<sup>-/-</sup> x C1q<sup>-/-</sup>) exhibit elevated systolic blood pressure ( $p<0.01$ ), diastolic blood pressure ( $p<0.01$ ), and mean arterial pressure ( $p<0.01$ ) when compared to ApoE<sup>-/-</sup> x C57 controls. Preeclampsia-like ApoE<sup>-/-</sup> females had larger heart weights ( $p=0.03$ ) and heart to body weight ratios ( $p=0.01$ ) compared to ApoE<sup>-/-</sup> controls. Further, preeclampsia-like ApoE<sup>-/-</sup> females exhibited vascular dysfunction evidenced by increased sensitivity to phenylephrine ( $p<0.03$ ) and blunted endothelial-dependent relaxation ( $p<0.01$ ).

**Conclusion:** Breeding hypercholesterolemic ApoE<sup>-/-</sup> female mice to C1q<sup>-/-</sup> male mice induces preeclampsia-like vascular dysfunction during pregnancy. This model provides a novel method to investigate preeclampsia in individuals with a predisposition toward poor cardiovascular health and may improve our understanding of preeclampsia subtypes. This project was supported by the American Heart Association Go Red for Women 16SFRN27810001.

**W-135**

**Blood Pressure and Hypertensive Disorders of Pregnancy at High Altitude: A Systematic Review and Meta-Analysis.** Imogen D Grant†, Dino A Giussani\*, Catherine E Aiken\*. *University of Cambridge, Cambridge, United Kingdom.*

**Introduction:** Exposure to high altitude ( $\geq 2500$ m) is associated with increased systemic blood pressure (Narvaez-Guerra et al., *Hypertension* 72:567-578, 2018). During pregnancy, even mild elevation of maternal blood pressure is associated with reduced birth weight and increased prevalence of pregnancy complications (Macdonald-Wallis et al., *Hypertension* 64:36-44, 2014). Here, we aimed to systematically assess the impact of altitude on maternal blood pressure at term and on the prevalence of hypertensive disorders of pregnancy.

**Methods:** PubMed, Ovid EMBASE, Cochrane Library, Medline, Web of Science and clinicaltrials.gov were searched (inception to 11/11/2020). Observational, cohort, or case-control studies were included if they reported a high-altitude and appropriate control pregnant population. Studies published >50 years ago were excluded. Two reviewers independently assessed articles for eligibility and risk of bias.

**Results:** At high altitude, maternal systolic and diastolic blood pressure at term was higher than at low altitude ( $4.4 \pm 2.2$  mmHg,  $p<0.05$ ,  $3.8 \pm 1.0$  mmHg,  $p<0.001$  respectively,  $n = 4140$ , 11 studies, Figure 1). Hypertensive disorders of pregnancy were more common at high altitude (Odds Ratio (OR): 1.31, 95% CI: [1.02, 1.67],  $I^2=55.0\%$ ,  $p<0.05$ ,  $n = 20,041$ , 5 studies). Prevalence of gestational hypertension was nearly twice as high at high altitude (OR: 1.90 [1.17, 3.07],  $I^2=62.3\%$ ,  $p<0.01$ ,  $n = 654,772$ , 6 studies) but there was no significant difference in prevalence of pre-eclampsia (OR: 0.64 [0.28, 1.48],  $I^2=99.8\%$ ,  $p=0.30$ ,  $n = 898,528$ , 8 studies). The likelihood of stillbirth was increased by 63% in pregnancies at high compared to low altitude (OR: 1.63 [1.15, 2.32],  $I^2=83.4\%$ ,  $p<0.01$ ,  $n = 564,489$ , 8 studies).

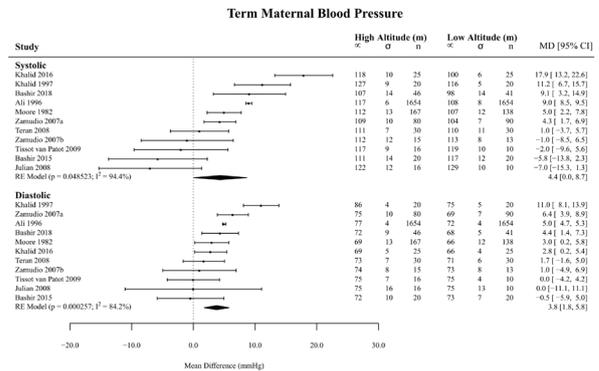


Figure 1: Blood pressure meta-analysis. Results expressed as mean difference (random effects model) with 95% CI. Black diamond represents overall effect.

**Conclusion:** Maternal blood pressure is higher at term in pregnancies at high compared to low altitudes, accompanied with increased risk of gestational hypertension but not pre-eclampsia. Risk of stillbirth at high altitude is also increased. With a growing population residing at high altitude worldwide, it is essential to clearly define the associated risk of adverse pregnancy outcomes.

**W-136**

**Cardiovascular Effects of Extra Virgin Olive Oil (EVOO) in Healthy Reproductive-Aged Women: A Randomized Controlled Trial.** Erin A Morris,<sup>1</sup> Carole A McBride,<sup>1</sup> Megan Boyer†,<sup>1</sup> Lorinda Roberts,<sup>1</sup> Joan Skelly,<sup>1</sup> Maurizio Mandalà,<sup>2</sup> Ira M Bernstein\*. <sup>1</sup>University of Vermont Larner College of Medicine, Burlington, VT, United States; <sup>2</sup>University of Calabria, Rende (CS), Italy.

**Introduction:** In individuals with metabolic risk factors, extra virgin olive oil (EVOO) intake has been associated with reduced risk of cardiovascular morbidity by improving lipoprotein profile, increasing insulin sensitivity and decreasing blood pressure, inflammation and oxidative stress. Studies in pregnant women show reduced risks of gestational diabetes, preeclampsia and fetal growth restriction in those who adhere to Mediterranean-based diets high in EVOO. We aimed to investigate the effects of EVOO consumption on cardiovascular function and laboratory markers of metabolic dysfunction in healthy reproductive-aged women.

**Methods:** Healthy nulliparous women were randomized to receive 45 ml (40 g) of EVOO high in oleic acid and phenolic content daily for 8 weeks or an identical dose of a control sunflower seed oil. Participants and study personnel were blinded to group assignment. Women underwent detailed cardiovascular assessment at baseline and following treatment, including assessment of blood pressure, cardiac output, pulse-wave velocity (PWV), calculation of arterial distensibility and beta stiffness, and response to volume challenge. Lipid profiles and markers of insulin sensitivity were assessed at both study visits. Group differences at baseline were assessed by Student's t-test and Fisher's exact test for continuous and dichotomous variables, respectively. Repeated measures ANOVA was used to assess the group by time interaction.

**Results:** 12 women were randomized to EVOO and 15 to the control oil. Women were similar at baseline, although women in the EVOO group were more likely to report a family history of hypertension (90%, 40%,  $p = .03$ ), had faster brachial PWV ( $8.8 \pm 1.4$  m/s,  $7.5 \pm 1.4$  m/s,  $p = .03$ ),

greater blood pressure response to volume challenge ( $160.6 \pm 82.5$ ,  $49.2 \pm 115.5$ ,  $p = .01$ ) and had lower HDL ( $53.2 \pm 10.3$  mg/dL,  $64.3 \pm 12.9$  mg/dL,  $p = .02$ ). Compliance with the prescribed volume of oil was high in both groups (EVOO 97.8%, control 99.5%). Following the 8 week intervention, women randomized to EVOO had relatively reduced fasting insulin ( $-0.31 \pm 0.52$  uU/mL,  $p = .03$ ), reduced HOMA-IR ( $-0.054 \pm 0.12$ ,  $p = .046$ ), but increased LDL cholesterol ( $+5.1 \pm 4.0$  mg/dL,  $p = .047$ ). Blood pressure was not different between groups, but there was a tendency toward slower PWV, lower blood pressure response to volume challenge and lower hemoglobin A1c in the women randomized to EVOO.

**Conclusion:** Dietary supplementation with EVOO high in oleic acid and phenols was well tolerated and resulted in improved insulin sensitivity, but higher LDL in our pilot study in healthy nulliparous women. A larger trial in pregnant women will determine whether this improved insulin sensitivity is associated with lower risks of pregnancy morbidity.

### W-137

**Continuous Core Body Temperature and the Onset of Parturition in Humans.** Elise N Erickson, Kierstyn Tuel, Leslie Myatt, Leonardo Pereira. Oregon Health and Science University, Portland, OR, United States.

**Introduction:** Clinicians have no reliable method for determining future onset of labor in term or preterm pregnancies with precision. The purpose of this study was to use continuous body temperature measurement in healthy pregnancies to determine if a temperature decrease occurs in the days prior to the onset of labor as is observed in other mammals. This method of labor prediction has been used for veterinary care (e.g. horse, sheep, dog); however, this has not been evaluated in human pregnancy.

**Methods:** Multiparous pregnant individuals at 36-38 weeks of gestation carrying a singleton pregnancy were included. We excluded individuals who have HTN, GDM, BMI >30.0, work night or rotating shifts. The thermometer was an axillary device worn in the inner aspect of the upper arm, held in place with adhesive patches, monitors were worn continuously for 24 hours and changed to the alternate side daily. Temperatures were recorded every 4 seconds, synced on a smartphone application and stored in cloud-based platform. Data averaged over one-minute intervals was downloaded and used as the unit of analysis. Temperatures were then standardized within each individual (calculated z-score for each temperature). A total of 685,198 temperatures were analyzed using mixed effects regression within each 24 hour period of time prior to the participant's report of when labor symptoms were noted (participant as the random effect). Data were further considered by day/night periods (6am-10pm/ 10pm-6am). The models were adjusted by the gestational age at which the measurement of temperature occurred across the study period and by night/day period. Comparisons of temperature across the study period were also made between participants who began laboring spontaneously and those who did not.

**Results:** Twenty-eight participants enrolled in the study and 25 completed data collection. Mean participant age was 29.3 (4.2) years and mean pregnancy body mass index was 23.5 (3.2) kg/m<sup>2</sup>. Vaginal birth occurred in 23 participants. Nine participants underwent a labor induction at a mean (SD) gestational age of 40.6 (0.72) weeks. The remainder of the sample began laboring spontaneously at a mean (SD) of 40.1(0.89) weeks. Models adjusted for gestational age and day/night measurement revealed that individuals had a significant decrease in body temperature in the 7 days prior to spontaneous labor onset with a nadir of day 3 before labor symptoms ( $-1.27^{\circ}\text{F}$ , 95%CI  $-1.37$ -  $-1.11$ ).

**Conclusion:** This pilot data demonstrate that human female body temperature decreases in the days preceding the onset of a physiologic labor similar to what is documented in various mammalian species. This pattern did not emerge from within the sample of individuals prior to perception of labor symptoms during induction of labor. Further study will determine if real-time temperature measurement and changes in temperature may be useful for prediction of future labor onset.

### W-138

**The Sodium-Dependent Phosphate Transporter Slc20a2 Protects the Placenta from Ectopic Calcification.** Ana Correia-Branco<sup>†</sup>,<sup>1</sup> Ciara Benson,<sup>2</sup> Nirmala Jayaraman<sup>†</sup>,<sup>1</sup> Olga Kashpur<sup>†</sup>,<sup>1</sup> Mary C. Wallingford<sup>\*</sup>,<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>University of Washington, Seattle, WA, United States.

**Introduction:** Mechanisms of maternal-fetal phosphate transport flux and the biomedical relevance of placental vascular calcification (calcium-phosphate mineral deposition) remain largely unknown. Expression of the placental phosphate transporter Slc20a2 is decreased in early preeclampsia (PE) while adenosine and expression of the adenosine receptor are both increased. Both Slc20a2 and adenosine signaling are described to mediate processes promoting vascular calcification. Furthermore, Slc20a2 knockout (KO) mice have been described as a model of placental calcification, thus providing a genetic link and preclinical model. We propose that Slc20a2 protects the placenta from ectopic calcification by maintaining homeostatic phosphate levels in balance with ATP metabolism and adenosine signaling and hypothesize that Slc20a2 loss will upregulate extracellular phosphate levels and adenosine signaling in placenta.

**Methods:** Phosphate P<sup>33</sup> uptake and extracellular calcium deposition were assessed on BeWo cells after cGMP induction by forskolin as a model of human syncytiotrophoblast. Placental gene expression was examined in human (n=200) and mouse (n=15) by microarray and RNAseq. The molecular requirement for placental Slc20a2 was assessed with the Slc20a2 KO mouse line by protein analyses after Slc20a2 loss was confirmed by in situ hybridization. The relationship between fetal skeletal ossification and ectopic placental calcification (E17.5) was evaluated by novel tissue clearing and Alizarin Red (AR) staining methods. Cell-type specific calcium deposition and immunohistochemistry patterns were evaluated in human placenta.

**Results:** P<sup>33</sup> uptake by BeWo cells was sodium-dependent and 2.4mM phosphoric acid induced extracellular calcium deposition. The sodium-dependent phosphate transporters Slc20a1 and Slc20a2 are the most highly expressed in mouse and human placenta. Increased placental calcification was observed and initiates in the chorionic plate and labyrinth; ongoing fetal ossification studies compare whole tissue AR findings to microCT-based quantitative analysis. Human placentae presented diverse microcalcification patterns.

**Conclusion:** We conclude that Slc20a2 is abundant in mouse and human placenta and presents a protective effect upon the chorionic plate and basal labyrinth from calcification in mouse. Ongoing work investigates the interaction of Slc20a2 with the adenosine pathway through proteomic approaches. We present the argument that this research will advance our understanding of the link between maternal phosphate homeostasis and placental calcification, improving clinical assessment of early onset placental disease.

### W-139

**Prolonged Hypoxia Inhibits HMSMC HIF1a-Related Contractility Expressions but Not Apoptosis, Possible Leading to Uterine Atony at Labor.** Yunshan Chen. Guangzhou Women and Children's Medical Center, Guangzhou, China.

**Introduction:** Uterine atony is often accompanied with labor arrest. Transient hypoxic state was considered to be one of the mechanisms ensuring myometrium contraction. And in prolonged labor, a long-term repeated hypoxic state might have some effect on myometrium and HMSMCs leading to uterine atony.

**Methods:** Expressions of hypoxia and contraction-related markers of clinical myometrium biopsy specimens in two groups (In-labor group and long-labor group) will be compared in vivo, and long-term hypoxia in vitro experiments, in which myometrium tension and HMSMCs changes, will be all observed at different levels.

**Results:** HIF-1 $\alpha$  expressions in Long-labor group were obviously decreased compared to In-labor group ( $p < 0.05$ ) in vivo, and OTR (Oxytocin receptor) and Cx43 expressions in myometrium both had a decreased trend in vivo. While in vitro experiment, as the duration of hypoxia time increased from 2h to 12h, the expressions of HIF-1 $\alpha$ , OTR and Cx43 all keep decreasing ( $P < 0.05$ ) similar to myometrium sample

of Long-labor group. Also, in myometrium strips tension experiment, with the prolongation of hypoxia time, the uterine muscle contraction amplitude, contraction frequency and area under curve all continued to decrease, and HMSMCs contraction state showed a gradual decline from 2-4 hours to 12 hours with hypoxia period prolonged, all with a significant statistically difference ( $p < 0.01$ ). While the autophagic level (number of autophagosomes, LC3B and Beclin-1 expressions) in the Long-labor group were all down-regulated, but apoptosis markers (Caspase-3, Caspase-9) and TUNEL Assay all showed no statistically difference ( $P > 0.05$ ). In vitro experiment, the prolongation of hypoxia did not cause changes in the expressions of apoptosis-related proteins (caspase3, 9) and Flow cytometry results on HMSMCs ( $P > 0.05$ ), which was also consistent with the trend of myometrium samples from Long-labor group.

**Conclusion:** Prolonged hypoxia in HMSMCs might inhibit HIF1 $\alpha$ -related contractility and self autophagy protection mechanism, but not enhanced apoptosis, this could be one of the internal mechanisms of uterine atony in long labor.

#### W-140

**Impaired Endothelium-Dependent Vascular Function in Female Mice with a History of a Pregnancy Complicated by Dyslipidemia.** Tamara Sáez†, <sup>1,2</sup> Abbey Pagée†, <sup>1,2</sup> Raven Kirschenman, <sup>1,2</sup> Floor Spaans, <sup>1,2</sup> Sandra T Davidge\*, <sup>1,2</sup> University of Alberta, Edmonton, AB, Canada; <sup>2</sup> Women and Children's Health Research Institute, Edmonton, AB, Canada.

**Introduction:** Women that experienced dyslipidemia during pregnancy are at risk for developing cardiovascular complications later in life. High circulating levels of oxidized low-density-lipoproteins (oxLDL) are associated with vascular dysfunction via oxidative stress, contributing to the pathophysiology of cardiovascular diseases. It has been shown that gestational dyslipidemia concomitants with maternal vascular dysfunction; however, whether this vascular dysfunction may persist postpartum is unclear. Therefore, we hypothesized that gestational dyslipidemia leads to later-life vascular dysfunction.

**Methods:** Pregnant C57BL/6 mice were fed a high-cholesterol diet (HCD) between gestational day (GD) 13.5 and term (GD19.5). Control pregnant mice were fed a standard chow diet (CD). After delivery, all females were on a CD for 3 months (3 months postpartum), after which aortas were isolated to assess ex vivo vascular function by wire myography ( $n=7-11$ ). Vascular responses to methacholine (MCh) were evaluated in the presence or absence of oxLDL (50  $\mu\text{g}/\text{mL}$ ) or L-NAME (nitric oxide synthase inhibitor; 100  $\mu\text{M}$ ), as well as vascular responses to sodium nitroprusside (SNP). Reactive oxygen species (ROS, oxidative stress marker) were evaluated in aortic sections by dihydroethidium staining ( $n=3-5$ ).

**Results:** Three months postpartum, a HCD during late pregnancy reduced maximal MCh-induced vasodilation ( $72.5 \pm 5.1$  vs  $90.7 \pm 2.1\%$ ;  $p=0.001$ ) and reduced nitric oxide contribution ( $177.1 \pm 24.7$  vs  $239.2 \pm 16.4$  AU;  $p=0.036$ ) versus females that were fed on a CD in pregnancy. In females that were on a HCD in late pregnancy, pre-incubation with oxLDL decreased maximal vasodilation to MCh compared to vessels without oxLDL ( $54.6 \pm 9.9$  vs  $72.5 \pm 5.1\%$ ;  $p=0.04$ ), while no effects of oxLDL were found in CD females. No differences between groups were found in the maximal SNP-induced vasodilation. Finally, HCD during late pregnancy tended to increase aortic superoxide levels 3 months postpartum compared to females on a CD during gestation ( $4.5 \pm 2.1$  vs  $3.6 \pm 1.1$  AU,  $p=0.06$ ).

**Conclusion:** A high-cholesterol diet during late pregnancy impairs maternal vascular function long-term, potentially via increased vascular responsiveness to oxLDL and oxidative stress formation, and lower nitric oxide contribution to vasodilation. Our study suggests that vascular dysfunction during gestational dyslipidemia persists after pregnancy, which could play a key role in developing long-term cardiovascular complications.

#### W-141

**Role of Monocyte Chemoattractant Protein 1 in Mesenteric Artery Remodeling Postpartum in Mice.** Kirtika Prakash, Rebecca I Fairchild, Nicole M DeLance, Natalia I Gokina, Elizabeth A Bonney\*. University of Vermont, Larner College of Medicine, Burlington, VT, United States.

**Introduction:** Pregnancy-induced vascular remodeling occurs postpartum (PP) and is regulated by maternal immunity and breastfeeding. In mesenteric vessels this begins in late pregnancy, peaks 2 to 4 weeks (wks) PP then resolves. Macrophages regulate vascular biology. Tissue macrophage presence is regulated by Monocyte chemoattractant protein 1 (MCP-1, CCL2). Blood levels of MCP-1 increase 3d and peak 2 wks PP. Vessels from MCP1<sup>-/-</sup> mice show impaired PP remodeling. We altered PP MCP-1 levels to determine the effect on vascular parameters

**Methods:** Adult (~4 months) C57BL/6 (WT) and MCP-1<sup>-/-</sup> females were mated with same-strain males or left virgin. WT mice were injected with 0.5  $\mu\text{g}$  MCP-1 or PBS (control) i.p. every 4 days from 2 to 4 wks PP. MCP-1<sup>-/-</sup> mice were similarly treated from 3d to 2 wks PP. WT mice were given 50  $\mu\text{g}$  MCP-1 blocking antibody every 4 days from 3d to 2 wks PP or from 2 to 4 wks PP. Serum from nursing and non-nursing females was assayed for MCP-1 by ELISA. Passive distensibility was assessed from arterial diameters measured post pressure increase from 3 to 120 mmHg. Here we report % increase in diameters at 80 mmHg relative to 3 mmHg. Passive arterial diameters or wall thickness ( $\mu\text{m}$ ) were measured at 50 mmHg. Statistical analysis of distensibility used two-way RM ANOVA. Analysis of structural data and MCP-1 levels used the t-test. Significance was set at  $p < .05$ .

**Results:** Serum MCP-1 levels were similar in nursing vs. non-nursing mice at 3d ( $p=0.13$ ) and 2wks ( $p=0.9$ ) PP. Vessels from WT virgins given MCP-1 ( $n=6$ ) vs. PBS ( $n=4$ ) for 2 wks exhibited similar distensibility ( $p=0.51$ ), passive lumen diameters ( $p=0.96$ ), and arterial wall thickness ( $p=0.36$ ). Similarly, vessels of WT mice given MCP-1 from 2 to 4 wks PP ( $n=8$ ) vs PBS ( $n=4$ ) did not show changes in these parameters ( $p=0.74$ , 0.44, 0.12, respectively). However, vessels from MCP1<sup>-/-</sup> mice given MCP-1 from 3d to 2 wks PP ( $n=12$ ) showed an increase in distensibility compared to vessels ( $n=12$ ) from control mice ( $90.1 \pm 2.7\%$  vs.  $78.2 \pm 1.9\%$ ,  $p < 0.001$ ). No significant difference in passive lumen diameters ( $\mu\text{m}$ ) ( $232.5 \pm 8.9$  vs.  $215.3 \pm 8.0$ ,  $p=0.17$ ) or arterial wall thickness ( $12.5 \pm 0.8$  vs.  $11.6 \pm 0.5$ ,  $p=0.24$ ) was found. Vessels from WT mice given anti-MCP-1 3d to 2 wks PP had similar distensibility ( $94.1 \pm 3$  vs.  $90.2 \pm 4.3$ ,  $p=0.43$ ) and passive lumen diameters ( $218.2 \pm 9.3$  vs.  $217.7 \pm 7.7$ ,  $p=0.97$ ) compared to controls. However, these vessels had increased wall thickness ( $12.7 \pm 0.6$  vs.  $10.5 \pm 0.3$ ,  $p=0.003$ ). Administration of anti-MCP-1 to WT mice 2 to 4 wks PP did not change these parameters.

**Conclusion:** Although MCP-1 is not modified by nursing, it likely plays a role in early PP vascular remodeling. However, MCP-1 probably does not play a critical role in this process late PP. Supported by NIH RO1HL141747

#### W-142

**Relationship between Low-Dose Epidural Analgesia and Obstetric Laceration Location and Severity.** Gillian Horwitz†, Megan Trostle†, Iffath Hoskins\*, Ashley S. Roman\*. NYU Langone Health, New York, NY, United States.

**Introduction:** While previous studies have characterized the relationship between epidural analgesia and severe perineal lacerations, the relationship between epidural analgesia, specifically low-dose epidural analgesia (LDEA) and all other obstetric lacerations has not been well studied. The objective of this study was to determine the association of LDEA with any perineal laceration. We hypothesized that women with LDEA would be less likely to have any perineal laceration.

**Methods:** This was a single-center, retrospective, cohort study of all vaginal deliveries of vertex, singleton gestations at 34 weeks or beyond in women with no prior vaginal deliveries from 7/2013 to 10/2018. We compared obstetric laceration location and severity between women with and without LDEA. The primary outcome was the rate of any perineal laceration. The secondary outcomes included rates of anterior vulvar, 1<sup>st</sup>-degree, 2<sup>nd</sup>-degree, severe perineal, sulcal, or cervical

lacerations. Fischer's exact test, Chi-square test, Mann Whitney U test, and multivariate regression were performed with  $p < 0.05$  considered statistically significant.

**Results:** Among 8,544 women meeting inclusion criteria, 7,316 had LDEA. Women with LDEA were at increased risk of perineal lacerations ( $p < 0.001$ ), and in particular 2<sup>nd</sup>-degree perineal lacerations ( $p = 0.001$ ). Multivariate analysis was performed to adjust for demographic and labor differences between the groups, and women with LDEA remained at increased risk of perineal lacerations (OR 1.40, 1.11-1.75). In the multivariate analysis, other significant factors associated with perineal laceration included birth weight, race and ethnicity. LDEA was associated with a lower risk of anterior vulvar laceration (OR 0.83, CI 0.71-0.96). There was no difference between groups in the rates of 1<sup>st</sup> degree, severe perineal, sulcal or cervical lacerations.

**Conclusion:** Women with LDEA were at increased risk of perineal lacerations, particularly 2<sup>nd</sup>-degree lacerations. LDEA was not associated with an increased risk of severe perineal laceration.

	Epidural; n=7316;N (%)	No epidural; n=1228;N (%)	OR(95% CI)	p-value
1 <sup>st</sup> degree	2003 (27.4)	353 (28.7)	0.93(0.82-1.07)	0.321
2 <sup>nd</sup> degree	4224 (57.7)	644 (52.4)	1.24(1.10-1.40)	<b>0.001</b>
3 <sup>rd</sup> degree	247 (3.4)	38 (3.1)	1.09(0.77-1.55)	0.611
4 <sup>th</sup> degree	20 (0.3)	2 (0.2)	1.68(0.39-7.20)	0.760
3 <sup>rd</sup> or 4 <sup>th</sup> degree	267 (3.6)	40 (3.3)	1.13(0.80-1.58)	0.494
Any perineal	6504 (88.9)	1042 (84.9)	1.43(1.20-1.70)	<b>&lt;0.001</b>

	aOR (95% CI)	p-value
Epidural	1.40 (1.11 - 1.75)	<b>0.004</b>
Ethnicity	0.73 (0.55 - 0.92)	<b>0.007</b>
Gestational age	0.95 (0.89 - 1.02)	0.186
2 <sup>nd</sup> stage duration	0.996 (0.92 - 1.07)	0.918
Birth weight	0.9992 (0.9990 - 0.9995)	<b>&lt;0.001</b>
Race	1.16 (1.08 - 1.26)	<b>&lt;0.001</b>

#### W-143

**Are Regulatory T-cells Involved in Attaining an Ongoing Uncomplicated Pregnancy after Unexplained Recurrent Pregnancy Loss?** Juliette Krop†, Hanneke Kapsenberg\*, Carin van der Keur\*, Marie-Louise van der Hoorn\*, Frits Koning\*, Frans Claas\*, Sebastiaan Heidt\*, Michael Eikmans\*. *Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** Maintenance of immunological tolerance towards the semi-allogeneic fetus is important for successful pregnancy outcome. However, some couples experience unexplained recurrent pregnancy losses (uRPL). It has been described that regulatory T-cell (Treg) percentages are decreased at the maternal-fetal interface (decidua) after miscarriage compared to elective termination of pregnancy. We hypothesized that in women with uncomplicated term pregnancy after a history of uRPL a higher level of immune regulation is required at the fetal-maternal interface compared to women with uncomplicated pregnancy without any miscarriage in history.

**Methods:** We developed two heavy metal-labelled antibody panels for in-depth immune profiling by mass cytometry using a total of 59 unique immune markers. The first panel focused on all immune lineages while the second had a focus on T cells, including intracellular markers. Immune cells from both the decidua basalis and the decidua parietalis were isolated shortly after delivery, and directly stained with the two antibody panels

together with a reference control for data normalization. We analyzed decidua of 6 women with historic uRPL (case group) and 3 women without miscarriage in history (control group).

**Results:** Analysis and visualization of the high-dimensional data was performed by applying HSNE and t-SNE for the case and control groups combined. For this first analysis, we focused on Tregs. In clustering analysis based on marker expression, FoxP3<sup>+</sup> and FoxP3<sup>-</sup> Treg-like cells (CD25<sup>+</sup>CD127<sup>-</sup>CTLA-4<sup>+</sup>) clustered together. We observed a significantly higher Treg-like cell percentage in the decidua parietalis of cases compared to controls (2.35% vs. 0.18% within CD45<sup>+</sup> cells,  $P = 0.024$ ). For both the decidua basalis and parietalis, 50% of cases had an increased Treg-like cell percentage compared to controls.

**Conclusion:** A proportion of women with a history of uRPL display increased Treg-like cell levels at the fetal-maternal interface, suggesting a possible compensatory role allowing a successful pregnancy. We aim to further elucidate the immunological context of this potential regulatory compensation mechanism by analyzing the other immune cell types in the decidua.

#### W-144

**Preterm Birth in Chronic Toxoplasma Gondii Infection.** Maureen Edith Groer\*,<sup>1</sup> Adetola Louis-Jacques\*,<sup>1</sup> Samia Dutra†,<sup>1,2</sup> Ming Ji.<sup>1</sup> <sup>1</sup>University of South Florida, Tampa, FL, United States; <sup>2</sup>University of Tennessee, Knoxville, TN, United States.

**Introduction:** *Toxoplasma gondii* is a Iapicomplexan unicellular parasite that infects most warm-blooded animals with more than 40 million infected people in the U.S. Chronic infection is marked by quiescent tissue cysts containing bradyzoites which are suppressed by adaptive and innate immune responses. Th1 immunity (IFN- $\gamma$  and IL-12) keeps the organisms confined in cysts as bradyzoites, but NK cells. The humoral immune system responds by producing immunoglobulins. Expert opinion and clinical practice guidelines are that women with chronic *T. gondii* infection are not at risk for reactivation. In pregnant women with there is potential for reactivation due to the immune changes in pregnancy. The goal of the study is to measure immune changes and pregnancy outcome in chronically infected women.

**Methods:** During prenatal visits 578 Hispanic women completed questionnaires, blood was drawn, and clinical data were obtained. All women were screened for *T.gondii* IgG titers. While a cohort is being followed through pregnancy, data presented here is about the women initially screened at the first prenatal visit and birth outcomes data were collected from the electronic health record. The *T.gondii* positive and negative groups were compared on gestational age (GA), preterm birth, miscarriage and small for gestational age (SGA).

**Results:** *T.gondii* chronic infection was much higher in this population than is typical in the U.S.. The mean gestational age at delivery was 36.5  $\pm$  6.4 for the *T. gondii* negative women and 34.1  $\pm$  9.1 for the *T. gondii* positive women. ( $t = 2.04$ ,  $p = .044$ ). Using the Cochran Mantel-Haenszel method (CMH) the analysis showed a significant difference between the *T. gondii* negative and *T. gondii* positive groups on preterm birth ( $S = 159$ ,  $p = .047$ ), miscarriage. ( $S = 190$ ,  $p = .05$ ), and GA at birth ( $S = 162$ ,  $p = .043$ ), when controlling for country of birth. There was also an association of *T.gondii* serotype with preterm birth and miscarriage. Women who miscarried and those who had preterm birth were more likely to have the non-reactive serotype. A chi square test for independence (with Yates Continuity Correction) indicated a significant difference between *T.gondii* status and SGA,  $\chi^2 (1, n = 246) = 5.64$ ,  $p = .02$ ,  $\phi = .166$ ). There was no evidence of *T.gondii* reactivation by PCR, or eye retinoscopic examination in the *T.gondii* positive women who were measured during their pregnancies.

**Conclusion:** Chronic infection with *Toxoplasma gondii* may represent a previously unrecognized risk for preterm birth, miscarriage and SGA. Mechanisms may be related to immune processes associated with pregnancy and with the chronic infection.

## W-145

**CBS-Derived Endogenous H<sub>2</sub>S Contributes to Estradiol-Induced Pregnancy-Dependent Uterine Artery Relaxation via Activation of BK<sub>Ca</sub> Channels in Human Uterine Artery Smooth Muscle Cells.** Yan Li†, Yihua Yang†, Sam Zhang†, Qianrong Qi†, Jin Bai†, Ronald R. Magness\*, Naoto Hoshi\*, Dongbao Chen\*. <sup>1</sup>University of California, Irvine, CA, United States; <sup>2</sup>University of South Florida, Tampa, FL, United States.

**Introduction:** Human pregnancy is a physiological state with elevated endogenous estrogens that increase uterine blood flow to provide nutrients and oxygen supplies to meet fetal demands. Estrogen-induced and pregnancy-associated uterine artery (UA) dilation is accompanied with significantly augmented UA smooth muscle cell (UASMC) production of a “newly” recognized UA vasodilator hydrogen sulfide (H<sub>2</sub>S) by selectively upregulating its biosynthetic enzyme cystathionine β-synthase (CBS), but not cystathionine γ-lyase (CSE) *in vivo*. Activation of UASM large conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (BK<sub>Ca</sub>) are shown to in part mediate both estrogen-induced and pregnancy-associated uterine vasodilation. However, it is unknown if endogenous H<sub>2</sub>S plays a role in estrogen-induced pregnancy-dependent UASM BK<sub>Ca</sub> activation or if this pathway plays a role in UA dilation. In this study using validated primary human UASMCs and organ cultures with UA rings from nonpregnant (NP) and pregnant (P) women, we tested if estradiol-17β (E<sub>2</sub>β) stimulates pregnancy-dependent activation of UASM BK<sub>Ca</sub> channels via CBS- vs. CSE- derived endogenous H<sub>2</sub>S production and if this plays a role in UA dilation.

**Methods:** Explant UA ring cultures were treated with 10 nM E<sub>2</sub>β with or without ICI 182,780 for 24 h for determining K<sup>+</sup> channel mRNA and protein expressions by RT-qPCR and immunoblotting, respectively. UASMC cell BK<sub>Ca</sub> activity was recorded by whole cell and single channel recordings using patch clamp. The role of BK<sub>Ca</sub> and endogenous H<sub>2</sub>S in UA dilation was determined by organ bath studies of phenylephrine-precontracted human UA rings with specific inhibitors and siRNAs.

**Results:** E<sub>2</sub>β stimulated CBS (not CSE) and BK<sub>Ca</sub> β-subunit mRNA and protein expression and H<sub>2</sub>S production in P and NP hUASMC *in vitro*, with ~1-fold greater (p < 0.05) responses in the P state. Baseline BK<sub>Ca</sub> activity was significantly higher in P vs. NP hUASMC, which was further stimulated by E<sub>2</sub>β or H<sub>2</sub>S donor (100 μM NaSH) alone in P vs. NP hUASMC, but with ~1-fold greater responses in the P state. Blockade of endogenous H<sub>2</sub>S biosynthesis using specific CBS inhibitor or siRNA reduced E<sub>2</sub>β (but not NaSH) -induced BK<sub>Ca</sub> activity; whereas specific CSE inhibitor or siRNA had no effect on E<sub>2</sub>β (but not NaSH) -induced BK<sub>Ca</sub> activation in hUASMC. Furthermore, E<sub>2</sub>β dose-dependently relaxed phenylephrine-precontracted human UA rings with greater response in P vs. NP state; E<sub>2</sub>β-induced UA relaxation was significantly inhibited by a specific CBS inhibitor and the BK<sub>Ca</sub> blockers tetraethylammonium and iberiotoxin.

**Conclusion:** Augmented CBS-derived endogenous H<sub>2</sub>S contributes to estrogen-induced pregnancy-dependent UA dilation via activation of UASM BK<sub>Ca</sub> channels (NIH RO1 HL70562 and R21 HD97498).

## W-146

**Factors Associated with Addition of Pharmacotherapy for Gestational Diabetes Mellitus Treatment.** Vishmayaa Saravanan,<sup>1</sup> Rachel Harrison,<sup>2</sup> Lauren Pavlik,<sup>1</sup> Anna Palatnik.<sup>1</sup> <sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>2</sup>Advocate-Aurora Medical Group, Chicago, IL, United States.

**Introduction:** Patient race is known to impact medical care. We sought to identify if race, among other maternal factors, was associated with pharmacotherapy initiation in women with Gestational Diabetes Mellitus (GDM).

**Methods:** Retrospective cohort study of women with GDM in a single healthcare system from 2011-2019. Hispanic and non-Hispanic Black women were compared separately from non-Hispanic white women. Women of other races were excluded due to low numbers. Those recommended to start pharmacotherapy were compared to those who were not via univariable and multivariable analyses. Factors noted to be different between groups (p < 0.05) in univariable analysis, including

maternal race, were input into a backward selection multivariable logistic regression to analyze the independent association of each factor with initiation of pharmacotherapy.

**Results:** Of the 819 subjects who met inclusion criteria, 464 (56.7%) were recommended pharmacotherapy. Those of Hispanic and non-Hispanic Black race and ethnicity were more likely to receive pharmacotherapy (22.6% vs 10.7% and 12.5% vs 10.7%, respectively, p<0.001), as were women with public insurance (34.3% vs 27.9%, p=0.022), higher BMI (35.0±8.4 vs 29.9±7.0 kg/m<sup>2</sup>, p<0.001), earlier GDM diagnosis (25.9±5.9 vs 28.1±4.2 weeks, p<0.001), and worse glucose tolerance testing (Table 1). Nulliparous women were less likely to receive pharmacotherapy (28.7% vs 39.7%, p=0.001). After including all variables that differed between groups with p<0.05 in the logistic regression, the independent association with pharmacotherapy initiation and maternal race and ethnicity did not persist. Higher BMI was modestly associated with pharmacotherapy (aOR 1.05, 95%CI 1.02-1.09). Nulliparity was noted to decrease likelihood of pharmacotherapy (aOR 0.58, 95%CI 0.38-0.89) (Table 2).

**Conclusion:** Maternal race and ethnicity were not associated with recommendation for pharmacotherapy after controlling for confounders. Nulliparity was associated with lower rates of receiving pharmacotherapy, whereas high BMI was associated with greater rates.

	GDM A1 (N=355)	GDM A2 (N=464)	p-value
Maternal age at delivery (years)	31.3 ± 4.7	31.8 ± 5.2	0.183
Maternal ethnicity/race			
Non-Hispanic White	279 (78.6%)	301 (64.9%)	<0.001
Non-Hispanic Black	38 (10.7%)	105 (22.6%)	
Hispanic	38 (10.7%)	58 (12.5%)	
Nulliparity	141 (39.7%)	133 (28.7%)	0.001
Insurance			
Private	238 (67.0%)	269 (60.0%)	0.022
Public	99 (27.9%)	159 (34.3%)	
None	17 (4.8%)	35 (7.5%)	
Unknown	1 (0.3%)	1 (0.2%)	
Marital Status			
Single	96 (27.0%)	149 (32.1%)	0.124
Married	229 (64.5%)	272 (58.6%)	
Divorced/Widowed	8 (2.3%)	17 (3.7%)	
Unknown	22 (6.2%)	26 (5.6%)	
Tobacco use in pregnancy	27 (7.8%)	44 (9.6%)	0.314
BMI early pregnancy (kg/m <sup>2</sup> )	29.9 ± 7.0	35.0 ± 8.4	<0.001
Chronic hypertension	22 (6.3%)	35 (7.5%)	0.473
50-g glucose test (mg/dl)	164.1 ± 31.3	174.9 ± 31.8	<0.001
3-hour results			
Fasting	87.5 ± 12.5	97.9 ± 15.0	<0.001
1 hour	189.8 ± 24.6	197.7 ± 28.4	0.003
2 hour	173.8 ± 26.8	178.2 ± 32.8	0.113
3 hour	133.4 ± 32.4	135.9 ± 32.9	0.464
Weeks gestation at diagnosis	28.1 ± 4.2	25.9 ± 5.9	<0.001
All data presented as N (%) or mean ± SD GDM: Gestational diabetes mellitus, BMI: body mass index			

**Table 2: Logistic regression for impact of maternal factors on treatment with pharmacotherapy**

	OR	aOR
Maternal age at delivery (years)	1.02 (0.99-1.05)	-
Maternal ethnicity/race	Ref	Ref
Non-Hispanic White	2.56 (1.71-3.84)	1.29 (0.66-2.50)
Non-Hispanic Black	-	-
Hispanic	1.41 (0.91-2.20)	1.23 (0.64-2.37)
Insurance	Ref	Ref
Private	1.42 (1.05-1.93)	1.21 (0.74-1.97)
Public	-	-
None	1.82 (0.99-3.34)	2.41 (1.00-5.83)
Marital Status	Ref	-
Single	0.76 (0.56-1.04)	-
Married	1.37 (0.57-3.30)	-
Divorced/Widowed	-	-
Nulliparity	<b>0.61 (0.46-0.82)</b>	<b>0.58 (0.38-0.89)</b>
Tobacco use in pregnancy	1.30 (0.78-2.15)	-
BMI early pregnancy (kg/m <sup>2</sup> )	1.09 (1.07-1.11)	1.05 (1.02-1.09)
Chronic hypertension	1.22 (0.70-2.13)	-
50-g glucose test (mg/dl)	1.01 (1.01-1.02)	1.01 (1.00-1.02)
3-hour results	-	-
Fasting	1.07 (1.05-1.09)	1.01 (1.00-1.04)
1 hour	1.01 (1.00-1.02)	1.01 (1.00-1.02)
2 hour	1.00 (1.00-1.01)	-
3 hour	1.00 (1.00-1.01)	-
Gestational age at diagnosis (weeks)	0.92 (0.89-0.95)	0.92 (0.84-1.01)
Maternal age at delivery (years)	1.02 (0.99-1.05)	-
Maternal ethnicity/race	Ref	Ref
Non-Hispanic White	2.56 (1.71-3.84)	0.91 (0.39-2.17)
Non-Hispanic Black	-	-
Hispanic	1.41 (0.91-2.20)	3.32 (0.91-12.14)
Insurance	Ref	Ref
Private	1.42 (1.05-1.93)	2.63 (1.23-5.63)
Public	-	-
None	1.82 (0.99-3.34)	2.82 (0.74-20.77)
Marital Status	Ref	-
Single	0.76 (0.56-1.04)	-
Married	1.37 (0.57-3.30)	-
Divorced/Widowed	-	-
Managing provider	Ref	Ref
MFM	9.66 (3.72-25.10)	12.61 (3.16-50.31)
OB/GYN	1.01 (0.67-1.52)	1.40 (0.72-2.75)
Endocrinology	-	-
Nulliparity	<b>0.61 (0.46-0.82)</b>	<b>0.77 (0.42-1.40)</b>
Tobacco use in pregnancy	1.30 (0.78-2.15)	-
BMI early pregnancy (kg/m <sup>2</sup> )	1.09 (1.07-1.11)	1.06 (1.01-1.11)
Chronic hypertension	1.22 (0.70-2.13)	-
50-g glucose test (mg/dl)	1.01 (1.01-1.02)	1.01 (1.00-1.04)
3-hour results	-	-
Fasting	1.07 (1.05-1.09)	1.02 (1.00-1.04)
1 hour	1.01 (1.00-1.02)	1.01 (1.00-1.02)
2 hour	1.00 (1.00-1.01)	-
3 hour	1.00 (1.00-1.01)	-
Gestational age at diagnosis (weeks)	0.92 (0.89-0.95)	0.92 (0.84-1.01)

## W-147

**The Developmental Mouse Placenta Proteome Identifies Dynamic Temporal Changes.** Olga Kashpur,<sup>1</sup> Ariel Mei,<sup>1</sup> Shiori Kuraoka,<sup>2</sup> Hideyuki Higashi,<sup>2</sup> Sasha Singh,<sup>2</sup> Elena Aikawa,<sup>2</sup> Mary C Wallingford\*.<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>Brigham and Women's Hospital, Boston, MA, United States.

**Introduction:** The placenta is a highly vascularized organ that supports maternal and fetal health during pregnancy. Placental dysfunction is associated with adverse clinical outcomes, such as preeclampsia, fetal growth restriction, and preterm birth. Herein, we employed tandem mass spectrometry proteomic analyses of mouse placenta collected between embryonic day (E) 8.5-E17.5. We hypothesized that proteins and pathways that regulate crucial processes such as specification of placental progenitors, or regulation of placental morphogenesis, maturation and aging would 1) be overrepresented at corresponding time points and 2) display common temporal abundance patterns.

**Methods:** To test this idea, we analyzed mouse allantois, chorion and placenta samples collected at 12h intervals between E8.5-E12.5 and 24h intervals between E12.5-E17.5 (n=3/independent litters per stage, N=45). Decidua was removed to enrich for placental progenitors and the developing maternal-fetal interface (MFI). Matched paraffin embedded placenta tissue sections were used to conduct a corresponding histological examination of major morphological features and cell types of interest. In total, 3792 distinct proteins were detected.

**Results:** Proteomes differed principally in accordance with gestational age. Protein abundance kinetics (Xina) identified groups of co-occurring proteins over the course of development. The complex abundance pattern of prolactins in the mouse placenta proteome were evaluated in order to compare the proteome to published literature. Functional enrichment analysis (GO and GSEA) identified biological processes, molecular functions and pathways that change during development of the placental labyrinth. Abundance patterns that fluctuated during the initial differentiation of placental structures (E8.5-E9.0), placental morphogenesis (E9.5-E13.5), and/or placental maturation (E13.5-E17.5) were identified. GSEA using Reactome pathway database identified that

FGF signaling was enriched early in MFI development, while VEGF, EPH/EPHRIN, and signaling by RHO GTPases was enriched late in MFI development. Finally, co-occurring temporal abundance patterns and kinetics of proteins associated with blood vessel formation and blood pressure were identified, such as cell-matrix adhesion, cell-cell junctions, cellular adhesion, and extracellular matrix constituents.

**Conclusion:** The presented study has defined novel developmental stage-specific protein networks that characterize mouse placentation. Future work will evaluate conserved targets which can be manipulated to alter placental vascular structure or function in new strategies for preventing or treating human placental dysfunction.

## W-148

**Identification of Subtype-Specific Markers for Preeclampsia Using Placental Pathology and RNAseq.** Mariko Horii, Cuong To, Rebecca Adami, Kathy Zhang-Rutledge, Leah Lamale-Smith, Louise C Laurent\*, Mana Parast\*. *University of California, San Diego, La Jolla, CA, United States.*

**Introduction:** Preeclampsia (PE) is a multifactorial disease for which no sensitive predictive biomarkers exist. This is at least partly due to lack of disease subclassification. Current clinical classification of PE is based on gestational age at diagnosis (early/EO- vs late onset/LO-) and severity of disease. However, PE placentas have heterogeneous findings on pathologic exam, even within these clinical categories. We previously used detailed placental pathologic data to further subclassify PE: EO-PE was further subdivided into "classic" (small placentas, with 2 of these 3 lesions: decidual vasculopathy, infarcts, and hypermaturity) +/- fetal thrombotic vasculopathy (FTV). LO-PE was further subdivided into "normal" (no findings) and "villitis" (mostly chronic villitis). Here, we combined RNA sequencing data with the above pathologic findings to identify markers specific to each subtype.

**Methods:** 47 PE and superimposed PE placentas (9 cases of classic+FTV, 10 classic-FTV, 16 normal, and 12 villitis), all with severe features, were used for this study. Clinical diagnosis was based on current ACOG criteria, and adjudicated by two maternal fetal medicine specialists; all placentas had gross and histologic examination performed by a single perinatal pathologist. RNAseq was performed using Illumina HS4000. Data was processed using our standard RNAseq pipeline; differential expression analysis (DEA) was done by DESeq2 (using Log<sub>2</sub>FC ≥ 1.5, padj ≤ 0.005 as cut-off) to detect markers of each pathological subclass.

**Results:** We first performed DEA of PE vs superimposed PE cases and noted similar gene expression patterns. Therefore, we combined PE and superimposed PE for further analysis. Six combinations of DEA from the pathology category were performed, and the EO-classic-FTV category was noted to have the highest number of differentially expressed genes (77-120 DEGs). Normal vs. villitis showed only 6 DEGs, consistent with the mostly focal nature of villitis in these placentas. Functional enrichment analysis of the unique genes among these DEGs identified altered Apelin pathway in EO-classic+FTV, and altered immune response pathways in LO-villitis.

**Conclusion:** Our findings suggest that severe PE can be characterized by combination of pathological findings and molecular profiles. Further detailed pathological subclassification will improve the identification of subclasses and molecular biomarkers.

## W-149

**Use of the Hypoxia-Inducible Factor (HIF)-2α Inhibitor PT2385 in Placental Dysfunction: New Intervention Addressing Fetal Growth Restriction and Preeclampsia.** Arthur Colson†, Isaline Lambert†, Christophe L Depoix\*, Corinne Hubinont\*, Pierre Sonveaux\*, Frédéric Debieve\*. <sup>1,2</sup> *Catholic University of Louvain, Brussels, Belgium;* <sup>3</sup> *Saint-Luc University Hospital, Brussels, Belgium.*

**Introduction:** It is established that insufficient remodeling of the uterine arteries could lead to a persistent low-oxygen environment in the placenta. Thus, chronic hypoxia persisting after the first trimester of pregnancy is thought to participate in the development of preeclampsia (PE) and fetal growth restriction (FGR). Our previous work demonstrated that low-oxygen tension impairs trophoblast differentiation and functions

through HIF-2 $\alpha$  signaling. Here, we studied the effects of a new, selective, and orally available HIF-2 $\alpha$  inhibitor on primary human cytotrophoblast (CTB) differentiating under a low-oxygen tension.

**Methods:** Primary human CTBs isolated from normal term placentas ( $N = 3$ ,  $n = 6$ ) were cultivated under 2.5% O<sub>2</sub> and exposed to increasing concentrations of PT2385 for 96 hours. The effects of the drug on differentiation and functions were evaluated by RNA sequencing, RT-qPCR, western blot, ELISA, and immunofluorescence. Statistical analyses included ANOVA one-way and Student's t-test and a  $P < .05$  was considered statistically significant.

**Results:** We first controlled that PT2385 efficiently inhibited HIF-2 $\alpha$  in our model of CTBs differentiation under 2.5% O<sub>2</sub>. As expected, low-oxygen tension significantly impaired CTB differentiation compared to 21% O<sub>2</sub>. However, increasing concentrations of PT2385 radically improved *CGB* gene expression (4.20-fold increase,  $P = 0.0037$ ) and hCG secretion (2.57-fold increase,  $P = 0.0095$ ) in comparison with vehicle-treated cells. In addition, PT2385 increased the expression of *GCM1* (2.45-fold increase,  $P < 0.0001$ ) and *syncytin-1* (1.81-fold increase;  $P = 0.0007$ ), two important syncytialization markers, as well as the fusion index (2.15-fold increase,  $P = 0.0002$ ). Finally, PT2385 increased placental growth factor (*PGF*) gene expression (2.71-fold increase,  $P = 0.0008$ ) and protein secretion (4.15-fold increase,  $P = 0.0040$ ) while decreasing soluble fms-like tyrosine kinase-1 (sFLT-1) secretion in comparison with vehicle-treated cells (2.66-fold decrease,  $P = 0.0499$ ), thus significantly inverting the angiogenic balance. The differential gene expression analysis confirmed that PT2385 enhanced the differentiation of CTBs. Interestingly, the gene set enrichment analysis revealed that the ovarian steroidogenesis and the mTOR signaling pathway sets (KEGG) as well as the placental development set (GO) were all significantly enriched by the treatment.

**Conclusion:** PT2385 improved CTB differentiation and functions, as well as the angiogenic balance under low-oxygen tension. These results confirm the benefit of targeting HIF-2 $\alpha$  in placental hypoxia and highlight the use of PT2385 as a potential therapy for placenta-related diseases such as FGR and PE.

## W-150

**Characterizing Sex-Specific Differences in Placental Epigenome Using DNase-Sequencing Data.** Yeon Mi Hwang<sup>†</sup>,<sup>1</sup> Alison G Paquette,<sup>1</sup> Paul Shannon,<sup>1</sup> Cory Funk,<sup>1</sup> Jocelynn Pearl,<sup>2</sup> Hanna Liao,<sup>2</sup> Yoel Sadovsky,<sup>3</sup> Leslie Myatt,<sup>4</sup> John Stamatoyannopoulos,<sup>2</sup> Louis Muglia,<sup>5</sup> Nathan D Price.<sup>1</sup> <sup>1</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>2</sup>Altius Institute for Biomedical Sciences, Seattle, WA, United States; <sup>3</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States; <sup>4</sup>Oregon Health Sciences University, Portland, OR, United States; <sup>5</sup>Cincinnati Children's Hospital, Cincinnati, OH, United States.

**Introduction:** Fetal sex is a key biological variable to include in omics-based analyses of the placenta. DNase-seq data is a type of epigenomic data that profiles the accessible chromatin landscape of the genome by detecting regions digested by DNase-I. Compared to other chromatin accessibility assays, such as ATAC-seq, it is more sensitive and its cleavage bias is better characterized. The purpose of this study was to analyze the sex-specific differences of placenta DNase-seq data to better understand the potential sex difference in placental epigenetic regulatory mechanisms.

**Methods:** Six female and six male placental samples from term, non-pathological pregnancies were collected from the OHSU Placental Biorepository. DNase-seq data of these samples were generated at the Altius Institute for Biomedical Sciences. We identified overlapping peak sets present in both sexes using DiffBind, and identified statistically different peaks using EdgeR (FDR  $q < 0.05$ ).

**Results:** Female samples have significantly more DNase-I Hypersensitive sites (DHSs) at the first exon than males ( $p = 2.95 \times 10^{-4}$ , t-test). We identified 27,745 peak sets unique to female samples and 16,972 unique to male samples. 90,655 overlapping peak sets appeared in both female and male samples. Out of 90,655 peak sets, 181 peaks (0.002%) were statistically different. A total of 105 peaks had stronger peak signals in female samples; 81 peaks at chromosome X and 24 peaks at autosomal chromosomes. The ten most DNase-I hypersensitive peaks in female samples were in the X chromosome.

**Conclusion:** To our knowledge, this is the first genome-scale assessment of sexual dimorphisms in the placental epigenome characterized using DNase-seq data of placenta tissue. We identified differential DHSs depending on the fetal sex qualitatively and quantitatively. The next steps include the integration of ChIP-seq data from matched samples. These additional data will further provide a clearer description of the sex-specific epigenetic regulation of gene expression.

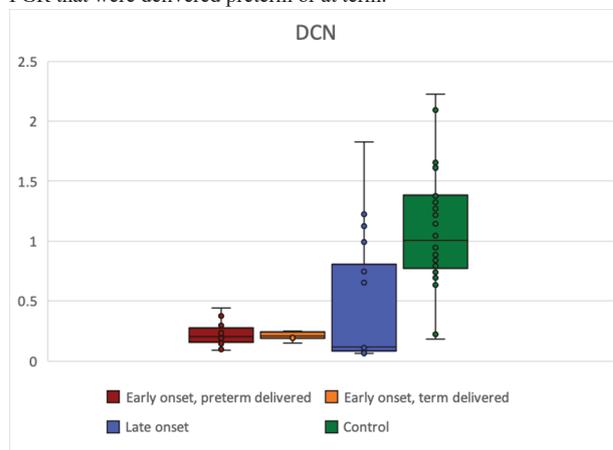
## W-151

**Reduced Placental Decorin Expression Is Associated with Pregnancies Affected by Fetal Growth Restriction Independently of Gestational Age at Delivery.** Kasia Maksym<sup>†</sup>,<sup>1</sup> Hannah EJ Yong,<sup>2</sup> Anna L David,<sup>1</sup> Amanda N Sferruzzi-Perri,<sup>3</sup> Sara L Hillman\*.<sup>1</sup> <sup>1</sup>University College London Institute for Women's Health, London, United Kingdom; <sup>2</sup>Agency for Science, Technology and Research, Singapore, Singapore; <sup>3</sup>Cambridge University, Cambridge, United Kingdom.

**Introduction:** Decorin (DCN) is a proteoglycan that can have pro-angiogenic or anti-angiogenic effects, depending on the molecular environment. DCN has previously been shown to be reduced in third trimester FGR placentas (1) and in first trimester chorionic villus samples from pregnancies with subsequently small for gestational age fetuses (2). Decreased DCN levels in early pregnancy are hypothesised to lead to uncontrolled cell proliferation and premature cytotrophoblast differentiation, thereby leading to impaired placentation and poor fetal growth.

**Methods:** Placenta tissues were collected at delivery from pregnancies affected by early-onset FGR <32 weeks ( $n=21$ ), late-onset FGR  $\geq 37$  weeks ( $n=9$ ) and AGA  $\geq 37$  weeks ( $n=15$ ). Early-onset cases were recruited as part of EVERREST study (3). FGR was defined as infants with birthweights <3<sup>rd</sup> centile. Two biopsies were sampled from each placenta using a standardised approach and then snap frozen within 30 minutes of delivery. Following RNA extraction and cDNA synthesis, DCR mRNA expression was determined by real time qPCR using Taqman probes, alongside housekeepers. Relative expression was calculated using the 2<sup>- $\Delta\Delta C_t$</sup>  method. Data were analysed by Student t-test, with  $p < 0.05$  considered significant.

**Results:** Overall, there was significantly lower expression of DCN in both early- and late-onset FGR compared to AGA placentas (control,  $p < 0.001$ ). No statistically significant difference was identified between early onset FGR that were delivered preterm or at term.



**Conclusion:** FGR placentas had decreased expression of DCN, compared with AGA placentas. Despite differences in gestational age at delivery among FGR cases, FGR cases had similar reductions in placental DCR expression at delivery, indicating that observed reduction was not purely a function of gestational age. Hence, lower DCN expression, particularly given its presence in early-onset FGR placentas, suggests an intrinsic role in development. However, further work is warranted to examine if low levels of DCN drive a placenta to function poorly or conversely, whether it is a consequence of placental failure. 1. *Reprod Fertil Dev.* 2010;22(6):949-955 2. *Placenta.* 2016; Sep;45:58-62 3. *BMC Pregnancy Childbirth.* 2017;17(1):43.

**W-152**

**Human Placenta and Trophoblasts Express a Novel Atypical Protein Kinase C- $\zeta$  Isoform.** Sumaiyah Zubair Shaha†, Khushali Patel†, Saba Saadat, Meghan Riddell\*. *University of Alberta, Edmonton, AB, Canada.*

**Introduction:** Atypical protein kinase Cs (aPKCs) are a family of kinases important for the regulation of cell polarity. The two major forms of aPKC present in humans are aPKC- $\iota$  and aPKC- $\zeta$ . Very recently, aPKCs have been found to initiate trophoblast polarization and establishment, and thus, placental formation in humans. Additionally, aPKC- $\iota$  specific knockdown in human trophoblast stem cells (TSCs) fail to form syncytiotrophoblast (ST), emphasizing its critical role in trophoblast differentiation. Compensation for aPKC- $\iota$  by aPKC- $\zeta$  isoforms has been described, yet, the expression and function of aPKC- $\zeta$  isoforms in trophoblasts and the placenta remains unknown. Thus, we examined the expression and localization of aPKC- $\iota$  and aPKC- $\zeta$  isoforms in human placenta and trophoblasts. Further, we assessed syncytialization of primary human trophoblasts in the presence of total aPKC inhibitors to address the importance of all aPKCs in trophoblast differentiation.

**Methods:** Placental tissue (1<sup>st</sup> and 3<sup>rd</sup> trimester) was stained using immunofluorescence (IF) for aPKC- $\iota$  and aPKC- $\zeta$ . Isolated primary trophoblasts and total placental lysate protein and mRNA were collected for western blotting and RT-PCR analysis of aPKC isoforms. Primary first trimester and term CT were stimulated to differentiate into ST with Br-cAMP +/- aPKC inhibitors (pseudosubstrate and aurothioglucose[ATG]) and fusion assessed with IF using anti- E-cadherin and nuclear stains.

**Results:** aPKC- $\iota$  and aPKC- $\zeta$  specific antibodies revealed largely overlapping expression patterns in CT and ST, and low expression in extravillous trophoblasts, in first trimester and term placentas. Surprisingly, aPKC- $\zeta$  western blots of placental lysates and trophoblasts displayed two bands; one corresponding to full length aPKC- $\zeta$  and a ~55kDa band. Using RT-PCR and isoform specific primers aPKC- $\zeta$  isoform 1 (full length) and isoform 3, which has a predicted molecular weight of ~55kDa, could be amplified. Thus, there are 3 isoforms of aPKC expressed in the human placenta, and specifically, the trophoblasts. Pseudosubstrate and ATG inhibitors did not prevent syncytialization of both primary first trimester and term CTs to ST.

**Conclusion:** We have shown for the first time that aPKC- $\zeta$  isoforms 1 and 3 are present in human placenta, specifically, the trophoblasts. Thus, placental trophoblasts express 3 isoforms of aPKCs that must be addressed when assessing placental function. Published aPKC- $\iota$  phenotypes do not address aPKC- $\zeta$  isoforms, however, their distinct and overlapping expression with aPKC- $\iota$  suggest both isoform specific functions and redundant roles. Unlike the published data that show inhibition of aPKC- $\iota$  prevents syncytialization in TSCs, inhibition of total aPKCs did not prevent syncytialization in primary CTs of the first trimester and term. Thus, aPKC isoform specific roles will be important to understand as their functions regulate pivotal trophoblast features.

**W-153**

**Differential DNA Methylation between Infection Associated and Idiopathic Spontaneous Preterm Birth.** Heather M Brockway\*, Jones N Helen\*. *University of Florida, Gainesville, FL, United States.*

**Introduction:** Preterm birth (PTB), birth before 37 weeks gestation, is a global public health concern with 70% of these PTB categorized as idiopathic spontaneous (isPTB). Yet little is known regarding the molecular mechanisms of isPTB. We previously identified transcriptomic signatures of placental maturation in a highly phenotyped cohorts. The aim of this project is to detect isPTB specific methylation signatures and to determine if they are indicative of hypermaturity.

**Methods:** Placental villous methylation levels were determined using the Illumina MethylationEPIC array for Acute Histological Chorioamnionitis Births (AHC, n=8), idiopathic spontaneous preterm birth (isPTB, n=11) and normal term births (TB, n=8). After quality control the final data matrix consisted of 758,210 methylation points. *DMRCate/limma* was used to identify significant differentially methylation regions (DMRs) across all pairwise comparisons. Functional assessment of gene associated with significant DMRs was conducted in Panther DB.

**Results:** In total 31,945 significant DMRs (minimum smoothed FDR <0.05) were identified: isPTB v TB = 56 DMRs, isPTB v AHC=12,883 DMRs and TB vs AHC = 19,006 DMRs. Only 7 DMRs with a specific isPTB hypomethylated signature were identified: *LINC0202*, *FAM186A*, *NOD2*, *UBL7-AS1*, *PDE9A*, *ZBTB4* and *STXB6*. No isPTB specific pathways were identified. In contrast, we identified 1,718 AHC specific DMRs with 801 DMRs hypermethylated and 917 hypomethylated in comparison to the TB and isPTB samples. Within the top 25 hyper/hypomethylated *GSE1*, *MLL1*, *GCK*, *CTSH*, *ACSS1*, and *ITGAX* were identified as genes of interest. Pathway analyses of the AHC specific gene associated DMRs identified WNT and Cadherin pathways as having significant over-representation of hypermethylated DMRs.

**Conclusion:** Overall, the isPTB methylation pattern at the DMR levels mimics the TB methylation pattern suggesting hypermaturity. Only 56 significant DMRs were identified with only 7 fitting the isPTB specific hypomethylation pattern indicating unusually high similarity between isPTB and TB DMRs. Interestingly, within this isPTB methylation signature was a DMR associated with *NOD2*, a NOD-like receptor, which has been previously identified in PTB genetic studies as a gene of interest and is expressed within the syncytiotrophoblast and villous stroma. In contrast, pathway analyses of the 1,718 AHC specific DMRs identified the hypermethylated DMRs in WNT and cadherin pathways which are known to be active in placenta development, with no methylation difference in isPTB vs TB samples. No hypomethylated pathways were identified. Taken together, these data suggest DNA methylation has a potential impact on placental hypermaturity and thus, birth timing.

**W-154**

**Rosiglitazone Restores Expression of HO1 in the Preeclamptic Placenta.** Brooke A Armistead†, Hamid-Reza Kohan-Ghadri\*, Sascha Drewlo\*. *Michigan State University, Grand Rapids, MI, United States.*

**Introduction:** Preeclampsia (PE) is a hypertensive disorder of pregnancy and is a major cause of maternal-fetal morbidity and mortality worldwide. Severe PE is associated with abnormal placentation including ischemia-reperfusion injury. These changes are accompanied with altered expression of key transcription factors, such as peroxisome proliferator activated receptor (PPAR)- $\gamma$ , which contribute to abnormal villous trophoblast differentiation. Furthermore, imbalances in cyto-protective molecules, such as heme oxygenase-1 (HO1) are part of the PE pathophysiology. Activation of PPAR $\gamma$  by Rosiglitazone has been shown to increase HO1 expression in animal models of PE, however this has not been shown in the human placenta, and the mechanism by which PPAR $\gamma$  regulates HO1 is not well understood. Here, we hypothesize that activating PPAR $\gamma$  with Rosiglitazone in the PE placenta can restore HO1 expression and this is accomplished through direct transcriptional regulation of HO1 by PPAR $\gamma$ .

**Methods:** PPAR $\gamma$  and HO1 protein expression was measured in PE (n=6) and gestation age-matched control (n=7) placentas by western blot. HO1 mRNA expression was measured by qPCR in the following experiments: 1. PE placentas (n=6) that were treated for 24 hours with 10 $\mu$ M Rosiglitazone or DMSO (vehicle) and 2. BeWo (representative villous trophoblast cell line) cultured under normoxia (36hrs 20% O<sub>2</sub>, n=3) or hypoxia-reoxygenation (24hrs 1.5% O<sub>2</sub> followed by 18hrs 20% O<sub>2</sub>, n=3) to mimic oxidative stress in ischemia-reperfusion injury and treated with 10 $\mu$ M Rosiglitazone or DMSO. Student's T test was used to determine if data was statistically significant after an F-test identified if datasets had equal variances. Groups treated with Rosiglitazone were normalized to DMSO. A p-value <0.05 was considered statistically significant.

**Results:** PPAR $\gamma$  and HO1 protein expression were significantly reduced in the PE placenta compared to gestation age-matched controls (p<0.01). Rosiglitazone increased HO1 mRNA expression in the PE placenta (p=0.0069) and during *in vitro* oxidative stress conditions (p=0.0015).

**Conclusion:** Our study is the first to show that activation of PPAR $\gamma$  by Rosiglitazone increases HO1 expression in the PE placenta. We have extended this to our *in vitro* model of ischemia-reperfusion injury which will be used in future studies to elucidate the mechanism underlying PPAR $\gamma$  activation on increased HO1 expression.

## W-155

**Lifelong Western Diet Exposure Impacts upon the Vasculature of Placental Labyrinth at Mid-Gestation in a Non-Obese Guinea Pig Model.** Takashi Hashimoto,<sup>1,2</sup> Flavien Delhaes†,<sup>3</sup> Karen Nygard,<sup>2</sup> Lanette J Friesen-Waldner,<sup>2</sup> Charles A McKenzie,<sup>2,4,5</sup> Barbra de Vrijer,<sup>2,4,5</sup> Bryan S Richardson,<sup>2,4,5</sup> Patti Kiser,<sup>2</sup> Timothy RH Regnault\*.<sup>2,4,5</sup> <sup>1</sup>Kagoshima City Hospital, Kagoshima City, Japan; <sup>2</sup>University of Western Ontario, London, ON, Canada; <sup>3</sup>Geneva University, Geneva, Switzerland; <sup>4</sup>Children's Health Research Institute, London, ON, Canada; <sup>5</sup>Lawson Health Research Institute, London, ON, Canada.

**Introduction:** Increasing numbers of women consume a 'Western diet' (WD), high in fats and sugars, which has been associated with maternal obesity and adverse pregnancy outcomes such as fetal growth restriction. However, WD alone, independent of maternal obesity, negatively impacts maternal metabolic health and is associated with adverse term placental and fetal outcomes, although impacts upon the placenta, especially earlier in pregnancy, are ill-defined. We postulated that WD exposure during pregnancy, in conjunction with markers of poor maternal metabolic health, impacts the vasculature of the placental labyrinth, independent of maternal obesity.

**Methods:** Female guinea pigs were weaned onto a Control or WD and mated to control males at six months (~25-30 human years) of age, then necropsied at 40 days gestation (term ~69 days) for gross and histologic examination (CD: n=9 (6 pregnancies), WD: n=12(7 pregnancies)). Placental histopathology was assessed using H&E and IHC. The fetal capillary area was detected by vimentin staining, and the maternal lacunae with pan-cytokeratin staining.

**Results:** While maternal and placental weights were unchanged between the two groups, WD fetal weights were 23% lighter (p=0.08) and fetal/placental weight ratio was significantly smaller than CD (p=0.02). In histologic assessment, 2 out of 12 WD placentae displayed large necrotic areas, comprising ~50% of the tissue area, compared to CD placentae which showed only small necrotic areas. Although the ratio of labyrinth/Interlobium area was unchanged between the two groups, the fetal capillary area in the labyrinth was significantly decreased (p<0.001) and the maternal sinus area in the labyrinth was significantly increased (p=0.02) in WD placentae.

**Conclusion:** Lifelong WD exposure, independent of maternal body weight, results in distinct abnormalities of late 2nd third placental architecture and decreased fetal capillary area. This study supports the concept that life-long maternal diet alone plays a significant role upon placental vascularization in association with markers of poor maternal metabolic health, independent of obesity. These resulting alterations may negatively impact later pregnancy placental hemodynamics and contribute to poor birth outcomes including growth restriction and stillbirth associated with a dysfunctional placenta.

## W-156

**Expression Quantitative Trait Loci Analysis in the Human Placenta.** Clara Apicella†,<sup>1</sup> Camino SM Ruano†,<sup>1</sup> Géraldine Gascoin\*,<sup>2</sup> Francisco Miralles\*,<sup>1</sup> Celine Méhats\*,<sup>1</sup> Daniel Vaiman\*.<sup>1</sup> <sup>1</sup>Institut Cochin, U1016 INSERM, Paris, France; <sup>2</sup>CHU Angers, Angers, France.

**Introduction:** The key role of placental dysfunction in the aetiology of numerous pregnancy pathologies, such as preeclampsia (PE) and intra-uterine growth restriction (IUGR) is now well established. Nonetheless, molecular mechanisms that orchestrate placental function and that are behind disease development are still incompletely understood. In this study, we seek to identify expression Quantitative Trait Loci (eQTLs) in healthy and pathological placentas. An eQTL describes the correlation between a change in sequence of a specific locus on the genome (eSNP) and levels of expression of a target gene (eGene). Therefore, these results can give insights into the regulation of the eGene in question, identifying potential key elements (e.g. transcription factor binding sites, enhancer regions). eQTL mapping will therefore help to clarify the functional landscape of the human placenta.

**Methods:** RNA and DNA were collected from 17 control, 6 PE, 3 PE+IUGR and 10 IUGR human placentas (n=36). SNP variants were genotyped with Infinium OmniExpress (Illumina) array, covering more

than 710K loci. Gene expression was measured at the exon-level by microarray (ClariomD, Affymetrix) and validated by qPCR. 10559 transcripts and 438590 SNPs (MAF > 0.15) were used as input for analysis with MatrixeQTL. 14 covariates (placental sex, gestational age, disease group, experimental batch and genotype principal components) have been accounted for by the software when performing multivariate linear regression for each gene-SNP pair. A False Discovery Rate (FDR) of 0.05 has been set as statistical significance threshold for cis-QTLs, according to previous studies.

**Results:** Preliminary results identified 177 cis-QTLs (FDR < 0.05) corresponding to 43 unique eGenes; 62.8% of these had been previously identified as associated to placental eQTLs, including the well described ERAP1 and ERAP2 genes, corroborating the robustness of our study design. Additionally, we describe 16 novel eGenes. Among these, TP53BP1 and ASCC3 are involved in DNA repair, and the latter in enhancing the NF-kappa-B-mediated inflammatory response, NT5E is necessary for correct lymphocyte differentiation, ACS3 in the synthesis of Acetyl-CoA.

**Conclusion:** Combining gene expression alterations with genetic variants opens the possibility to gain insights into key molecular hierarchies governing complex tissues. Here, we further validate previous findings and we identify novel eQTLs in the healthy placenta that might highlight regions of the genome that are relevant for placental function. Having access to disease samples (PE and IUGR), we wish now to harness this validated study design to investigate placental disease-specific eQTLs, that would shed light on key regulatory mechanisms in placental dysfunction.

## W-157

**Long Noncoding RNA XIST Regulates Fetal Growth by Acting as a Molecular Augment of miR-424 to Modulate MEK1 and FGFR1 Expression.** Lu Huang. *The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University, Wuxi, China.*

**Introduction:** To test a hypothesis that long noncoding RNA-XIST may act as a molecular augment of miR-424, which may consequently associate with intrauterine fetal growth through modulation of cellular proliferation, invasion, and migration of human trophoblasts, and down-regulation of MEK1 and FGFR1 expression in human placenta.

**Methods:** Human placentas collected from normal pregnancies and from fetal growth restriction (FGR). Quantitation of mRNA expressions with quantitative real time PCR (qRT-PCR) and of proteins with Western blot were applied for measurement of expression of lncRNA XIST, MEK1 and FGFR1 in human placenta tissues, as well as in cultured HTR-8/SVneo trophoblasts. Functional analysis of lncRNA XIST, including regulatory process between lncRNA XIST and miR-424, was performed.

**Results:** Quantitative measurement documented the significant increase of lncRNA-XIST and miR-424 in FGR placenta tissues and HTR-8/SVneo cells. Function studies revealed that overexpression of lncRNA-XIST and miR-424 inhibited proliferation, migration, and invasion in HTR-8/SVneo cell. MEK1 and FGFR1 was up-regulated in HTR-8/SVneo cell, was inversely correlated with lncRNA-XIST/miR-424 expression. The expression level of the MEK1 and FGFR1 were inhibited by overexpression lncRNA-XIST/ miR-424. Overexpression of lncRNA-XIST/miR-424 efficiently suppressive effect on proliferation, migration and invasion in the HTR-8/SVneo cell. Furthermore, a positive relationship between lncRNA-XIST and miR-424 was found. MEK1 and FGFR1, a direct target of miR-424, could mediated the biological effects that lncRNA-XIST/ miR-424 exerted.

**Conclusion:** lncRNA XIST is up-regulated and associated with the proliferation, migration, and invasion of trophoblasts, and the newly identified lncRNA-XIST/miR-424/ MEK1/FGFR1 axis could be a potential biomarkers or therapeutic targets for the FGR. These results provide insight into the mechanism underlying the directed proliferation and migration of HTR-8/SVneo cells. Further research should address and to explore the correlation about with lncRNA XIST/ miR-424 expression and may inactivating transcript of MAPK signal pathway.

**W-158**

**Exposure to  $\Delta$ -9-Tetrahydrocannabinol Disrupts Mitochondrial Function and Inhibits Syncytialization in BeWo Cells.** O'Llenecia Walker, Harmeet Gurm†, Linda L May, Rehginald Ragos†, Mariah Lapierre†, Sandeep Raha\*. *McMaster University, Hamilton, ON, Canada.*

**Introduction:** Cannabis use during pregnancy is associated with a variety of obstetrical outcomes, including preeclampsia, preterm labour and low birth weight. The molecular mechanisms underpinning these outcomes are not well understood but may be attributed, in part, to the actions of the psychoactive cannabinoid,  $\Delta$ -9-tetrahydrocannabinol (THC). In the placenta, mononucleated cytotrophoblasts (CT) syncytialize/fuse to form the multinucleated syncytiotrophoblast (ST) layer at the maternal-fetal interface. Furthermore, the placenta is rich in mitochondria and these organelles are known to play an important role in the development and function of this tissue. We hypothesized that THC would compromise the process of trophoblast syncytialization and alter trophoblast function, in part, as a result of mitochondrial dysfunction.

**Methods:** BeWo cells, as CTs or STs, were treated with 20  $\mu$ M THC for 48 hours. We quantified markers of trophoblast fusion (*GCM1*, *ERVW-1*, *ERVFRD-1*, *CG*), and function (placental lactogen (*PL*), insulin like growth factor 2 (*IGF2*) and progesterone (*P4*)) using RT-PCR and ELISA. We quantified mitochondrial stress (*HSP60*, *HSP70*), mitochondrial ETC function (respirometry), cellular ROS (DCFDA assay) and mitochondrial membrane potential (JC-1 fluorescence). We also evaluated the contribution of cannabinoid receptor 1 (CB1R) or cannabinoid receptor 2 (CB2R) on mitochondrial fragmentation (*OPA1*, *MFN1*, *MFN2*, *DRP1*) using inhibitors specific to each of these receptors (AM281 and AM630, respectively).

**Results:** Treatment with 20  $\mu$ M THC caused a significant elevation of cellular ROS (80% increase), reduction in ATP production (by 50%) as well as an attenuation of the mitochondrial membrane potential along with a, more than 2-fold, increase in the expression of (*HSP60*, *HSP70*). These changes were paralleled with an observed reduction in trophoblast fusion (reduced mRNA levels of *GCM1*, *ERVW-1*, *ERVFRD-1*, *CG*). We also observed reduced hCG and P4 (3-fold reduction) secretion, reduced expression of PL and IGF2 transcripts concomitant with a significant increase in mitochondrial fission (decreased *OPA1* and *MFN2*, increased *DRP1* transcripts). These markers of mitochondrial fission, resulting from THC treatment, were reversed when cultures were pretreated with 1 $\mu$ M AM281 and not with 1  $\mu$ M AM630.

**Conclusion:** Taken together, our data suggest THC evokes mitochondrial dysfunction in syncytiotrophoblasts and impacts mitochondrial dynamics, a process known to be linked to cellular health. These changes appear to be mediated, in part, through CB1R. Importantly, these changes in mitochondrial function are associated with the reduced production of hormones which are important for fetal growth and may support a plausible mechanism by which THC exposure during pregnancy leads to adverse fetal outcomes.

**W-159**

**Influence of Placental Matrix Metalloproteinase-9 Protein Expression on the Branching Architecture of Chorionic Blood Vessels of Human Placenta.** Sara Oraee†, Jayasri Basu\*, Yingyi Wu†, Diana Encalada†, Lara Molina†, Magdy Mikhail. *BronxCare Health System, Bronx, NY, United States.*

**Introduction:** Human placenta is marked on the maternal side by the basal plate and on the fetal side by the chorionic plate. Blood vessels branching from the umbilical arteries and vein traverse along the chorionic plate as chorionic blood vessels. Two different branching patterns of chorionic vessels exist. In the dispersal type, the chorionic blood vessels undergo successive divisions with gradually diminishing caliber as the vessels traverse towards the placental margins. In the magistral type, the blood vessels traverse towards the placental margins without appreciable decrease in vessel diameter. Since extracellular matrix needs to be continually degraded to modify the structural scaffold as these chorionic blood vessels evolve, we hypothesize, that matrix degrading enzymes secreted by cytotrophoblasts may dictate the branching patterns of the

chorionic vessels. We, therefore, investigated if placental expression of matrix metalloproteinase-9 (MMP-9) protein would differ between the two vascular patterns of chorionic blood vessels of human placenta.

**Methods:** In this IRB approved study, full-term placentas were obtained from women with uncomplicated pregnancy. All placentas were photographed, the vascular patterns were determined and chorionic villi were isolated. Chorionic villi MMP-9 protein expression was analyzed using human MMP-9 monoclonal antibody based ELISA kit from R&D Systems, MN. Independent t test was used for statistical analysis.  $P < 0.05$  was considered significant.

**Results:** 26 full term placentas were collected. All women delivered normal healthy newborns. The branching pattern of the chorionic vessels was 54% of the dispersal type, and 46% magistral. In placentas with dispersal type of branching pattern, MMP-9 protein expression was significantly higher ( $p=0.006$ ). Women who delivered placentas with magistral type of branching pattern, the mean newborn weight was found to be significantly higher ( $p=0.039$ ).

**Conclusion:** Our findings underscore the importance of chorionic villi MMP-9 protein expression in molding the branching architecture of chorionic blood vessels of human placenta. In the magistral type, the calibers of the chorionic blood vessels from the point of insertion of the umbilical cord to the placental margin, remain unchanged. We suggest, that this unchanged caliber of blood vessels in the magistral type, may have allowed better nourishment and oxygen to be catered to the fetus; thereby resulting in fetal weight gain.

**Differences between the branching patterns of chorionic blood vessels**

Chorionic Blood Vessels	N	MMP-9		p value
		(ng/ 100 mg tissue)	SD	
Dispersal Type	14	26.98	14.70	0.006
Magistral Type	12	17.04	6.63	
		Newborn Weight (gms)		0.039
Dispersal Type	14	3257	305	
Magistral Type	12	3450	531	

**W-160**

**Leptin, IGFBP3 and IGFBP7 Are Potential Serum Biomarkers of Placenta accreta Spectrum.** Bradley H. Sipe†, Ozlem Guzeloglu-Kayisli, Kellie Larsen, Xiaofang Guo, Asli Ozmen, Nihan Semerci, Charles J. Lockwood, Umit A. Kayisli\*. *University of South Florida Morsani College of Medicine, Tampa, FL, United States.*

**Introduction:** Placenta accreta spectrum (PAS) is a significant cause of intrapartum hemorrhage, maternal morbidity and mortality. The increasing rates of uterine surgery including cesarean deliveries enhance PAS incidence. Clinical history and antenatal ultrasound serve as the mainstays of antepartum diagnosis for suspected PAS; however, these methods have limitations. Incorrect diagnoses may lead to iatrogenic preterm deliveries in those with suspected PAS found to have normal placentation at delivery, or may lead to unexpected hemorrhage and maternal morbidity/mortality in those whose PAS is discovered at delivery. Thus, we sought to identify placental/decidual secretory proteins as serum biomarkers in patients with suspected PAS to improve its prenatal diagnosis.

**Methods:** Global RNA-seq analysis of primary decidual cell (n=3) and cytotrophoblast (n=3) cultures were used to identify transcription levels of secretory proteins. RNA isolated from decidual and villus tissues (including invasive and non-invasive areas) from PAS specimens (n=9) and from gestational age (GA)-matched controls (n=9) were analyzed by qPCR using TaqMan gene specific assays for leptin (LEP), Insulin Like Growth Factor Binding Protein 3 (IGFBP3), and IGFBP7. Serum obtained

from 13 PAS complicated pregnancies at time of cesarean hysterectomy and from 13 GA-matched controls at routine prenatal visits, were analyzed by ELISA to determine LEP, IGFBP3, and IGFBP7 protein levels.

**Results:** Global RNA-seq identified highly expressed transcripts of secretory proteins, including LEP, IGFBP3 and IGFBP7 in decidual cell and/or trophoblast cultures. Compared to GA-matched controls, invasive and non-invasive villous tissues from PAS specimens expressed significantly lower mRNA levels of LEP (mean  $\pm$  SEM:  $2.08 \pm 0.79$  vs.  $0.40 \pm 0.13$  and  $0.59 \pm 0.20$ , respectively,  $p < 0.05$ ) and IGFBP7 ( $2.54 \pm 0.57$ ,  $0.32 \pm 0.06$  and  $0.62 \pm 0.009$ , respectively,  $p < 0.05$ ). Similarly, compared to GA-matched controls, invasive and non-invasive placental villous site of PAS specimen displayed lower IGFBP3 mRNA levels, without attaining a statistical significance ( $1.45 \pm 0.47$  vs.  $0.59 \pm 0.12$  and  $0.57 \pm 0.22$ , respectively,  $p = 0.10$ ). ELISA analysis revealed significantly lower serum protein levels (pg/ml) of LEP (mean  $\pm$  SEM  $4.2 \times 10^4 \pm 7.3 \times 10^3$  vs.  $7.3 \times 10^4 \pm 1.3 \times 10^4$ ,  $p = 0.036$ ), IGFBP3 ( $1.2 \times 10^7 \pm 3.2 \times 10^6$  vs.  $8.3 \times 10^7 \pm 5.6 \times 10^7$ ,  $p = 0.03$ ) and IGFBP7 ( $6.92 \times 10^4 \pm 6.6 \times 10^3$  vs.  $1.1 \times 10^5 \pm 2.2 \times 10^4$ ,  $p = 0.005$ ) in PAS patients vs. GA-matched controls.

**Conclusion:** Reduced LEP, IGFBP3 and IGFBP7 mRNA levels in placenta are likely responsible for their decreased protein levels in the maternal serum of PAS. Moreover, serum LEP, IGFBP3 and IGFBP7 levels can be used as screening biomarkers in pregnant women to improve clinically diagnosed PAS.

## W-161

**Study on Role of Hypoxia-Inducing Factor 1 $\alpha$  Mediated Aquaporin1 in Trophoblast Invasion.** Xiaoyan Sha<sup>†</sup>, Binsheng Wu, Kaimin Guo<sup>†</sup>, Junjie Bao, Huishu Liu\*. *Guangzhou Women & Children Medical Center, Guangzhou, China.*

**Introduction:** Abnormal trophoblast invasion can result in abortion, pre-eclampsia, fetal growth restriction and placenta implantation. A large number of studies have shown that AQP1 not only regulates the water permeability of various cells, but also mediates cell migration and invasion during pathophysiological changes. It was reported that hypoxia-induces AQP1 mRNA expression, and it is also known that pathological situations presenting tissue hypoxia stimulate the transcriptional expression of AQP1. Participation of HIF-1 $\alpha$  in the transcriptional regulation of AQP1 has recently been demonstrated. We aimed to explore HIF-1 $\alpha$  mediated AQP1 regulate trophoblast invasion.

**Methods:** AQP1-siRNA silenced was constructed and transfected to human trophoblast cell line HTR8/SVneo, and RT-PCR was used to detect the expression of AQP1 in trophoblast cells after transfection. Real-time cell analysis (RTCA) was used to detect the invasion and proliferation of wild-type trophoblast cells and trophoblast cells transfected with AQP1-siRNA. MTT assays the cell viability after AQP1 siRNA transfection. Trophoblast cells were cultured in a hypoxia environment (1%O<sub>2</sub>, 94%N<sub>2</sub>, 5%CO<sub>2</sub>) for 24 hours to detect the expression changes of AQP1 and HIF-1 $\alpha$ , and to detect the trophoblast invasion changes after hypoxia culture by Real-Time Cell Analysis.

**Results:** The expression of AQP1 was identified in HTR8/SVneo and the AQP1 expression decreased after siRNA transfection. The invasion and proliferation of trophoblast was reduced after AQP1 siRNA transfection. The AQP1 siRNA transfection cell viability was decreased by MTT assay. The expression of HIF-1 $\alpha$  and AQP1 were increased under hypoxia condition, and trophoblast invasion was also increased.

**Conclusion:** This study revealed AQP1 promoted the invasion, proliferation and cell viability of trophoblast, and HIF-1 $\alpha$  mediated AQP1 promoting the invasion of human trophoblast in hypoxic condition.

## W-162

**VEGFA and FGF2 Alter Protein Phosphorylation Profiles in Human Fetoplacental Endothelial Cells.** Chi Zhou,<sup>1,2</sup> Xin-wen Chang<sup>†</sup>,<sup>3</sup> Jing Zheng\*. <sup>1</sup>University of Wisconsin-Madison, Madison, WI, United States; <sup>2</sup>University of Arizona, Tucson, AZ, United States; <sup>3</sup>Tonji University Hospital, Shanghai, China.

**Introduction:** Maintaining normal fetoplacental endothelial functions is critical to the success of the pregnancy as it is required to support the rapidly growing fetus. Disruption of fetoplacental endothelial functions may lead

to pregnancy complications such as preeclampsia. Vascular endothelial growth factor-A (VEGFA) and fibroblast growth factor-2 (FGF2) regulate endothelial functions primarily via inducing phosphorylation of their receptors and downstream proteins. Thereby, determining the VEGFA- and FGF2-induced protein phosphorylation profile changes in fetoplacental endothelial cells will advance our understanding of signaling mechanisms regulating fetoplacental endothelial functions.

**Methods:** Human umbilical cord vein endothelial cells (HUVECs) isolated from healthy pregnant women at term and cultured till passages 4-5. After serum starvation, cells were treated with 10 ng/ml VEGFA, FGF2, or ECM-b (control) for 10 min. Cell lysates were prepared using a lysis buffer containing protein phosphatase inhibitors, followed by chemical cleavage. After determination of protein concentrations, the cell lysates were subjected to antibody-based protein phosphorylation array analysis (Kinexus Bioinformatics). Western blotting was used to confirm phosphorylation of proteins of interest.

**Results:** Compared with the control, 10 min of VEGFA treatment elevated ( $\geq 2$  fold) the phosphorylation of 48 proteins including many proteins that are important to endothelial functions (e.g., STAT2, AKT1, and ERK1/2), and suppressed ( $\geq 2$  fold) phosphorylation of 88 proteins including many proteins in the VEGFA signaling pathway (e.g., MEK1, VEGFR1, and VEGFR2). FGF2 increased phosphorylation of 18 proteins including STAT4 and Src, while it decreased phosphorylation of 19 proteins including RSK1 and SIK3. VEGFA-induced AKT1 and ERK1/2 phosphorylation was confirmed by Western blotting.

**Conclusion:** This is the first report of the VEGFA- and FGF2-induced protein phosphorylation profile changes in HUVECs. These data indicate that VEGFA and FGF2 rapidly and dramatically alter the signaling cascades in fetal endothelial cells, which is much more complicated than we expected before. More importantly, these signaling molecules could be potential therapeutic targets for VEGFA- and FGF2-regulation associated endothelial dysfunction in pregnancy complications such as preeclampsia. **NIH PO1 HD38843 (JZ).**

## W-163

**Is Extracellular Outflow of Transcription-Related Factor High-Mobility Group A1 Protein Involved in the Pathogenesis of Preeclampsia?** Yuka Uchikura, Keiichi Matsubara, Yuko Matsubara, Takashi Sugiyama. *Ehime University, Toon, Japan.*

**Introduction:** High-mobility group A1 (HMGA1) protein, a transcription-related factor, generally fulfill its function in nuclei of cells. It is known that HMGA1 is highly expressed in cancer cells and undifferentiated cells early in pregnancy, and is lowly expressed in differentiated cells. Although we have reported the involvement of HMGA1 in the pathogenesis of preeclampsia (PE), there are few reports on its function so far. We investigated the role of HMGA1 in terms of the pathogenesis of PE.

**Methods:** The expression of HMGA1 on the maternal side of preeclamptic placenta and in the uterus 3 days after fertilization in PE model mice was observed by immunohistochemistry. In order to study the effects of extracellular HMGA1, extravillous trophoblast cells (EVTs; HTR8 / SVneo) was cultivated with deoxycholic acid (DCA) and we performed wound healing assay / Matrigel transwell migration assay. HMGA1 concentration in the culture supernatant was measured by ELSA under low oxygen or with DCA. Exosomes in the culture supernatant were collected and HMGA1 expression in exosomes was analyzed using western blotting method and DIA proteome analysis.

**Results:** HMGA1 was translocated outside the nuclei of EVT in preeclamptic placenta and a part of it was found extracellularly early in pregnancy. HMGA1 was also expressed around zygote in PE model mice. The migration and invasion ability of EVT was suppressed by promoting nuclear export of HMGA1 caused by DCA. The exosomal HMGA1 released from EVT was induced by DCA.

**Conclusion:** HMGA1 generally exists in the nuclei of EVT in normal pregnancy and promotes the proliferation, invasion, and migration. However, HMGA1 is translocated from the nuclei into the cytoplasm and a part of it is released extracellularly in the form of exosomes resulted in reduced EVT's invasion and migration in PE.

## W-164

**The Impact of Maternal Diabetes on Fetal-Placental Size at Birth and Umbilical Cord Oxygen Values.** Sheryl Choo†,<sup>1</sup> Barbra de Vrijer,<sup>1</sup> Hilary Brown,<sup>2</sup> Larry Stitt,<sup>1</sup> Timothy RH Regnault,<sup>1</sup> Bryan S. Richardson,<sup>1</sup> <sup>1</sup>Western University, London, ON, Canada; <sup>2</sup>University of Toronto, London, ON, Canada.

**Introduction:** Studies of diabetic mothers show increased glucose to be causative for fetal macrosomia with enhanced placental growth and up-regulation of glucose transporters. However, whether fetal oxygenation is increased through enhanced placental diffusion as seen in LGA infants from non-diabetic (ND) pregnancies (AJOG 2001;185:674-82), is unknown. We examined associations between maternal diabetes and newborn birth/placental weight ratios as measures of placental development, and umbilical vein and artery PO<sub>2</sub> as measures of fetal oxygenation to test the hypothesis that these parameters will be altered in diabetic vs ND pregnancies with implications for regulatory mechanisms. **Methods:** A tertiary hospital database was used to obtain maternal diabetes status, birth and placental weights, umbilical cord gases, and other pregnancy/labor-related information for all patients delivering at more than 34 completed weeks between Jan 1, 1990 and Jun 15, 2011 (N=69,854). The effect of maternal diabetes on birth/placental weight ratios and umbilical cord PO<sub>2</sub> values was examined controlling for pregnancy and labor covariates using ANCOVA. Data are presented as percentages and means ± SD, as applicable.

**Results:** There were 66,552 ND, 2,676 gestational-diabetic (GD), and 626 overt diabetic mellitus (DM) patients available for study. Diabetic patients were older, had increased BMI, hypertension and preterm birth, and increased use of regional anesthesia and cesarean delivery (all p<.01). GD and DM patients had a stepwise increase in LGA infants at 20.2% and 42.2% vs the NDs at 9.2% (all p<.01); and a decrease in the birth/placental weight ratio at 4.99±0.83 (p<.01) and 4.94±0.83 (p<.05) indicating disproportionately large placentas vs the NDs at 5.24±0.85. DM patients had lower umbilical vein PO<sub>2</sub> at 25.4±7.1 vs the NDs at 27.5±6.7 mmHg (p<.05) and lower umbilical artery PO<sub>2</sub> at 14.6±5.7 vs the NDs at 15.3±5.5 mmHg, although NS after adjusting for covariates, while GD values were little changed.

**Conclusion:** GD and DM patients had increased LGA infants and disproportionately large placentas consistent with enhancement of nutrient supply to the placenta leading to up-regulation in nutrient transport and thereby placental and fetal growth. While little changed in the GDs, umbilical vein and artery PO<sub>2</sub> were decreased in DM infants, likely involving aberrant placental development with diffusional impairment of oxygen and increased utilization with the larger size in these infants, respectively. Accordingly, oxygen is unlikely to be a primary promoter for fetal growth in these pregnancies as it is in ND pregnancies, with a change in the balance of nutritional cues whereby oxygenation is lower, with implications for longer term development.

## W-165

**Cardiovascular, Anti-Angiogenic, Metabolic and Mitochondrial Signatures of Preeclampsia in Hypoxic Pregnancy.** Wen Tong†,<sup>1,2</sup> Kirsty L. Brain,<sup>1</sup> Beth J Allison,<sup>1</sup> Kimberley J Botting,<sup>1</sup> Youguo Niu,<sup>1,2</sup> Sage G Ford,<sup>1</sup> Tess A Garrud†,<sup>1</sup> Peter F.B. Wooding,<sup>1,2</sup> Olga V Patey,<sup>1</sup> Qiang Lyu,<sup>1</sup> Lin Zhang,<sup>1</sup> Caroline J Shaw,<sup>1</sup> Katie A O'Brien†,<sup>1</sup> Alice P Sowton†,<sup>1</sup> Tereza Cindrova-Davies,<sup>1,2</sup> Hong W Yung,<sup>1,2</sup> Graham J Burton\*,<sup>1,2</sup> Andrew J Murray\*,<sup>1</sup> Dino A Giussani\*,<sup>1,2</sup> <sup>1</sup>Department of Physiology, Development and Neuroscience, University of Cambridge, United Kingdom; <sup>2</sup>Centre for Trophoblast Research, Cambridge, United Kingdom.

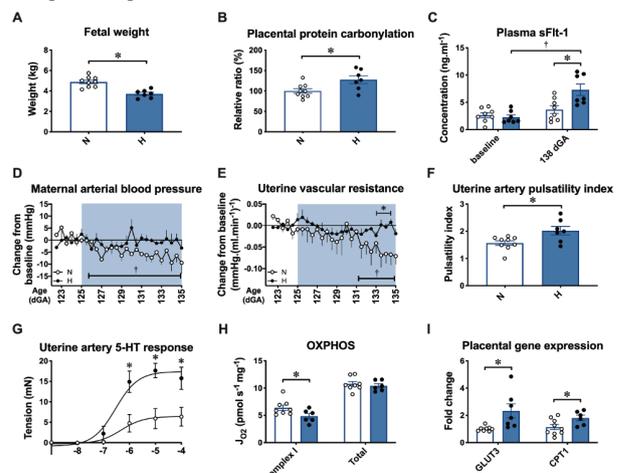
**Introduction:** Preeclampsia is a serious pregnancy complication, but underlying mechanisms remain uncertain. By combining experiments *in vivo* with those at the cellular and sub-cellular levels, we investigated whether hypoxic pregnancy in sheep promotes maternal cardiovascular and placental dysfunction, as in preeclampsia.

**Methods:** Pregnant ewes were exposed to normoxia (N; n=10) or 10% hypoxia (H; n=7) from 105 to 138 days gestational age (dGA; term 145 dGA) in isobaric chambers. Maternal blood samples were taken before and at the end of exposure. At 138 dGA, *in vivo* uterine artery pulsatility index (PI) was determined by Doppler. *Post mortem*, molecular analyses

and respirometry were carried out in placentomes, and uterine artery reactivity was assessed by myography. A separate cohort of pregnant ewes was surgically instrumented at 120 dGA with Transonic flow probes and catheters to record cardiovascular function *via* wireless data acquisition. Five days later, ewes were exposed to N (n=5) or H (n=5) for 10 days. Data were compared statistically via Two-way RM ANOVA or the Student's *t* test.

**Results:** Hypoxic pregnancy led to fetal growth restriction (FGR), increasing placental protein carbonylation and maternal serum sFlt-1 (Figure, A-C). The fall in maternal blood pressure and uterine vascular resistance measured in control ewes with advancing gestation did not occur in hypoxic ewes, and this was associated with increased uterine PI and enhanced vasoconstrictor reactivity to serotonin (5-HT; Figure, D-G). Hypoxic pregnancy decreased placental mitochondrial complex I-dependent respiration (OXPHOS) with no effect on total OXPHOS, but increased placental transcription of glucose transporter 3 (GLUT3) and carnitine palmitoyl transferase 1 (CPT1; Figure, H-I).

**Conclusion:** Gestational hypoxia leading to FGR in sheep increases placental oxidative stress and sFlt-1 in the maternal circulation. This may link alterations in placental glucose and fatty acid metabolism with uteroplacental vascular function causing downstream adverse effects on fetal growth and upstream adverse effects on maternal blood pressure, as in preeclampsia.



**Figure.** Values are mean ± S.E.M. for (A) fetal weight, (B) the relative ratio of placental protein carbonylation compared to control, (C) maternal plasma levels of sFlt-1 at baseline and 138 dGA, (D) the change from baseline in maternal arterial blood pressure, (E) the change from baseline in uterine vascular resistance, (F) maternal uterine artery PI, (G) dose-dependent uterine artery constriction in response to 5-HT, (H) placental OXPHOS dependent on complex I and total OXPHOS and (I) the relative placental fold change of GLUT3 and CPT1. Groups are N (○, n=5-9) and H (●, n=5-7). Significant differences (p<0.05) are \*N vs. H or † vs. baseline; Student's *t*-test for unpaired data or two-way RM ANOVA, where appropriate.

## W-166

**Long Chain Polyunsaturated Fatty Acids Are Predominantly Incorporated into Phospholipids in Uterine and Umbilical Circulations in Pregnant Sheep.** Stephanie S Chassen, Stefanie Raymond-Whish, Stephanie R Wesolowski, Paul J Rozance, Theresa L Powell\*. *University of Colorado, Aurora, CO, United States.*

**Introduction:** Fatty acids (FA), particularly the long chain polyunsaturated fatty acids (LCPUFA) arachidonic (AA) and docosahexaenoic acid (DHA), are critical for development of the fetal brain and retina and must be transferred across the placenta from the mother. This process is altered in pregnancy complications, including intrauterine growth restriction. The pregnant sheep is a well-established model for investigating placental nutrient exchange in normal and pathologic pregnancy conditions; however, *current* studies investigating FA transfer in sheep are lacking. In this study we determined the maternal and fetal plasma lipidomic profile in healthy pregnant sheep. We hypothesized that maternal to fetal FA concentration differences in sheep would indicate significant transplacental transfer toward the fetus.

**Methods:** Studies were conducted in Columbia-Rambouillet sheep (n=7) with singleton pregnancies. Indwelling catheters were surgically placed

at gestational day 120 (term 147 days) to allow simultaneous sampling of blood from the maternal femoral artery supplying the uterine artery, uterine vein, fetal abdominal artery (representing umbilical artery, UA), and umbilical vein (UV). Following recovery, fetal and maternal plasma samples were collected and FA were extracted as total lipids and in 4 lipid classes [cholesterol ester (CE), free fatty acids, triglyceride, phospholipid (PL)]. Targeted lipidomic analyses were performed using GC-MS. Statistical analysis of maternal-fetal (maternal artery-umbilical artery), maternal A-V and fetal v-a gradients were assessed using paired t test.

**Results:** UV plasma concentrations of the essential FA linoleic (2 ng/uL) and alpha-linolenic (0.01 ng/uL) in total lipids were minimal compared to their corresponding maternal artery counterparts (140 ng/uL and 26 ng/uL, respectively). In contrast, UV AA (12 ng/uL) and DHA (15 ng/uL) concentrations in total lipids were half the maternal artery values (26 ng/uL and 25 ng/uL, respectively). All measured LCPUFA in the 4 vessels were preferentially incorporated into the PL class in both maternal and umbilical circulations, while the essential FAs were predominantly incorporated into CE. No significant differences were found in the maternal A-V or fetal v-a gradients.

**Conclusion:** Comparison of maternal and umbilical FA concentrations indicates that the sheep placenta provides a substantial barrier for lipid transfer. Interestingly, a concentration of LCPUFA derivatives, including AA and DHA, rather than their essential FA precursors can be found in the fetal circulation. AA and DHA are required for brain development and are incorporated predominantly into PL in both maternal and fetal plasma, suggesting placental PL uptake, metabolism and transfer are likely critical for fetal LCPUFA delivery.

#### W-167

**Pathologic Lesions in Placentas of Fetuses with Left Ventricular Outflow Tract Obstruction.** Rachel L. Leon, Kavita Sharma, Imran N. Mir, Lina F. Chalak\*. *University of Texas Southwestern Medical Center, Dallas, TX, United States.*

**Introduction:** The placenta and heart are both vascular organs of fetal origin that develop concurrently in early pregnancy. Hemodynamic disturbances to the extra-embryonic circulation can result in cardiac anomalies, particularly lesions characterized by left ventricular outflow tract obstruction (LVOTO). Placental microstructure is known to be disrupted in pregnancies complicated by congenital heart disease (CHD), but studies using accepted classification systems of placental histopathology in LVOTO and non-LVOTO CHD are lacking. In this study, we sought to test the hypothesis that fetuses with LVOTO have higher rates of placental pathology and lower placenta-to-birth weight ratios compared to fetuses with non-LVOTO CHD.

**Methods:** In this single-center cohort study of neonates with CHD, we determined the prevalence of pathologic lesions of the placenta in those with LVOTO and non-LVOTO CHD. We used the Redline classification system to characterize placental lesions into four main categories: 1) maternal stromal-vascular lesions, 2) fetal stromal-vascular lesions, 3) acute inflammatory/ immune processes, and 4) chronic inflammatory/ immune processes. Additionally, we quantified placentas with umbilical cord abnormalities, large for gestational age (>90<sup>th</sup> percentile), and small for gestational age (<10<sup>th</sup> percentile). Placentas in each group with any pathology and those with multiple pathologies were also determined. Prevalence of each lesion type, presence of any pathology, and presence of multiple pathologies were compared using Chi square analysis. We determined differences in placental weight to birth weight ratios between the two groups using linear regression analysis.

**Results:** A total of 272 pregnancies complicated by CHD were assessed for inclusion and 196 had placental pathologic examination performed (LVOTO n=60, non-LVOTO n=136). Pathologic lesions were present in 82% of placentas from fetuses with CHD (LVOTO 88% vs. non-LVOTO 79%; p=0.13) and multiple pathologies were present in 55% (LVOTO 52% vs. non-LVOTO 56%; p=0.58). No specific pathology was significantly more common in LVOTO compared to non-LVOTO fetal CHD. Specifically of interest were fetal stromal-vascular lesions which were found in 30% and 19% of fetuses with LVOTO and non-LVOTO

CHD, respectively (p=0.09) and umbilical cord abnormalities (LVOTO 22% vs. non-LVOTO 17%; p= 0.43). There was no significant difference in placenta-to-birth weight ratios between groups (p=0.99).

**Conclusion:** Pathologic lesions of the placenta are highly prevalent in fetal CHD irrespective of type of structural heart disease and no specific category of placental pathology predominates. Pathologic examination of the placenta should be considered in all pregnancies complicated by fetal CHD.

#### W-168

**Sexual Dimorphism in Cytotrophoblast and Syncytiotrophoblast Energetics.** Matthew Bucher†, Leslie Myatt\*. *Oregon Health & Science University, Portland, OR, United States.*

**Introduction:** The placenta is highly metabolically active and uses various fuels to generate energy to support transport and endocrine functions. Progenitor cytotrophoblasts (CTBs) are metabolically distinct from differentiated syncytiotrophoblasts (STBs). We have previously shown utilization of the three major fuel sources; glucose, fatty acids and glutamine changes in a sexually dimorphic manner in STBs from pregnancies complicated with obesity or GDM. In this study, we investigate sexual dimorphism in glycolysis, mitochondrial respiration and fuel utilization of CTBs versus STBs.

**Methods:** Placentas were collected at delivery from term C-sections of normal pregnancies with no labor (n=8, 4 males, 4 females). CTBs were isolated by enzymatic digestion followed by density gradient purification. The Agilent Seahorse XFe96 analyzer measured glycolysis (glyco stress test) and mitochondrial respiration (mito stress test) in CTBs (24hr culture) and STBs (96hr culture). Additionally, we measured mitochondrial capacity to utilize glucose, glutamine and long-chain fatty acids to generate energy using combinations of the specific inhibitors UK5099, BPTES and Etomoxir to block utilization of each of these fuels.

**Results:** With data of both sexes combined, CTBs display increased maximum glycolytic capacity (p=0.05) and glycolytic reserve (maximum - glycolytic rate) (p=0.07) vs STBs. STB mitochondria have increased basal (p=0.03), ATP-linked (p=0.01), maximal (p=0.01), non-mitochondrial respiration (p=0.01) and spare capacity (maximal - basal) (p=0.06) vs CTBs. STBs also increase the oxidation of fatty acids (p=0.08) to maintain basal respiration and glutamine (p=0.01) to maintain spare capacity (ability to respond to a stress) when the other two pathways are inhibited. When the effect of fetal sex is considered, male CTBs have increased non-glycolytic acidification (p=0.06), glycolytic capacity (p=0.07) and glycolytic reserve (p=0.06) vs male STBs. In the mito stress test, female STBs show increases in every parameter (p=0.06) except proton leak vs female CTBs. Compared to male STBs, female STBs have increased spare capacity (p=0.06) and non-mito respiration (p=0.06). Both male and female STBs increase fatty acid oxidation (p=0.06) to maintain basal respiration while male STBs increase glutamine (p=0.06) and female STBs increase glucose (p=0.06) utilization to maintain spare capacity when the other two pathways are inhibited.

**Conclusion:** As previously described, proliferative CTBs are more glycolytic while STBs predominately use oxidative phosphorylation. A clear sexual dimorphism exists in choice of fuels supporting placental cellular bioenergetics. Female STBs maintain a larger spare capacity vs male STBs due to an increased capacity to utilize glucose in response to a stress. This strategy may put males at a disadvantage if asked to respond to a stress and may lead to higher rates of adverse pregnancy outcomes.

## W-169

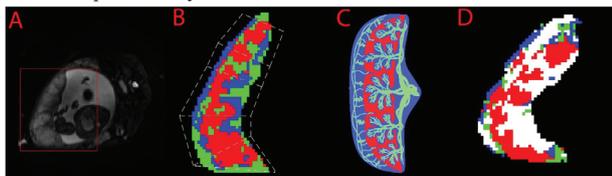
**Detangle the Anatomical Heterogeneity of the Human Placenta and Identify the Placental Lesions Using Multi-Contrast Magnetic Resonance Imaging.** Zhexion Sun<sup>†,1,2</sup>, Wenjie Wu<sup>†,1,2</sup>, Hui Wang,<sup>1,2</sup> Xiao Ma,<sup>1</sup> Hansong Gao,<sup>1,2</sup> Sicheng Wang,<sup>1,2</sup> Peinan Zhao,<sup>2</sup> Zichao Wen,<sup>2</sup> Robert McKinstry,<sup>2</sup> D. Michael Nelson,<sup>2</sup> Pamela Woodard,<sup>2</sup> Qing Wang,<sup>2</sup> Alison G Cahill,<sup>3</sup> Yong Wang.<sup>2</sup> <sup>1</sup>Washington University in St. Louis, St. Louis, MO, United States; <sup>2</sup>Washington University in St. Louis, School of Medicine, St. Louis, MO, United States; <sup>3</sup>University of Texas at Austin, Austin, TX, United States.

**Introduction:** The critical role of the placenta is to deliver adequate oxygen and nutrition to support fetal development. Despite its importance, we know little about it. Due to the heterogeneous nature of the placenta, the organ-level average measurements, as commonly performed in most placental magnetic resonance imaging (MRI) analysis, are not region-specific and often fail to represent the regional variation. To address this challenge, we developed an automatic intra-placenta segmentation technique using multi-contrast MRI.

**Methods:** A fuzzy clustering algorithm based on a Gaussian mixture model was employed. While the number of clusters equal to three as estimated by Calinski-Harabasz criterion, there are a total of four groups, as a built-in outlier detection session sorted voxels similar to neither of the clusters into the abnormal group. The parameters needed for the fuzzy clustering algorithm were extracted from T2\* and diffusion MRI by mono-exponential fitting and DTI fitting, representing the features of each voxel. The MRI data was collected from 22 patients with normal pregnancy, and 5 patients with confirmed placental pathologies. Elastix was employed to conduct image registration.

**Results:** The three separated compartments were automatically identified as intervillous space (IVS), placental vessel (PV), and placental tissue (PT). The spatial distribution of each compartment closely matches the biology of the placenta. We found that the placenta can be reliably segmented into intra-placental compartments, and we found a remarkable difference in the volumes of abnormal placental regions in normal and abnormal pregnancies ( $p=0.003$ ). Percentages of abnormal region (the 4<sup>th</sup> group) volume against total placental volume in normal patients was lower than 3%, while the percentage in five abnormal patients are 14%, 16%, 17%, 23%, and 38% respectively.

**Conclusion:** The automatic intra-placental segmentation achieved by our method allows detailed spatial evaluations of three placental compartments, enhancing *in vivo* visualization and compartment-specific quantifications of the heterogeneous organ as well as providing an early marker of placental dysfunction.



**Fig.1 A)** structural MRI of one representative patient, the image is in axial view. The placenta is located in the left half of the image, within the red box. **B)** The cluster map of the placenta from the same patient, with IVS marked in red, placental vessel marked in green, and placental tissue marked in blue. The IVS (red) have a partially connected pattern, which is similar to the shape of placental cotyledon, the functional units of material exchange. Five units are depicted by the dashed line. We assume these units correspond to five cotyledons. **C)** a placenta diagram, with the same color-encoding as that in B). Due to resolution limitation (3mm x 3mm), only a few vessel structures in the chorionic villous tree (<1.2mm) can be seen. **D)** Placenta from another patient later confirmed with placenta local edema. Areas regarded abnormal by the model were marked white. The pattern of IVS is almost preserved; however, the majority of the outer layer marked white, indicating the edema mainly affects placenta parenchyma. The patient has a 20.1% total placenta marked abnormal.

## W-170

**Sexual Dimorphism in Placental IGF-II Expression in Hypoxia-Induced Fetal Growth Restriction.** Emad Elsamadicy<sup>†</sup>, Loren P. Thompson\*. *University of Maryland School of Medicine, Baltimore, MD, United States.*

**Introduction:** Chronic hypoxia can cause fetal growth restriction (FGR) through placental dysfunction. Insulin-like growth factors (IGFs), such as IGFII, play a role in placental growth and preservation of function via vasculogenesis and nutrient transport. We hypothesize that hypoxia decreases placental IGFII protein expression leading to FGR.

**Methods:** Pregnant guinea pigs (GPs) were randomly assigned to normoxia (NMX, 21% O<sub>2</sub>) or hypoxia (HPX, 10.5% O<sub>2</sub>) during the last 14d of pregnancy (term=65d). At term, fetuses were removed via hysterotomy. Fetal body, organ, and placenta weights (wt) were recorded. Asymmetric FGR was classified as fetal wt <25% and brain:liver wt ratio >0.66. Effects of HPX and sex differences were evaluated by Western immunoblot on separate gels. IGFII band densities were normalized to  $\beta$ -actin, and groups (NMX/ HPX and males/females) were compared using Student's t-tests ( $p < 0.05$ ).

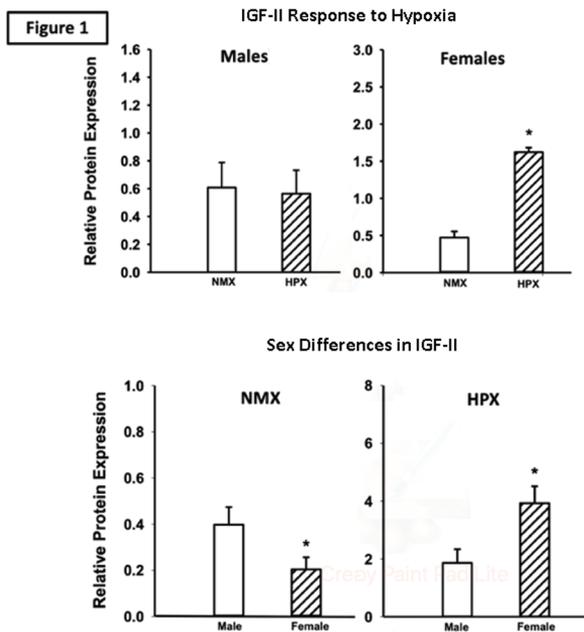
**Results:** Gestational ages were similar between the NMX and HPX cohorts of both males and females (**Table 1**). Fetal body, brain, and liver wts were less ( $p < 0.05$ ) in HPX vs. NMX controls for both sexes. HPX increased ( $p < 0.05$ ) relative placenta wt (placental wt/fetal body wt ratio) by ~30% and brain:liver wt ratios by ~40% (**Table 1**). HPX had no effect on IGFII protein levels in males but increased ( $p=0.047$ ) in females (**Figure 1, top**). With NMX, females had lower IGFII levels than males ( $0.39 \pm .07$  vs  $0.20 \pm .05$ ,  $p=0.029$ ). With HPX, females had higher IGFII levels than males ( $1.86 \pm .48$  vs  $3.9 \pm .59$ ,  $p=0.049$ ) (**Figure 1, bottom**).

**Conclusion:** This study identified two specific effects of IGFII expression in GP placenta, contrary to our hypothesis. HPX female placentas exhibit an enhanced response in IGFII expression compared to males. This may be a compensatory mechanism for protecting against further placental dysfunction. Moreover, there is an inherent sex difference in the expression of IGFII, which may indicate a reserve capacity for IGFII synthesis in females. Thus, male placentas may be more vulnerable to worsening placental function under conditions of hypoxia due to their limited capacity to generate IGFII. (NIH HL 126859)

**Table 1: Fetal Characteristics**

	Control	HPX	p-Value
<b>Males</b>			
N	7	7	
GA (d)	63.83 $\pm$ 0.54	63.2 $\pm$ 0.66	0.21
Fetal Body Weight (g)	87.13 $\pm$ 2.17	57.04 $\pm$ 1.93	<.005*
Placenta Weight(g)	4.45 $\pm$ 0.34	3.71 $\pm$ .23	.065
Brain: Liver Ratio	0.62 $\pm$ 0.04	0.86 $\pm$ .02	<.005*
Relative Placenta Wt	.051 $\pm$ .003	.067 $\pm$ .005	.014*
<b>Females</b>			
N	7	7	
GA (d)	63.71 $\pm$ 0.47	63.28 $\pm$ 0.18	.208
Fetal Body Weight (g)	93.89 $\pm$ 2.23	59.45 $\pm$ 1.98	<.005*
Placenta Weight(g)	4.33 $\pm$ 0.15	3.84 $\pm$ .11	.016*
Brain: Liver Ratio	0.59 $\pm$ 0.02	0.84 $\pm$ .04	<.005*
Relative Placenta Wt	.046 $\pm$ .001	.065 $\pm$ .002	<.005*

\*statistical significance  $p < .05$



### W-171

**Urinary Cell-Free Transcriptomic Signature Potentially Provides a Modality for Non-Invasive Detection of Adverse Outcomes during Pregnancies.** *Giorgia Del Vecchio*<sup>†</sup>,<sup>1</sup> *Shanthie Thamotharan*\*,<sup>1</sup> *Fang Wei*\*,<sup>2</sup> *Kyunghyun Sung*\*,<sup>1</sup> *Carla Janzen*\*,<sup>1</sup> *Sherin U Devaskar*\*,<sup>1</sup> <sup>1</sup>*David Geffen School of Medicine at UCLA, Los Angeles, CA, United States;* <sup>2</sup>*University of California Los Angeles, Los Angeles, CA, United States.*

**Introduction:** We recently reported on placenta-specific cell-free (cf) DNA methylation and cell-free transcriptomics during early gestation, providing predictive possibilities for pregnancies with adverse outcomes, even prior to the emergence of characteristic clinical and laboratory features. We next extended our early gestation detection capabilities towards exploring maternal urine cell-free (cf) RNA signatures along with the electric field-induced release and measurement - liquid biopsy (eLB-EFIRM) technology with a rapid turnaround time, for non-invasive detection of adverse pregnancy outcomes (APOs), such as preeclampsia (PreX), gestational diabetes mellitus (GDM) and fetal growth restriction (FGR).

**Methods:** We analyzed cfRNA content of 192 maternal urine samples collected prospectively and temporally for each subject from the first trimester until delivery. We examined urine cfRNA by RNA-seq analysis using SaVanT, a web-based signature visualization tool, in order to assess the tissues/cells-of-origin for the detected cfRNAs. Our focus was on the first and second trimesters of pregnancy, and detection of differentially expressed transcripts/genes (DEGs), along with validation of these DEGs by the eLB-EFIRM technology.

**Results:** Using the RNA-seq analysis at all trimesters of pregnancy and at delivery, and SaVanT, urine cfRNA was deconstructed to arise from sixteen different tissues-of-origin. Placenta-specific cfRNA signature increased with advancing gestation while trending lower than normal in pregnancies with adverse outcomes. Moreover, using cfRNA sequencing, we were able to detect 3710 differentially expressed genes (DEG) in the first trimester of pregnancy, 472 of them GDM-specific, 410 Preeclampsia-specific and 90 for FGR. Using elastic regularization and prediction modeling with eLB-EFIRM technology we plan on a panel of 3-5 reproducible DEGs in early gestation that are specific for PreX, GDM and FGR, prior to the emergence of classical clinical features. We tested and validated the ability of the eLB-EFIRM technology to detect one placenta-specific DEG, namely the human placental alkaline phosphatase in urine samples from subjects in our cohort.

**Conclusion:** Our preliminary studies support the possibility of employing urinary cell-free transcriptomics for the potential prediction of APOs that develop subsequently. This non-invasive approach coupled with the eLB-EFIRM technology of detection will pave the way for accessible and convenient diagnostic testing during pregnancies. Supported by NICHD.

### W-172

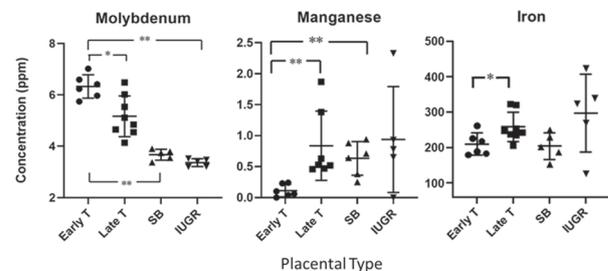
**X-Ray Fluorescence Microscopy Reveals Disruptions in Placental Element Homeostasis: An Indicator of Dysregulated Oxidative Stress Pathways?** *Vladimira Foteva*<sup>†</sup>,<sup>1</sup> *Roger Smith*,<sup>1</sup> *David Paterson*,<sup>2</sup> *Michael Jones*,<sup>3</sup> *Lee Dedman*,<sup>4</sup> *Kaushik Maiti*.<sup>1</sup> <sup>1</sup>*Hunter Medical Research Institute, Newcastle, Australia;* <sup>2</sup>*Australian Synchrotron, Clayton, Australia;* <sup>3</sup>*Queensland University of Technology, Brisbane, Australia;* <sup>4</sup>*University of Newcastle, Newcastle, Australia.*

**Introduction:** Placental dysfunction is strongly associated with obstetric complications such as intrauterine growth restriction (IUGR), preterm birth, prolonged pregnancy, and stillbirth. Oxidative stress is implicated in these pathologies and there is evidence this phenomenon is dependent upon appropriate metal homeostasis. Synchrotron technology is an unambiguous method for the identification and quantification of elements in biological tissue, forming a novel investigation of the placental metallome, and therefore the aetiology of gestational morbidities. Accordingly, our study aim was to elucidate the changes in concentration of metals in early term, late-term, IUGR and stillbirth placentas by Synchrotron X-Ray Fluorescence Microscopy (XFM) analysis.

**Methods:** Experimental groups consisted of 6 early term (37-39 wks), 8 late-term (41+ wks), 5 IUGR, and 5 stillbirth pregnancies. XFM imaging was used to spatially resolve the concentration of metals in 24 frozen, unfixed, 60  $\mu$ m placental tissue sections. Metal concentrations were measured via GeoPIXE software.

**Results:** Statistically significant differences in element concentrations (parts per million, ppm) were found between different placental sets. Mn concentrations were elevated in stillbirth placentas in comparison to healthy early term. Fe and Mn concentrations were both elevated in late term placentas. Molybdenum concentrations were significantly lower in late term, stillbirth and IUGR placentas in comparison to early term.

**Conclusion:** XRF is a unique tool in the field of placental physiology, and has opened avenues for nucleic and protein-based research. In this study, significant differences were observed in essential elements between pathological conditions. It is as yet unclear whether this represents differences in the uptake of metals from the maternal circulation or differences in the transport of metals to the fetus or a combination of these mechanisms. However, it is established that molybdoenzymes remove sulphites from the body, while iron is involved in catalase-mediated radical scavenging, and manganese is a cofactor of radical-scavenging family superoxide dismutase. We therefore postulate these results indicate dysregulation of the antioxidant response to oxidative stress in the placenta.



## W-173

**Regulation of Insulin-Like Growth Factor 1 (IGF1) Signaling in the Guinea Pig Placenta Following Nanoparticle Delivery of Human IGF1 Is Dependent on Fetal Phenotype.** Rebecca L Wilson<sup>†</sup>,<sup>1</sup> Kendal Stephens,<sup>2</sup> Kristin Lampe,<sup>2</sup> Helen Jones\*.<sup>1</sup> <sup>1</sup>University of Florida, College of Medicine, Gainesville, FL, United States; <sup>2</sup>Cincinnati Children's Hospital and Medical Center, Cincinnati, OH, United States.

**Introduction:** Abnormal placentation and function underlies many obstetric diseases such as stillbirth and fetal growth restriction (FGR). We have previously shown transgene expression of human insulin-like growth factor 1 (*hIGF1*) via nanoparticle in guinea pig placentas. Here, we investigated the effect of NP-*hIGF1* treatment on IGF1 signaling, analyzing mTOR signaling components, and downstream IGF1 targets, in a guinea pig model of FGR.

**Methods:** Female Hartley guinea pigs were fed either an *ad libitum* control diet ( $n=11$ ) or a restricted diet (maternal nutrient restriction, MNR - 70% of control,  $n=12$ ) prior to pregnancy and up to day (GD) 30-35. At GD30-35, ultrasound guided, transcutaneous, intraplacental injection of NP-Cyp19a-*hIGF1* or PBS (Sham-injected) was performed. Animals were sacrificed 5 days post-injection, fetuses and placentas weighed, and samples snap frozen for analysis of mTOR signaling proteins (Deptor, Raptor and Rictor) by Western Blotting or gene expression (*Plgf*, *Vegf*, *Pdgfr*, *Pdgfr*) by qPCR. Data was analyzed using generalized estimating equations including diet and treatment as main effects, maternal ID as a random effect and gestational day as a covariate. Statistical significance was considered at  $P<0.05$ .

**Results:** Fetal weight was reduced 20-25% in MNR compared to control fetuses ( $P<0.001$ ). There was no change in fetal weight with NP-Cyp19-*hIGF1* when compared to sham-injected ( $P>0.05$ ). Rictor expression was increased in the labyrinth (36-40%;  $P=0.045$ ) and Raptor in the sub-placenta/decidua (52-63%;  $P<0.01$ ) with NP-Cyp19-*hIGF1* treatment compared to sham-injected, irrespective of diet. Labyrinth expression of Deptor was differentially regulated by NP-Cyp19-*hIGF1* treatment: increased in control-NP but reduced in restricted-NP when compared to sham-injected ( $P=0.001$ ). Analysis of sub-placenta/decidua expression of IGF1 targets, showed in controls, NP-Cyp19-*hIGF1* treatment decreased expression of *Plgf*, *Vegf* and *Pdgfr* compared to sham (33%:  $P=0.001$ , 30%:  $P=0.048$  and 29%:  $P=0.028$ , respectively). In contrast, in restricted sub-placenta/decidua, mRNA expression of *Vegf* and *Pdgfr* was increased with NP treatment compared to sham (72%:  $P<0.001$  and 51%:  $P=0.016$ , respectively).

**Conclusion:** We have shown placenta NP-Cyp19-*hIGF1* treatment is capable of modifying intracellular IGF1 signaling and target gene expression. Expression of the mTOR inhibitor Deptor differed between diets and enhanced placenta signaling and expression under MNR conditions with IGF1 to potentially enhance fetal growth but reduced signaling under control conditions to maintain homeostasis and prevent potential overgrowth.

## W-174

**Evidence-Based View of Safety and Effectiveness of Prokineticin Receptors Antagonists during Pregnancy Prokineticin Receptors Antagonists during Pregnancy.** Deborah Reynaud<sup>†</sup>,<sup>1</sup> Frédéric Sergent\*,<sup>1</sup> Roland Abi Nahed<sup>†</sup>,<sup>1</sup> Wael Traboulsi<sup>†</sup>,<sup>2</sup> Constance Collet<sup>†</sup>,<sup>1</sup> Padma Murthi\*,<sup>3</sup> Nadia Alfaidy\*,<sup>1</sup> Mohamed Benharouga\*.<sup>1</sup> <sup>1</sup>INSERM U1292, Grenoble, France; <sup>2</sup>Georgetown University, Georgetown, WA, United States; <sup>3</sup>Monash University, Victoria, Australia.

**Introduction:** Endocrine gland derived vascular endothelial growth factor (EG-VEGF) is a canonical member of the prokineticin (PROKs) family. It acts via the two G-protein coupled receptors, namely PROKR1 and PROKR2. We have recently demonstrated that EG-VEGF is highly expressed in the human placenta; contributes to placental vascularization and growth and that its aberrant expression is associated with pregnancy pathologies including preeclampsia and fetal growth restriction. These findings strongly suggested that antagonization of its receptors may constitute a potential therapy for the pregnancy pathologies.

**Methods:** Two specific antagonists of PROKR1 (PC7) and for PROKR2 (PKRA) were reported to reverse PROKs adverse effects in other systems.

In the view of using these antagonists to treat pregnancy pathologies, a proof of concept study was designed to determine the biological significances of PC7 and PKRA in normal pregnancy outcome. PC7 and PKRA were tested independently or in combination in trophoblast cells and during early gestation in the gravid mouse.

**Results:** Both independent and combined treatments uncovered endogenous functions of EG-VEGF. The independent use of antagonists distinctively identified PROKR1 and PROKR2-mediated EG-VEGF signaling on trophoblast differentiation and invasion; thereby enhancing fetoplacental growth and pregnancy outcome.

**Conclusion:** Our study provides evidence for the potential safe use of PC7 or PKRA to improve pregnancy outcome.

## W-175

**COVID-19 in Pregnancy Is Associated with Increased Natural Killer Cell and Macrophage Infiltration at the Maternal-Fetal Interface.**

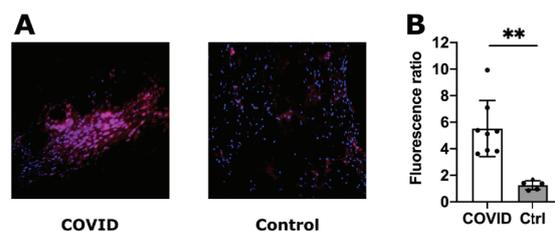
Lillian Juttukonda<sup>†</sup>,<sup>1</sup> Wachman Elisha\*,<sup>2</sup> Yoel Benarroch<sup>†</sup>,<sup>3</sup> Jeffery Boateng<sup>†</sup>,<sup>2</sup> Elizabeth Taglauer\*.<sup>1</sup> <sup>1</sup>Boston Children's Hospital, Boston, MA, United States; <sup>2</sup>Boston Medical Center, Boston, MA, United States; <sup>3</sup>Boston University School of Medicine, Boston, MA, United States.

**Introduction:** Maternal COVID-19 infections are common in pregnancy, yet rates of infant SARS-CoV-2 transmission are low. As the interface between mother and fetus during pregnancy, the placenta may form a protective barrier against vertical transmission of COVID-19. Major immune populations at the maternal-fetal interface include macrophages and natural killer (NK) cells which can mount cellular and inflammatory cytokine responses against viral infections. Understanding whether these cells respond to COVID-19 in the placenta may help understand intrauterine mechanisms preventing fetal transmission of SARS-CoV-2 in pregnancy.

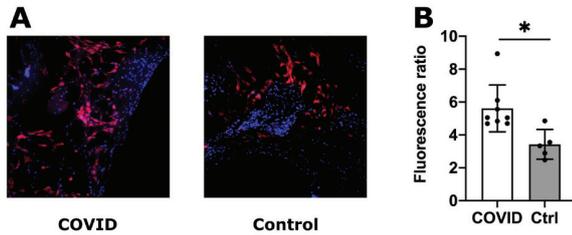
**Methods:** Placental biopsies (including decidual tissue) were obtained from 8 SARS-CoV-2 positive pregnancies (COVID) and 5 contemporary SARS-CoV-2 negative pregnancies (Control) during a peak period of COVID-19 admissions at Boston Medical Center. Formalin-fixed cryosections were stained with primary antibodies (CD14 or CD56) and fluorescently labeled secondary antibodies. Immunofluorescence images of decidual tissue were obtained by automated acquisition and used to calculate a fluorescence ratio (FR) of corrected total cell fluorescence (CTCF) of target antigens over secondary-only negative background. FR of COVID and Control specimens were compared using independent t-tests.

**Results:** CD14+ macrophages and CD56+ NK cells were observed in all decidual tissues examined. Quantitative microscopy revealed significantly greater macrophage infiltrates in COVID samples compared to Control samples ( $p<0.01$ , Figure 1). There were also significantly greater NK cell infiltrates in COVID samples compared to Controls ( $p<0.05$ , Figure 2).

**Conclusion:** Our data demonstrate that NK cells and macrophages accumulate at the maternal-fetal interface in pregnancies complicated by COVID-19, suggesting these leukocyte populations may be of interest for ongoing studies examining intrauterine responses to SARS-CoV-2 during pregnancy.



**Figure 1.** (A) Representative images (200x) of CD14 immunofluorescence. (B) Graphical analysis of comparative fluorescence quantitation. Placental tissues from pregnant women who were SARS-COV-2 positive (COVID,  $n = 8$ ) or negative (Control/Ctrl,  $n = 5$ ) upon admission screening ( $n = 8$ ). \*\*  $p < 0.01$ .



**Figure 2.** (A) Representative images (200x) of CD56 immunofluorescence. (B) Graphical analysis of comparative fluorescence quantitation. Placental tissues from pregnant women who were SARS-COV-2 positive (COVID, n = 8) or negative (Control/Ctrl, n = 5) upon admission screening. \* p < 0.05.

W-176

**The Immune Checkpoint PD-1/PD-L1 Expression Differs in Mouse Placenta in Acute and Sub-Chronic Maternal Inflammation.** Yang Liu†, Jin Liu†, Anguo Liu†, Jun Lei\*, Irina Burd\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Immune checkpoints pathway PD-1/PD-L1 (Programmed cell death 1/Ligand programmed cell death 1) in maternal-fetal interface has recently emerged as an exciting and effective prognostic and therapeutic strategy. PD-1 and PD-L1 have immunosuppressive functions. We hypothesized that PD-1/PD-L1 expression are different in acute (aMI) and sub-chronic (cMI) maternal inflammatory states.

**Methods:** Timed-pregnant CD-1 mice were utilized to two mouse models of MI, including acute local inflammation (ALI) and sub-chronic systemic inflammation (CSI). To mimic ALI, 25 µg lipopolysaccharide (LPS) in 100µl phosphate buffered saline (PBS) or 100µl PBS was administered into each dam via IU injection at embryonic (E) day 17. To establish the model of CSI, 0.5 µg IL-1β in 100µl PBS or 100µl PBS was IP injected into each pregnant dam from E14-17. After 6 hour post injection (hpi) and 24hpi of LPS injection and 3hpi and 24hpi of IL-1β administration, placentas were harvested. Flow cytometry was performed to characterize the PD-1 and PD-L1 expression on placenta leukocytes and subsets. Standard statistical analyses were employed.

**Results:** In the aMI model, we found that LPS significantly increased the number of leukocytes in the placenta at 24hpi compare to PBS group (p<0.01), while the PD-L1 expression in all placental leukocytes significantly increased at 6hpi (p<0.01) and 24hpi (p<0.05, Figure 1A). LPS had the tendency to increase the macrophages at 6hpi and have no differences at 24hpi compared to PBS, while PD-L1 expression in placenta macrophages was increased at 6hpi (p<0.05) and 24hpi (p<0.05, Figure 1B). T cells in placenta were decreased at 24hpi (p<0.05) compare with PBS, but the PD-1 expression in placenta T cells was significant increased at 24hpi (p<0.05, Figure 1 C). In the cMI model, the placenta leukocyte numbers significantly increased at 3hpi (p<0.01) and 24hpi (p<0.05), and the PD-1(p<0.01) and PD-L1 (p<0.01) expression increased in the leukocyte at 24hpi (Figure 2A,B).

**Conclusion:** Our study reveals that different course of maternal inflammation leads to a variable PD-1/PD-L1 expression, with a different intensity across the placenta. Future studies are needed to demonstrate the role of PD-1/PD-L1 pathway expression in evaluation of chronic vs acute maternal inflammation in clinical scenario.

Figure 1

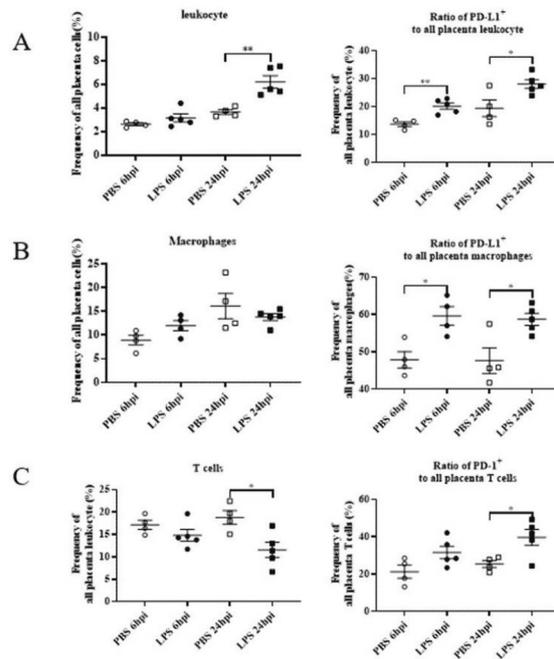
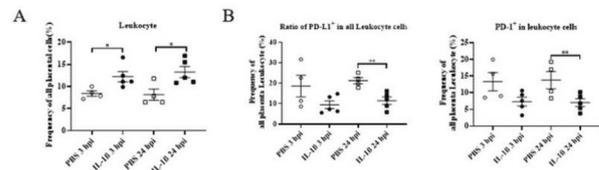


Figure 2



W-177

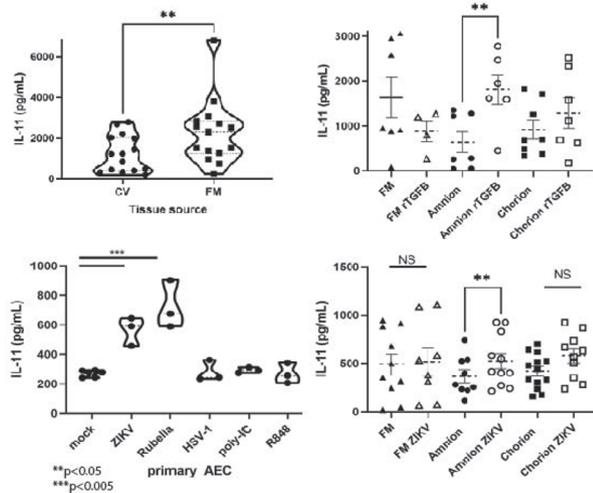
**IL-11 Is Regulated by TGFβ in the Human Amnion and Is Induced by Teratogenic Viruses.** Christina Megli, Pallavi Karunakaran, Azia Evans, Carolyn Coyne\*. *University of Pittsburgh School of Medicine, Pittsburgh, PA, United States.*

**Introduction:** The fetal membrane acts as an important immunologically protective barrier between the mother and fetus. We have previously demonstrated that innate immune mechanisms exist in placental villi to resist infection and select viruses preferentially infect the fetal membrane. Our prior work has also demonstrated that chorionic villi (CV) and fetal membrane (FM) explants have distinct cytokine/chemokine profiles. We identified interleukin-11 (IL-11) as a cytokine predominantly secreted by fetal membrane. IL-11 is in the IL-6 family and is required for successful embryo implantation. IL-11 regulation in non-reproductive tissues has been demonstrated to be tissue specific. In this study, we investigated the tissue specificity of IL-11 production by the fetal membrane and the induction of IL-11 in response to various infectious stimuli.

**Methods:** Fetal membranes were collected at cesarean section in the absence of labor or infection. Intact fetal membrane or separated amnion and chorion were incubated overnight in DMEM F12 supplemented with 10% FBS and penicillin-streptomycin. Primary amniotic epithelial cells (AEC) and immortalized amniotic epithelial cells were also used and cultured as previously described. TGF-β, Lipopolysaccharide (LPS), poly-I:C, cyclic GAMP, R848, herpes simplex virus (HSV)-1, *Listeria monocytogenes*, *Streptococcus agalactiae*, Zika (ZIKV) virus, and Rubella virus were used to infect or expose explants and primary cells. Culture supernatants were harvested and cytokine responses were measured by ELISA and Luminex profiling. Cells or tissues were lysed and RNA was isolated. IL11 transcription was measured by qPCR.

**Results:** IL-11 is constitutively secreted by intact fetal membrane, amnion and chorion explants. TGF- $\beta$  controls IL-11 secretion in the amnion. In amniotic epithelial cells inhibition of this signaling reduces IL-11 levels. Moreover, addition of TGF $\beta$  enhances IL-11 secretion in amnion and cells. IL-11 is not induced by synthetic ligands of antiviral pattern recognition receptor signaling, infection with HSV-1, LPS, or infection of *L. monocytogenes* or *S. agalactiae*. However, IL-11 is robustly induced by ZIKV virus and Rubella virus infections, which occurs early during replication.

**Conclusion:** IL-11 secretion is regulated by TGF- $\beta$  in the amnion layer of the fetal membrane and is produced at high amounts in comparison chorionic villi. IL-11 is secreted in response to teratogenic viruses and may be important in the response to vertical infections in the fetal membrane



### W-178

**Viral Infection Followed by Lipopolysaccharide Exposure Upregulates *IP-10* and *ITAC* in Human First Trimester Decidual Cells: Implications for Preeclampsia Pathogenesis.** Bradley H. Sipe†, Xiaofang Guo, Ozlem Guzeloglu-Kayisli, Nihan Semerci, Asli Ozmen, Kellie Larsen, Frederick Schatz, Umit A. Kayisli, Charles J. Lockwood\*. *University of South Florida Morsani College of Medicine, Tampa, FL, United States.*

**Introduction:** Preeclampsia, a significant cause of maternal morbidity and mortality is linked to reduced decidual natural killer (NK) cell recruitment. Exposure of cultured human first trimester decidual cells (FTDCs) to lipopolysaccharide (LPS) and interferon  $\gamma$  (IFN $\gamma$ ) synergistically upregulates both mRNA and protein levels of IFN-inducible protein 10 kDa (IP-10) and IFN-inducible T cell alpha chemoattractant (ITAC), potent NK cell chemoattractants. However, elevated IP-10 and ITAC downregulate their cognate NK cell receptors impairing NK cell recruitment to the decidua. Since viral infections induce IFN $\gamma$ , we tested the hypothesis that viral infections of FTDCs, followed by bacterial infection are responsible from synergistic upregulation of IP-10 and ITAC expression.

**Methods:** FTDCs cultures (n=4) were primed in basal medium containing 10% stripped calf serum with  $10^{-8}$  M estradiol +  $10^{-7}$  M medroxyprogesterone for 7 days. FTDCs were then passaged in 6-well culture plates treated with the following experimental conditions for 48 h: 1) Control medium; 2) Zika virus (ZIKV) infection at multiplicity of infection (MOI) of 1; 3) LPS treatment (5 $\mu$ g/ml); 4) 24 h ZIKV infection followed by LPS treatment; 5) 24 h LPS treatment followed by ZIKV infection; and 6) ZIKV infection + LPS co-incubation. After a 48 h incubation, FTDCs were collected for RNA isolation and qPCR using TaqMan gene specific assay was performed to measure *IP-10* and *ITAC* mRNA expression in cell lysates.

**Results:** In FTDCs, either ZIKV infection or LPS alone or in combination or sequentially increased *IP-10* and *ITAC* mRNA expression compared to controls. ZIKV infection induced higher *IP-10* and *ITAC* expression

(Mean $\pm$  SEM: 11.75 $\pm$  2.2 and 18.11 $\pm$ 3.4, respectively,  $P<0.05$ ) vs. LPS alone (3.41 $\pm$ 0.2 and 6.56 $\pm$ 0.5, respectively,  $P<0.05$ ). LPS treatment following ZIKV infection synergistically increased *IP-10* and *ITAC* mRNA levels (22.48 $\pm$ 4.5 and 29.82 $\pm$ 2.1, respectively,  $P<0.05$ ) compared to ZIKV infection or LPS-treatment alone. However, ZIKV infection following LPS treatment failed to further increase *IP-10* or *ITAC* mRNA levels (11.10 $\pm$  2.9 or 16.20 $\pm$ 2.9, respectively) compared to ZIKV infection alone. **Conclusion:** ZIKV infection followed by LPS derived from gram (-) bacteria in early pregnancy synergistically increases decidual cell expressed TLR4 signaling resulting in a significant increase in IP-10 and ITAC levels. The resulting synergistic increased in IP-10 and ITAC levels reduce CXCR3<sup>+</sup>NK cell recruitment to the decidua, leading to shallow placentation, thereby increasing the risk of preeclampsia.

### W-179

**Role of Androgen in GBS Induced Innate Immune Response and Subsequent Neurobehavioral Impairments.** Seline Yasmine Vancolet†, Ayash Taghreed†, Bernard Robaire\*, S ebire Guillaume\*. *McGill University, Montreal, QC, Canada.*

**Introduction:** Group B Streptococcus (GBS) is a leading cause of maternal infection during pregnancy. An exaggerated innate immune response leads to placental inflammation (chorioamnionitis). Chorioamnionitis is associated with an increased risk of autism spectrum disorders (ASD), a neurobehavioral disability that is more prominent in males than females. Although pre-clinical findings show that GBS-induced maternal immune activation occurs with more prominent inflammation and ASD-like behaviours in males than females, the mechanism underlying this sex difference is not understood. This knowledge gap raises the question of the role of sex steroids in the immune response. Our hypothesis is that testosterone up-regulates the placental innate immune response. Our objective is to test the effect of the androgen blockade in alleviating the molecular (pro-inflammatory cytokines) and cellular (PMN) responses in GBS-induced placental injury and subsequent neurobehavioral impairment of males.

**Methods:** Lewis dams were injected every 24 h from gestational day (G) 18 to G21 with corn oil (vehicle) or flutamide (anti-androgen) administered at a dose of 50 mg/kg body weight. On G19, dams were injected with saline or GBS serotype Ia suspended in saline. The four experimental groups were: (1) vehicle/saline, (2) flutamide/saline, (3) vehicle/GBS, (4) flutamide/GBS. Dams underwent C sections on G22, with respect to their injection time. Maternal, fetal sera and placentas were collected for protein assays and *in-situ* analyses. Specifically, ELISA to measure key placental and fetal blood inflammatory mediators and immunohistochemistry to specify the distribution and quantify the amount of these inflammatory molecules.

**Results:** Our preliminary results showed that the dose of flutamide we used counteracted the androgen effect as shown by a significant decrease in anogenital distance of male pups from flutamide compared to vehicle dams. An expected trend of decreased maternal weight gain was associated with maternal GBS infection. A significant decrease in IL-1 $\beta$ , IL-6 and TNF- $\alpha$  concentration was observed in placentas of the GBS/flutamide group compared to the GBS group only.

**Conclusion:** Flutamide is effective in inducing the androgen blockade. Furthermore, our results indicate that the androgen blockade results in a reduced GBS-induced placental innate immune response.

### W-180

**Saturated Fatty Acids and Group B *Streptococcus* Synergize to Induce Proinflammatory Responses in Gestational Membranes.** Alison J Eastman†, Lisa M Rogers, David M Aronoff\*. *Vanderbilt University Medical Center, Nashville, TN, United States.*

**Introduction:** Prepregnancy obesity (PPO) is increasingly common and linked to pregnancy complications. PPO is associated with rectovaginal colonization by Group B *Streptococcus* (GBS), chorioamnionitis and preterm, prelabor rupture of membranes (PPROM).

**Methods:** Decidual stromal cells (DSC, the cell line THESC) or cytotrophoblasts (CTB, primary human placental trophoblasts or the cell line JEG3) were co-cultured with macrophages (MO, primary human

placental macrophages or macrophage-like monocyte cell line THP.1), and incubated with the obesity-related saturated fatty acid palmitic acid (PA, 200  $\mu$ M) and GBS for 24 h, then assayed for proinflammatory cytokine and MMP production by ELISA. Full thickness human gestational membrane punches were similarly exposed to PA or GBS. Cell death was assayed by lactate dehydrogenase assay.

**Results:** PA induced cell death in MO cultured alone, which was ameliorated by co-culture of MO with DSC or CTB. PA did not kill DSC or CTB cultured alone. Without GBS, PA significantly induced GM-CSF, TNF $\alpha$ , MMP9, IL-1 $\beta$ , and IL-6 by DSC and CTB individually or in co-culture with MO, while PA did not significantly induce MO proinflammatory responses. Co-cultures treated with GBS alone induced GM-CSF, TNF $\alpha$ , MMP9, IL-1 $\beta$ , and IL-6. Co-cultures of MO with CTB or DSC induced the greatest inflammatory response when exposed to both GBS + PA. MO-DSC co-cultures induced a greater magnitude of proinflammatory cytokines than MO-CTB co-cultures. There was high variability in primary human gestational membranes, but synergistic induction of MMP9 during PA + GBS exposure was observed.

**Conclusion:** The saturated fat PA induced a proinflammatory response from DSCs, CTBs, and MO, both alone and in co-culture. Induction of proinflammatory cytokines during co-culture was often synergistic. A greater magnitude proinflammatory response from DSC-MO co-cultures suggests that DSCs are the structural cell type of the gestational membranes driving the proinflammatory response to PA. PA + GBS induced additive or synergistic increases in proinflammatory mediators. This study may explain why GBS-related complications are more common among pregnancies complicated by PPO.

## W-181

**Downregulation of WNT Signaling Is Critical for Extravillous Trophoblast Cell Differentiation.** Vinay Shukla<sup>†</sup>,<sup>1</sup> Kaela M. Varberg,<sup>1</sup> Marija Kuna,<sup>1</sup> Anna Galligos,<sup>1</sup> Khurshed Iqbal,<sup>1</sup> Michael J. Soares\*,<sup>1,1,2</sup>  
<sup>1</sup>University of Kansas Medical Center, Kansas City, KS, United States;  
<sup>2</sup>Children's Mercy, Kansas City, MO, United States.

**Introduction:** Trophoblast stem (TS) cell expansion and differentiation dictate the development and functionality of the hemochorial placenta. TS cell differentiation is characterized by the development of specialized cells entering the uterus termed extravillous trophoblast (EVT) cells and specialized multi-nucleated cell layers termed syncytiotrophoblast. In this project, we investigate the importance of modulating WNT signaling for regulating the trophoblast stem state and EVT cell differentiation.

**Methods:** Human TS cell lines were used to investigate the maintenance of the stem state and regulation of EVT cell differentiation. Beta catenin (CTNBN1) expression was used as a measure of canonical WNT signaling. Activation and loss-of-function manipulations were used to investigate WNT signaling. Cellular responses were assessed using morphological, gene and protein expression analyses, and the assessment of EVT cell invasive behavior.

**Results:** Canonical WNT signaling was evident in the TS cell stem state but not following EVT cell differentiation, suggesting that WNT signaling is downregulated during EVT cell differentiation. Furthermore, we found that exposure to a WNT activator (CHIR99021) inhibited EVT cell differentiation, as shown morphologically and by transcript expression profiles. Interestingly, EVT cell differentiation is characterized by the dramatic upregulation of NOTUM. NOTUM is a WNT antagonist. We proposed that the upregulation of NOTUM could be pivotal to diminishing WNT signaling leading to EVT cell differentiation. Inhibition of NOTUM expression using specific short hairpin RNAs increased canonical WNT signaling and interfered with EVT cell differentiation. Phenotypic TS cell responses were similar following exposure to the WNT activator or to knockdown of the WNT antagonist as determined by morphological and molecular indices, including global transcript profiles, and invasive cell behavior.

**Conclusion:** Canonical WNT signaling is pivotal to the maintenance of human TS cell stemness and prevention of EVT cell differentiation. NOTUM is identified as a critical liberator of canonical WNT dominance and development of EVT cells. Thus, the appropriate modulation of canonical WNT signaling is critical to placental development and its

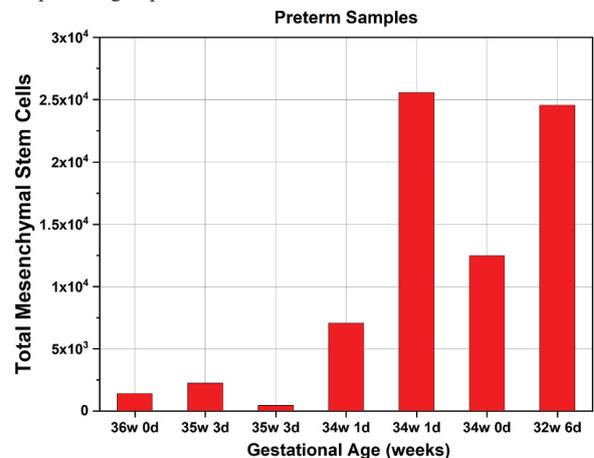
dysregulation a potential factor in placental disease. [Supported by KUMC BRTP & K-INBRE QW864667 (VS); F32HD096809 (KMV), GM103418 (MK); NIH grants HD020676, HD099638; Sosland Foundation]

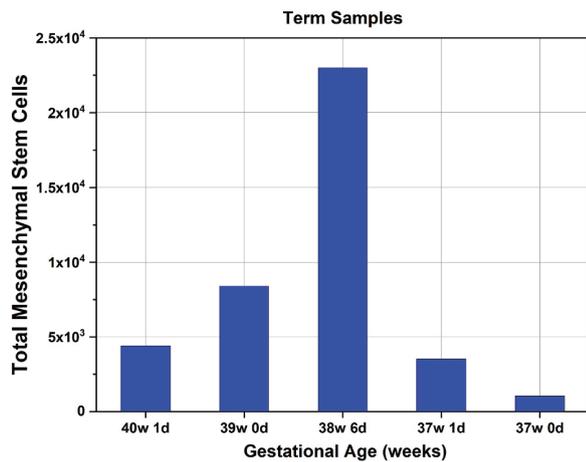
## W-182

**The Feasibility of Isolation, Identification and Quantification of Mesenchymal Umbilical Cord Blood Stem Cells in the Delayed Cord-Clamping Era.** Emily Rose Smith\*,<sup>1</sup> William Curtin\*,<sup>2</sup> Kevin Yeagle<sup>†</sup>,<sup>3</sup> Nurgul Carkaci-Salli\*,<sup>4</sup> Serdar Ural\*,<sup>3</sup> <sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>2</sup>Penn State Health, Reading, PA, United States; <sup>3</sup>Penn State Health, Hershey, PA, United States; <sup>4</sup>Penn State University, Hershey, PA, United States.

**Introduction:** Mesenchymal stem cells (MSC) are multipotent cells which have been shown to possess the ability to differentiate into multiple cell types. These MSCs can be obtained from umbilical cord blood (UC). This is especially important for potential treatment in hypoxic-ischemic brain injury, which is shown to have significant morbidity and mortality. MSCs have a promising role in the treatment of hypoxic ischemic encephalopathy.

**Methods:** 3 mL of umbilical cord blood were obtained at various gestational ages after delayed cord clamping. Peripheral blood mononuclear cells (PBMCs) separated by gradient centrifugation within 4 hours of delivery were passed through magnetic bead micro-columns to exclude the CD34 positive hematopoietic stem cells. The CD34 negative populations were cryopreserved for later processing. The samples were incubated with fluorescent-tagged mesenchymal cell marker antibodies CD 29, CD44, CD73, CD105, and hematopoietic cell marker CD45. Live cells were assessed with 7-Aminoactinomycin-D (7-AAD). The cell populations were analyzed by fluorescence-activated cell sorting (FACS). Descriptive statistics were used with results of the overall samples to test the hypothesis that stem cell number would be higher in the preterm group. <0.05.





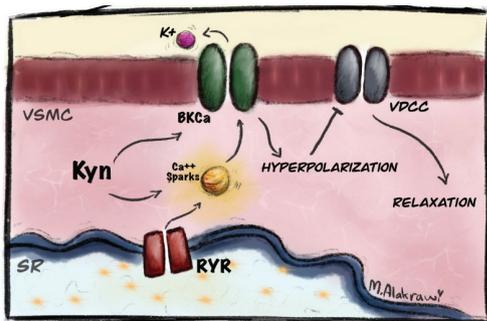
**Results:** A total of 12 samples were analyzed, ranging between 32w6d and 40w1d. All samples across gestational age demonstrated MSC populations by FACS. The percent of the live cell population in the term group was 0.3 - 0.7%. The percent of the live cell population in the preterm group varied between: 0.3 - 8.9%. MSC were isolated in all 12 cord samples with a mean count of 5740 (456- 25,584) cells. The mean number of mesenchymal stem cells isolated was higher in the preterm group, at 7,080 cells versus 4,400 cells in term group. *p* value of 0.81.

**Conclusion:** Mesenchymal stem cells can be isolated in as small a sample of umbilical cord blood as 3 mL across gestational ages.

#### W-183

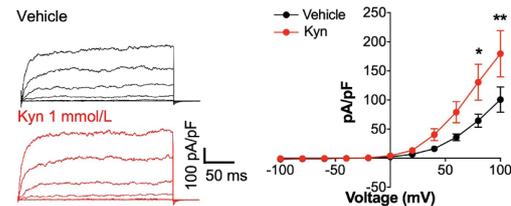
**The Putative Antihypertensive, Kynurenine, Activates BK<sub>Ca</sub> Channels Directly and Increases Ca<sup>2+</sup> Sparks.** Stephanie A Worton†, Harry AT Pritchard†, Susan L Greenwood\*, Mariam Alakrawi†, Adam Greenstein\*, Jenny E Myers\*. *University of Manchester, Manchester, United Kingdom.*

**Introduction:** The tryptophan metabolite, kynurenine (Kyn), is a potent relaxant in arteries for women with PE, and we have proposed kynurenine as a novel therapeutic for vascular dysfunction in PE. Kyn causes relaxation via activation of large-conductance calcium-activated potassium channels (BK<sub>Ca</sub>) on vascular smooth muscle cells (VSMCs). BK<sub>Ca</sub> are responsible for spontaneous transient outward currents (STOCs), and in the resting state, STOCs frequency is determined by the release of Ca<sup>2+</sup> sparks from ryanodine receptors. **Aim:** To determine the mechanism of Kyn-induced BK<sub>Ca</sub> activation.



**Methods:** Using the ruptured whole-cell patch clamp technique, BK<sub>Ca</sub> currents were assessed in VSMCs from women with normal pregnancy following treatment with Kyn 1 mmol/L or vehicle. STOCs were recorded in the absence or presence of Kyn (1 mmol/L) via a whole-cell perforated patch clamp approach. Intact resistance arteries (<400 μm) were mounted on the pressure myograph and loaded with Fluo-4-AM to image Ca<sup>2+</sup> sparks pre- and post-treatment with Kyn (1 mmol/L) or mounted on the wire myograph to assess the vasorelaxant effects of Kyn (0.05-3 mmol/L) in precontracted arteries ± inhibition of Ca<sup>2+</sup> sparks (ryanodine).

**Results:** In ruptured VSMCs, Kyn increased the BK<sub>Ca</sub> current (*n*=5-8, *p*=0.022), suggesting a direct effect on BK<sub>Ca</sub> channels. Following Kyn treatment, the amplitude of STOCs were increased (*n*=6, *p*=0.031) but STOCs frequency were unchanged (*n*=6, *p*=0.563). In intact arteries, Kyn did increase Ca<sup>2+</sup> spark frequency (*n*=8, *p*=0.031), but inhibiting Ca<sup>2+</sup> sparks with ryanodine did not affect Kyn-induced vasorelaxation (*N*=7, *p*=0.482).



**Figure 1:** Paxilline sensitive BK<sub>Ca</sub> currents in isolated vascular smooth muscle cells (VSMCs) recorded using the ruptured whole cell configuration of the patch clamp technique (-100 to +100 mV). VSMCs treated with vehicle or Kyn 1 mmol/L. **Left:** Representative currents recorded in response to voltage-steps in individual VSMCs. **Right:** Paxilline sensitive (BK<sub>Ca</sub>) current-voltage curves (*n*=5-8, *N*=4). Mean ± SEM. Curves compared by 2-way RM ANOVA with Sidak's multiple comparison tests. \**p*<0.05, \*\**p*<0.01.

**Conclusion:** Data indicate that Kyn activates BK<sub>Ca</sub> directly, and also increases the frequency of calcium sparks. The vasorelaxant effects of Kyn were not dependent on Ca<sup>2+</sup> spark activity, which suggests the direct effect of Kyn on BK<sub>Ca</sub> is the predominant effect. Interestingly, the increase in Ca<sup>2+</sup> sparks was not translated into increased STOCs frequency, suggesting pregnancy may induce weak coupling of Ca<sup>2+</sup> sparks and BK<sub>Ca</sub> activation.

#### W-184

**Progesterone Induced Blocking Factor Attenuates Hypertension, Placental and Endothelial Cell Mitochondrial Dysfunction and Reactive Oxygen Species in Response to sFlt-1 during Pregnancy.** Lorena M Amaral, Evangeline Deer†, Kyleigh Comley, Denise C Cornelius, Jalisa Jones, Tarek Ibrahim, Ramana Vaka, Michael Franks, Babbette LaMarca\*. *University of Mississippi Medical Center, Jackson, MS, United States.*

**Introduction:** Preeclampsia (PE) is characterized by new onset hypertension in association with placental ischemia, reduced fetal weight, elevated soluble fms-like tyrosine kinase-1 (sFlt-1) and placental mitochondrial (mt) dysfunction and oxidative stress (ROS). Infusion of sFlt-1 causes hypertension and other characteristics of PE in pregnant rodents. However a role for sFlt-1 in causing mt dysfunction and ROS is unknown. Progesterone induced blocking factor (PIBF), is a product of progesterone signaling which serves to lower inflammatory processes. We have shown that PIBF lowers blood pressure in a rat models of PE. This study was designed not only examine the role of mt mediated ROS in sFlt-1 induced hypertension during pregnancy but to also examine the effect of PIBF to improve mt function and hypertension in response to sFlt-1 during pregnancy.

**Methods:** sFlt-1 was infused into normal pregnant (NP) Sprague-Dawley rats (3.7 μg·kg<sup>-1</sup>·day<sup>-1</sup> for 6 days, gestation days 13-19) in the presence or absence of PIBF (2.0 μg/mL) administered intraperitoneal on gestation day 15 to sFlt-1 induced hypertensive pregnant rats. Mean arterial blood pressure (MAP), placental and endothelial mt ROS and function were measured on gestation day 19.

**Results:** Infusion of sFlt-1 into NP rats increased MAP to 112 ± 2 (n=11) compared with control NP rats 98 ± 2 mmHg (n=15, *p*<0.05). Administration of PIBF reduced MAP to 100 ± 1 mmHg in the presence of sFlt-1 (n=5, *p*<0.05). Mt ROS in placenta was 108 ± 6 in NP (n=4), 429 ± 32 in NP+ sFlt-1 (n=3) and reduced to 234 ± 15 in NP+ sFlt-1+ PIBF (n=3). State 3 respiration, which is indicative of ATP production, was reduced in placentas of sFlt-1 infused rats versus NP, but was improved with PIBF. Moreover, sera from NP+sFlt-1 treated with PIBF attenuated endothelial cell mtROS (29 ± 8 % gate) compared with sera from NP+sFlt-1 (54 ± 15 % gate) (n=4).

**Conclusion:** Overall, our study indicates a role of sFlt-1 induced hypertension during pregnancy to reduce placental and endothelial cell mt function. Importantly, supplementation of PIBF improved mt function

and ROS which were associated with improved blood pressure in sFlt-1 induced hypertensive pregnant rats indicating the efficacy of improved progesterone signaling as a potential therapeutic for PE. *This study was funded by NIH grants RO1HD067541-06, P20GM121334 and AHA 19CDA34670055*

**W-185**

**Aldosterone Levels in Pregnancy with Aspirin Prophylaxis for the Prevention of Pre-Eclampsia in High and Low Risk Women.** Katherine Vignes†, Robin Shoemaker, Hong Huang, Aarthi Srinivasan, Aric Shadler, Zachary Stanley†, Cynthia Cockerham, Brittany McKinley†, Erin MacLeod†, John Bauer\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*

**Introduction:** Normal pregnancy is associated with dramatic rise in serum aldosterone, while pre-eclampsia has been associated with a much lower increase. Our objective was to associate serum aldosterone levels with blood pressure profiles in pregnancy and determine aldosterone variation over gestation in high and low risk women.

**Methods:** Our data was derived from a cohort study and randomized trial. The cohort study recruited women at low risk for pre-eclampsia by USPSTF criteria. In the randomized trial, patients defined as high risk for pre-eclampsia were randomized to 81mg versus 162mg of aspirin for prophylaxis prior to 16 weeks gestation. Serum samples were obtained at two time points: 11-16 weeks (prior to aspirin) and 28-32 weeks gestation. In a convenience subgroup, serum aldosterone levels were assessed by liquid chromatography with tandem mass spectrometry. Analyses were completed with 2-way ANOVA with pairwise analysis.

**Results:** 78 low and high risk women were included. 7 women developed preterm pre-eclampsia. Serum aldosterone was significantly increased (2.5 fold) in the third vs first trimester in low risk patients who did not develop disease (n=35, P<0.0001). In the high-risk cohort treated with aspirin, a significant, (reduced compared to control) (1.69 fold) increase in serum aldosterone concentrations between trimesters was noted (Combined data 81+162, n=36, P<0.05). Across all groups, the change in aldosterone (delta, Late-Early) was negatively correlated with third trimester systolic blood pressure (p=0.007, r= -.305) and diastolic blood pressure (p=0.013, r= -.287). Serum aldosterone was not increased in patients who developed preterm preeclampsia (n=7, P=0.393).

**Conclusion:** Our data indicate the relevance of the rise in aldosterone for blood pressure regulation in pregnancy and a potential alteration in a subset of high-risk and pre-eclamptic patients. More study is needed to determine if aspirin alters this pathway in high risk women.

Figure 1. Serum Aldosterone levels by USPSTF category

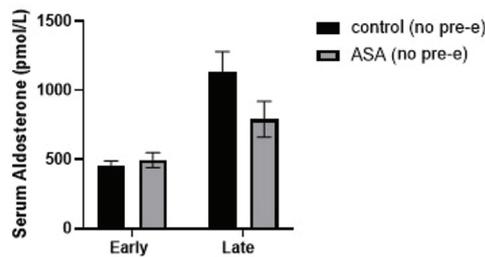
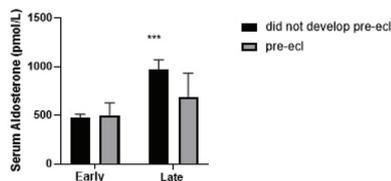


Figure 2: Serum aldosterone levels in women stratified by development of pre-eclampsia



**W-186**

**Aspirin Is Associated with Changes in Serum Aldosterone in High Risk Pregnancies.** Katherine Vignes†, Robin Shoemaker, Hong Huang, Aarthi Srinivasan, Aric Shadler, Zachary Stanley†, Cynthia Cockerham, Brittany McKinley†, Erin MacLeod†, John Bauer\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*

**Introduction:** The effect of aspirin on important physiologic pathways such as the renin-aldosterone system to prevent pre-eclampsia has not been sufficiently studied. Our objective was to compare serum aldosterone concentration in patients undergoing aspirin prophylaxis.

**Methods:** This is a secondary analysis from a randomized trial which recruited women indicated for aspirin prophylaxis by USPSTF criteria. Subjects were randomized to 81mg versus 162mg of aspirin starting prior to 16 weeks gestation. Serum samples were obtained at 11-16 weeks (prior to aspirin initiation) and 28-32 weeks. In a convenience subgroup, serum aldosterone levels were assessed by liquid chromatography with tandem mass spectrometry. Analyses were completed with 2-way ANOVA with pairwise analysis and sample t-tests.

**Results:** 42 women were included (n=20, 81 mg), (n=22, 162 mg). Common indications for enrollment were pre-existing DM (n=13) and chronic hypertension (n=19). 6 women (14%) developed preterm pre-eclampsia. The baseline aldosterone was not different based on dosing group. For women on 81mg, third trimester aldosterone was not significantly increased; however, women who received 162mg aspirin had a statistically significant increase in aldosterone in third trimester versus baseline (p=0.01) (Figure 1). For our subgroup analyses, women with pre-gestational diabetes had no difference in their change in aldosterone (delta, late-early) but in the non-diabetics, the aldosterone significantly changed (p=0.04). In women with chronic hypertension, no differences were observed in their third trimester values or change related to aspirin dose. In obese women (n=27), regardless of risk profile, the late aldosterone was significantly higher in women on 162mg versus 81mg (p=0.011), while early aldosterone levels were similar (Figure 2).

**Conclusion:** Higher aspirin dose was associated with an increase in serum aldosterone in the third trimester in high risk women. This association was stronger in obese women but the clinical risk profile also influenced serum aldosterone.

Figure 1: Serum Aldosterone levels between Aspirin Prophylaxis Regimens

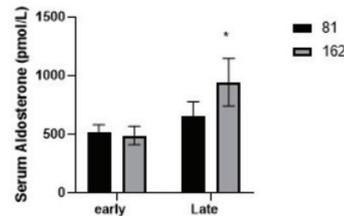
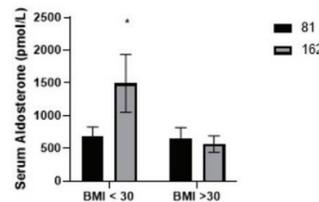


Figure 2: Comparison of Serum Aldosterone by Diagnosis of Obesity and Aspirin dose



**W-187****Placental Hypoplasia and Maternal Organic Vascular Disorder in Pregnant Women with Hypertensive Disorders of Pregnancy.** Kazushi Watanabe, Akihiko Wakatsuki. *Aichi Medical University School of Medicine, Nagakute, Japan.*

**Introduction:** Hypertensive disorders of pregnancy (HDP) is classified into preeclampsia (PE), gestational hypertension (GH). Although endothelial dysfunction is a basic pathologic event that occurs in pregnant women with HDP, it is believed that PE and GH are differ in etiology and pathology. We hypothesize that the etiology and pathology of PE is the two-stage disorder from uteroplacental dysfunction by abnormal implantation and placentation and those one of GH is inherent in organic vascular disorder. This study aimed to investigate these differences between PE and GH.

**Methods:** We examined serum parameters of oxygen free radicals (d-ROMs), maternal angiogenic factor (PIGF) and antiangiogenic factor (sFlt-1), placental hypoxic change and oxidative DNA damage, and maternal organic vascular disorder in 23 women with PE (PE group), 13 with GH (GH group), and 16 with uncomplicated pregnancies (control group). Intima-media thickness (IMT) of the carotid artery was assessed as a marker for maternal organ vascular disorder. Immunohistochemical analysis was performed to measure the proportion of placental trophoblast cell nuclei staining positive for hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), which is a marker of hypoxic change and 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a marker of oxidative DNA damage.

**Results:** Maternal serum d-ROM concentrations were significantly increased in both PE and GH groups relative to the control group. Maternal serum sFlt-1 concentrations, ratio of sFlt-1/PIGF, and proportions of HIF-1 $\alpha$ -positive nuclei and 8-OHdG-positive nuclei were significantly higher in the PE group compared to GH and control groups. IMT was significantly greater in PE and GH groups compared to the control group and was higher in the GH group compared to the PE group.

**Conclusion:** Our findings demonstrate that placental hypoxic changes and oxidative DNA damage are severe in patients with PE and accompanied by an increase in antiangiogenic factors. Moreover, maternal organ vascular disorder was more severe in patients with GH compared to those with PE, as assessed by IMT.

**W-188****Multiple Kinases Mediate TNF $\alpha$ - Induced Endothelial Damage in Uterine Artery Endothelial Monolayers; Could Complexity = Opportunity?** Rachel L Dahn<sup>†</sup>, Luca Clemente\*, Amanda C Ampey\*, Jason A Austin\*, Ian M Bird\*. *University of Wisconsin, Madison, WI, United States.*

**Introduction:** Preeclampsia (PE) is marked by the failure of pregnancy enhanced vasodilation and the loss of endothelial monolayer integrity giving rise to hypertension and proteinuria. PE is also associated with elevated TNF $\alpha$ , and local uteroplacental production of VEGF. We have previously shown that TNF $\alpha$  acts long term to disrupt cell-cell connectivity and this can in part be reversed by Src and MEK/ERK (M/E) kinase inhibitors PP2 and U0126, but the rescue effects are not always complete. Others have shown endothelial monolayers may be controlled by p38 MAPK and in 2007 Bain. et. al. showed U0126 has a 23% cross reactivity to p38MAPK $\alpha$ . **Objective:** 1) To verify the specificity of the rescue action of U0126 on TNF $\alpha$  mediated monolayer damage by comparison to other MEK/ERK kinase inhibitors. 2) To identify the role of p38MAPK in TNF $\alpha$  effects on monolayer integrity to better understand potential therapeutic targets for those effected by preeclampsia.

**Methods:** Uterine Artery Endothelial cells from late pregnant sheep (Ovine P-UAEC) were grown to confluence in 96-well ECIS impedance sensing plates which measures monolayer resistance. The higher the resistance, the better the monolayer integrity. Cells were serum starved and treated alone with different doses (0.1, 1, and 10 $\mu$ M) of (SB203580 (SB), BIRB0796 (BB), PP2, U0126, PD98059 (PD98), and PD0325901 (PD03)) and then +/- TNF $\alpha$  (10ng/mL) for 20 hours. We then followed with combinations of SB with either PP2, U0126, or PD98 to see if their rescue effects could be improved.

**Results:** While PP2, SB (at high doses), and U0126, alone had positive effects on the monolayer, PD98 and PD03 were only effective at very low doses and were toxic at 10  $\mu$ M. With TNF $\alpha$ , PP2 alone showed monolayer rescue. Combination of p38 and Src inhibition (SB and PP2) did not provide added rescue to PP2 alone. For MEK/ERK inhibitors, 10 $\mu$ M U0126 showed the greatest rescuing ability. To mimic the known cross specificity effect of U0126 on the M/E and p38 pathways, PD98 and SB were used in combination (neither one with cross specificity for the other). PD98/SB combinations at submaximal doses did indeed provide rescue although, not improved over PD98 alone at the higher doses. SB was also able to alleviate some of the basal high dose PD98 toxicity.

**Conclusion:** In addition to Src and MEK/ERK, p38MAPK appears to also regulate monolayer integrity in P-UAEC in both the basal and TNF $\alpha$  driven state. Further, the U0126 unique rescuing ability over PD98, PD03, and the improved action of lower PD98/SB dose combinations, suggesting U0126 is effective due to its cross reactivity with both MEK/ERK and p38MAPK. Together our data reveals the complexity of kinase signaling that controls both basal and TNF $\alpha$  impaired monolayer integrity and suggests multiple drug targets may exist which could be used alone or in combination to treat vasodilation dysfunction observed in PE. Funded by T32 HD041921.

**W-189****Finding the Missing Connection to Preeclampsia GWAS.** Jaeyong Choi,<sup>1</sup> Seung Mi Lee,<sup>2</sup> Hee Jin Ham,<sup>2</sup> Young Mi Jung,<sup>2</sup> Chan-Wook Park,<sup>2</sup> Jong Kwan Jun,<sup>2</sup> Jong-Il Kim,<sup>2</sup> Joong Shin Park,<sup>2</sup> <sup>1</sup>Medical Research Center, Seoul National University, Seoul, Korea, Republic of; <sup>2</sup>Seoul National University College of Medicine, Seoul, Korea, Republic of.

**Introduction:** Preeclampsia (PE) is one of the major complications during pregnancy, and defective placental development has been suggested as the key triggering factor in the pathophysiology of PE. Studies on expression quantitative trait loci (eQTLs) has been evaluated in various human tissues, but eQTL research in placenta has been relatively lacking. To determine the gene regulatory mechanism in preeclamptic placenta, we conducted the eQTL analysis in placentas.

**Methods:** The mRNA extracted from 83 placentas obtained from cesarean section performed in Seoul National University Hospital during Nov. 2018 to March 2020 were sequenced with NGS. Matching maternal and fetal DNA were analyzed with Korean Biobank array. Linear regression model was applied using matrix eQTL. Cell type proportions using single cell transcriptome was used as covariate to identify cell type specific eQTL.

**Results:** We found 4542 genes that contain at least one eQTL with statistical significance (FDR < 0.01). 40% of genes had eQTL located in placental regulatory element annotated by ENCODE, and another 10% were in coding region. 34% (1560) of eQTL had matching result with GTEx database in variant level. We found 14 novel genes that did not have any eQTL reported in multi-tissue analysis data from GTEx or all previously published placenta eQTL studies. Among these, 9 novel genes were annotated as highly placenta specific. No result was significant for FLT1 or rs4749613, which were reported to be highly associated with preeclampsia in previous GWAS studies.

**Conclusion:** We expanded the knowledge base for regulatory elements of placenta, and found 14 novel eQTL regulated genes. We suggest a more advanced technology which can target extra-villous trophoblast to further reveal cell specific regulation.

**W-190****Uterine Artery Endothelial Cell Proliferation during Pregnancy Can Be Elevated by Exogenous and Endogenous Catecholamines.** Ronald R. Magness,<sup>1</sup> Maja Okuka,<sup>1</sup> Omar S Jobe.<sup>2</sup> <sup>1</sup>University of South Florida, Tampa, FL, United States; <sup>2</sup>University of Wisconsin-Madison, Madison, WI, United States.

**Introduction:** Pregnancy exhibits dramatic rises in uterine blood flow (UBF) resulting from elevated angiogenesis and vasodilation, adaptations dysfunctional in pathological pregnancies (e.g. preeclampsia). Ovine uterine artery endothelial cells of pregnancy (P-UAECs) express  $\beta$ -adrenergic receptors (ARs). The structural catechol 3,4 di-OH-A ring of catecholestrogens stimulate P-UAEC proliferation via  $\beta$ -ARs.

**Hypothesis:**  $\beta$ -AR proliferative P-UAEC angiogenesis responses are stimulated by both exogenous and endogenous stimulation, the latter via autocrine catecholamine-induced signaling mechanisms.

**Methods:** Evaluated in P-UAECs: 1) expression of catecholamine biosynthetic enzymes, tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), dopamine  $\beta$ -hydroxylase (D $\beta$ H), and phenylethanolamine N-methyltransferase (PNMT) by Western Blotting; 2)  $\beta$ -AR Norepinephrine (NE) and Epinephrine (Epi) stimulation (0.1-100nM) of proliferation; 3) angiogenesis in P-UAECs is stimulated by the catecholamine substrate L-Tyrosine via  $\beta$ -ARs indicating *de novo* catecholamine biosynthesis, comparing to physiologic doses (100nM-10mM) of L-Tyrosine, L-Arginine, L-Leucine, and L-Threonine. Inhibition of  $\beta$ -ARs was performed using Propranolol (10 $\mu$ M; pretreatment 1h), then treatment with optimized concentrations of NE, Epi [0.1-1nM], or L-Tyrosine, L-Arginine, L-Threonine, and L-Leucine [1-10mM.] Proliferation was evaluated using direct cell counting and BrdU incorporation.

**Results:** P-UAECs express all catecholamine biosynthetic enzymes including TH, DDC, D $\beta$ H, and PNMT. NE and Epi dose dependently increased ( $P < 0.01$ ) P-UAEC proliferation (0.1nM, 1.78 $\pm$ 0.028- and 1.0nM, 1.89 $\pm$ 0.035-fold, respectively) which was abrogated (99.8%;  $P < 0.01$ ) by Propranolol. Catecholamine substrate L-Tyrosine dose dependently increased ( $P < 0.05$ ) P-UAEC proliferation with maximum proliferation (2.6 $\pm$ 0.13-fold) at 10mM. Nitric Oxide Substrate L-Arginine did not induce proliferation. By contrast, "nutritive" amino acids (L-Threonine and L-Leucine) responses were not dose dependent and these responses (1.9 $\pm$ 0.13- and 2.1 $\pm$ 0.13-fold, respectively) were less ( $P < 0.05$ ) than L-Tyrosine responses. Propranolol inhibited L-Tyrosine proliferation by 65.6%, but had no effect on L-Arginine, L-Threonine, or L-Leucine responses. Therefore 34.4% of L-Tyrosine-induced proliferation was non- $\beta$ -AR and likely was the tropic nutrient effect of amino acid supplementation.

**Conclusion:** We demonstrate for the first time that P-UAECs express functional biosynthetic catecholamine enzymes (TH, DDC, D $\beta$ H, and PNMT) and catecholamine-induced P-UAEC proliferation can occur via *de novo* endothelial synthesis via an autocrine intramural signaling mechanism regulating P-UAEC proliferation during pregnancy that may be dysfunctional in preeclampsia.

## W-191

**Galectin-7: A Novel Placental-Released Driver of Preeclampsia.** Ellen Menkhorst<sup>†</sup>,<sup>1</sup> Wei Zhou<sup>†</sup>,<sup>1</sup> Leilani Santos<sup>†</sup>,<sup>1</sup> Teresa So,<sup>1</sup> Sarah Delforce,<sup>2</sup> Argyro Syngelaki,<sup>3</sup> Kristy Pringle,<sup>2</sup> Kypros Nicolaides,<sup>3</sup> Yves St-Pierre,<sup>4</sup> Eva Dimitriadis\*.<sup>1</sup> <sup>1</sup>The University of Melbourne, Melbourne, Australia; <sup>2</sup>Hunter Institute of Medical Research, Newcastle, Australia; <sup>3</sup>King's College Hospital, London, United Kingdom; <sup>4</sup>Institut National de la Recherche Scientifique, Laval, QC, Canada.

**Introduction:** Preeclampsia (PE) is a pregnancy-induced disorder defined by sudden onset maternal hypertension  $> 20$  weeks gestation. The etiology of PE is poorly understood, however substantial evidence suggests the underlying cause is poor placentation. Galectins are animal (soluble) lectins which bind to surface glycoproteins and regulate many cell functions important for placentation. Galectin-7 is abnormally elevated in 1<sup>st</sup> trimester serum of women who go on to develop PE. We aimed to determine whether galectin-7 was central to the etiology of PE.

**Methods:** Female C57BL/6J mice were subcutaneously injected with recombinant human galectin-7 (400 $\mu$ g/kg/day) from embryonic day (E)8-12 of pregnancy (n=6) to investigate the effect of galectin-7 on systolic blood pressure [sBP], proteinuria, placental formation and fetal/pup growth. Galectin-7 production by human 1<sup>st</sup> trimester chorionic villous samples (CVS) from pregnancies which were uncomplicated vs those which had PE was compared (n=3-6). The effect of recombinant human galectin-7 (1 $\mu$ g/ml) and ADAM12S (2 $\mu$ g/ml) on 1<sup>st</sup> trimester placental villous gene expression (n=3-5) and outgrowth (n=3-5) was determined.

**Results:** *In vivo*, galectin-7 treatment induced the classic features of PE in pregnant mice: elevated sBP (E14-17) and proteinuria (E12-15). There was no effect in non-pregnant mice. Galectin-7 treatment decreased E13 placental weight ( $p < 0.05$ ), labyrinth vascular branching ( $p < 0.05$ ), and spiral artery remodeling ( $p < 0.05$ ) and altered E13 and E17 placental gene

expression ( $\uparrow$ IL6,  $\downarrow$ IL10,  $\downarrow$ ADAM12). Galectin-7 also dysregulated renin-angiotensin system components in the E13 and E17 placenta, decidua and kidney (angiotensinogen, prorenin and angiotensin II type 1 receptor). Pups from galectin-7 treated pregnancies were significantly smaller from post-natal day (P)1 to P21 ( $p < 0.05$ ). In women, *galectin-7* mRNA and immunostaining was increased in 1<sup>st</sup> trimester CVS from pregnancies that went on to have preterm PE compared to uncomplicated pregnancies ( $p < 0.05$ ). *In vitro*, treatment of 1<sup>st</sup> trimester human placental villous with galectin-7 impaired placental villous outgrowth ( $p < 0.05$ ), induced *sFlt-1-e15a* production ( $p < 0.05$ ) and inhibited *ADAM12* production ( $p < 0.05$ ) and *ADAM12S* and angiotensinogen secretion ( $p < 0.05$ ).

**Conclusion:** Galectin-7 is a placental-produced factor which plays a significant role in the initiation of PE, via its actions which impair placental formation, cause placental inflammation, and alters placental production of sFlt-1 and renin-angiotensin system components. Therapeutics to target galectin-7 may be useful to prevent placental damage leading to PE.

## W-192

**Peripheral Blood Monocytes Subsets Are Dysregulated in Women with Reproductive Failure.** Lujain AlSubki<sup>†</sup>, Nayoung Sung<sup>†</sup>, Shahrukh Syed<sup>†</sup>, Giovanni Jubiz, Xiuhua Yang, Wen-Juan Wang, Qiaohua He, Gloria Deutche, Svetlana Dambaeva, Alice Gilman-Sachs\*, Kenneth Beaman\*, Joanne Kwak-Kim\*. *Rosalind Franklin University of Medicine and Science, Vernon Hills, IL, United States.*

**Introduction:** Reproductive failures (RF), including infertility and recurrent pregnancy losses (RPL), have been attributed to inflammation and immune dysregulations. Innate immunity mediated by monocytes impacts the local and systemic immune responses in the reproductive system through the activation of the inflammatory cascade. Based on their surface expression of the pattern recognition receptors, various monocyte subsets have been identified. However, monocytes and their subsets have not been investigated well in women with RF. In this study, we aim to investigate peripheral blood monocyte subsets in women with RF.

**Methods:** A total of 51 women with RF were prospectively recruited at the University Clinic. Controls were 16 fertile women. Flow cytometric analysis was performed to assess monocytes, natural killer cells, and Th1/Th2 cells in the peripheral blood prior to any treatment. CD14 and CD16 surface markers were used to identify monocytes and three monocyte subsets, including classical (CD14<sup>++</sup>CD16<sup>-</sup>), intermediate (CD14<sup>++</sup>CD16<sup>+</sup>), and non-classical (CD14<sup>+</sup>CD16<sup>++</sup>) monocytes. Statistical analysis was performed by using the Student t-test and Pearson's correlation analysis.

**Results:** There were no significant differences in the patient's characteristics, including age, body mass index, and gravidity, between RF patients and controls. When comparing the proportion of monocyte subpopulations, classical monocytes were not different between the patients and control (92.45%  $\pm$  7.78 vs. 94.06%  $\pm$  2.08,  $P > 0.05$ ). The proportion of intermediate monocytes was increased in women with RF as compared to controls (5.27%  $\pm$  7.46 vs. 2.31%  $\pm$  1.20,  $P = 0.009$ ), and non-classical monocytes were decreased in patients as compared to controls (2.28%  $\pm$  1.26 vs. 3.63%  $\pm$  1.63,  $P = 0.001$ ). In women with RF, there was a negative correlation between classical monocytes and intermediate monocytes ( $r = -0.987$ ,  $p < 0.001$ ) and non-classical monocytes ( $r = -0.330$ ,  $p = 0.018$ ). In fertile controls, there was a negative correlation between classical monocytes and intermediate monocytes ( $r = -0.625$ ,  $p = 0.010$ ) and non-classical monocytes ( $r = -0.819$ ,  $p < 0.001$ ).

**Conclusion:** These findings indicate that women with RF have decreased anti-inflammatory immune response and dysregulated vascular homeostasis by having decreased non-classical and increased intermediate monocytes in the peripheral blood.

## W-193

**Biologic Differences between Preterm and Term Preeclampsia Revealed by Transcriptomic Analyses.** Olesya Plazyo, Ashley Hesson, Langen Elizabeth, Joseph Kirma, Santhi Ganesh, Johann Gudjonsson. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** Preeclampsia (PE) is an incompletely understood vascular endothelial disorder characterized by gestational-induced hypertension that affects 1 in 25 pregnancies in the United States and can lead to serious perinatal complications and death as well as impose life-long health risks. Although immune dysregulation at the maternal-fetal interface and inadequate remodeling of spiral arteries are known to contribute to PE, better understanding of the molecular mechanisms in PE pathogenesis is urgently needed, as highlighted by the current lack in FDA-approved therapies for this disease.

**Methods:** To investigate the PE-specific transcriptomes, we performed RNA-sequencing on formalin-fixed paraffin-embedded placenta samples obtained from PE patients (n=43) and gestational age-matched normal controls (n=41) using the Illumina NovaSeq S4-150 platform.

**Results:** The comparison revealed 259 up- and 90 down-regulated genes with statistical significance of  $p \leq 0.05$ , including those that have been previously linked to PE (*IGFBP1*, *PRL*, *DKK1*, *MAP3K7CL*) as well as those that were less known (*DDX20*, *OPTN*, *ZNF716*, *VW48*). PANTHER analysis of molecular functions showed enrichment in MHC class II receptor activity, SMAD binding, TGF- $\beta$  receptor binding, and Wnt-protein binding. Ingenuity Pathway Analysis revealed NFAT5,  $\alpha$ -Catenin, and PTCH1 among upstream regulators. When comparing differentially expressed genes (DEGs) in preterm PE (n=20 patients) to those in term PE (n=23 patients), enriched biological processes included cellular response to IFN- $\gamma$ , cytochrome complex assembly, and antigen processing and presentation via MHC class II in preterm PE. Enriched processes in term PE included metabolic processes, cellular component biogenesis, and extracellular matrix organization. Upstream regulators included KLF7, PDIA4, and LMTK3 in preterm PE and HOXA10, BTG2, and KITLG in term PE.

**Conclusion:** Collectively, these data provide transcriptome characterization of placenta alterations in term and preterm PE and serve as a gateway for further interrogation of signaling in PE pathogenesis. Currently other transcriptomic approaches including single-cell RNA-Seq and spatial Seq are being employed by our group to further characterize the expression signatures identified by these data.

## W-194

**Associations between Circulating sFlt1 and PlGF and Preeclampsia with Severe Maternal Complications, or Eclampsia.** Roxanne Hastie,<sup>1</sup> Lina Bergman,<sup>2</sup> Susan Walker,<sup>1</sup> Tu'uhevaha Kaitu'u-Lino,<sup>1</sup> Natalie J Hannan,<sup>1</sup> Fiona Brownfoot,<sup>1</sup> Alesia Harper,<sup>1</sup> Ping Cannon,<sup>1</sup> Cathy A Cluver,<sup>3</sup> Stephen Tong.<sup>1</sup> *<sup>1</sup>University of Melbourne, Heidelberg, Australia; <sup>2</sup>Uppsala University, Uppsala, Sweden; <sup>3</sup>Stellenbosch University, Cape Town, South Africa.*

**Introduction:** The angiogenic factors soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF) are believed to directly contribute to the pathophysiology of preeclampsia. If this is the case, then levels should be increasingly altered with severity of disease.

**Methods:** Maternal plasma samples were obtained from 348 women with preeclampsia prospectively recruited at Tygerberg Hospital, South Africa from 2016 - 2019. Plasma concentrations of sFlt-1 and PlGF were measured among women diagnosed with preeclampsia without severe features, and compared to those who had preeclampsia plus the following severe features: 1) haemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, disseminated intravascular coagulation (DIC) or severe renal involvement (creatinine  $\geq 120 \mu\text{M/L}$ ) 2) eclampsia, 3) pulmonary edema, and 4) severe hypertension (systolic blood pressure  $\geq 160$ /diastolic blood pressure  $\geq 110$  mmHg). Angiogenic levels were measured using a commercial platform (Roche) and expressed fold change relative to preeclampsia without severe features.

**Results:** Compared to 125 women with preeclampsia without severe features, those with preeclampsia and any of HELLP syndrome, DIC or severe renal involvement (ie 25 with exceptionally severe disease) had

the greatest change in angiogenic levels: a 2.63-fold increase in sFlt-1 (95% confidence interval [CI] 1.81-3.82;  $p < 0.001$ ), and 10.07-fold (95% CI 5.36-18.91;  $p < 0.001$ ) increase in the sFlt-1/PlGF ratio. Accordingly, PlGF levels were lowest among this group (fold change 0.26 [95% CI 0.18-0.39];  $p < 0.001$ ). Compared to those with preeclampsia and no severe features, eclampsia was associated with a 2.02-fold increase in sFlt-1 (95% CI 1.32-3.09;  $n=36$ ;  $p=0.001$ ), a 63% reduction in PlGF (0.43 [95% CI 0.27-0.68]) and a 4.71-fold increase (95% CI 2.30-9.66) in the sFlt-1/PlGF ratio. Interestingly, angiogenic levels among 15 women with preeclampsia and pulmonary edema were no different to women with preeclampsia without severe features. When we regrouped our cohort according to the number of adverse outcomes (1, 2 or  $\geq 3$ ), those who experienced two adverse outcomes had the greatest change in angiogenic levels (sFlt-1 2.54-fold change [95% CI 1.85-3.48]; PlGF 0.42 [95% CI 0.29-0.58]; sFlt-1/PlGF ratio 6.12 [95% CI 3.53-10.61]).

**Conclusion:** Circulating sFlt-1 and PlGF are associated with disease severity among women with preeclampsia. Given their roles in the pathophysiology of preeclampsia these strong correlations between sFlt-1 and PlGF and serious adverse maternal outcomes are biologically plausible. Our findings provide strong evidence supporting sFlt-1 as a central driver of disease pathogenesis.

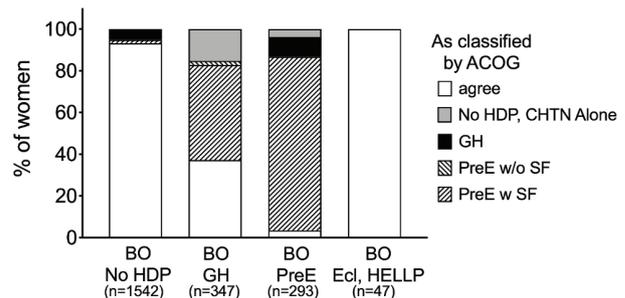
## W-195

**ACOG Guidelines Identify More At Risk Pregnancies in Bolivia.** Litzzi Lazo-Vega,<sup>1</sup> Lilian Toledo-Jaldin,<sup>1</sup> Alison Larrea,<sup>2</sup> Colleen G Julian,<sup>3</sup> Lorna G Moore\*.<sup>3</sup> *<sup>1</sup>Hospital Materno Infantil, La Paz, Bolivia, Plurinational State of; <sup>2</sup>University of Colorado Denver, La Paz, Bolivia, Plurinational State of; <sup>3</sup>University of Colorado Denver, Aurora, CO, United States.*

**Introduction:** Hypertensive disorders of pregnancy (HDP, i.e., gestational hypertension [GH]; preeclampsia [PreE]; HELLP syndrome or eclampsia [Ecl]) are leading causes of maternal deaths, especially in low- to middle-income countries (LMIC) such as Bolivia. The American College of Obstetricians and Gynecologists (ACOG) has updated its guidelines to distinguish between PreE without (w/o) or with (w) severe features (sf) to improve prediction of maternal deaths. We assessed (1) concordance between Bolivian and ACOG diagnostic criteria and (2) whether ACOG criteria improved prediction of adverse outcomes.

**Methods:** We reviewed prenatal and hospital records for all 731 cases with and 1641 controls without an HDP discharge diagnosis over 1 year at the 3 largest hospitals in La Paz/El Alto, Bolivia (3600-4100 m). 687 cases and 1542 controls met age and singleton-pregnancy inclusion criteria. We recorded their health history, systolic and diastolic BPs, HDP diagnoses, diagnostic symptoms, lab values, delivery and infant characteristics. Comparisons between diagnostic categories were made using t-tests, Chi squared or ANOVA, and logistic regression as appropriate.

**Results:** All BO HELLP or Ecl cases met ACOG criteria for PreE w sf. But 46% of BO-GH and 84% of BO-PreE women had severe hypertension (BPs  $\geq 160/110$ ), abnormal labs (platelets  $< 100\text{k IU}$ , serum creatinine  $\geq 1.1$  mg/dL, or liver transaminases  $\geq 80$  IU/L) or diagnostic symptoms (severe headache, blurred vision, dyspnea, or right epigastric pain), and hence met ACOG criteria for PreE w sf (figure).



35% of BO-HELLP/Ecl but only 6% of ACOG-PreE with sf diagnoses occurred postpartum. After controlling for risk factors, the odds of adverse maternal (C/s, ICU admission, prolonged hospitalization or death  $< 7$

days postpartum) or infant outcomes (preterm, SGA, 5-min APGAR <7, intubation, supplemental O<sub>2</sub>, NICU admission, death <7 da) rose with HDP severity and overall were greatest in BO HELLP/Ecl cases. Women with BO-GH that would have been PreE w/o or w sf by ACOG criteria had more frequent C/s, any adverse outcome, SGA or NICU admissions. **Conclusion:** Bolivian HELLP/Ecl diagnoses identify women with the most adverse maternal or infant outcomes, but ACOG criteria recognize more at increased risk. Using ACOG criteria may aid in identifying HDP earlier and directing scarce health-care resources to those at most risk.

**W-196**

**Clinical Outcome after First Trimester Pre-Eclampsia Screening-Directed High Dose Aspirin Therapy in High Risk Patients.** Nicole Rose Gavin†, Jena Miller\*, CiCi McShane†, Mara Rosner\*, Ahmet Baschat\*. *The Johns Hopkins Hospital, Baltimore, MD, United States.*

**Introduction:** The risk assessment by ACOG stratifies women at risk for preeclampsia (PEC) based on history. First trimester (T1) PEC screening calculates a multimarker derived personalized risk. A randomized trial has shown benefit of high dose aspirin (ASA) in women at high PEC risk after T1 PEC screen. We evaluated the clinical value of this approach in a high risk clinical population by ACOG criteria.

**Methods:** Retrospective cohort study of high risk patients referred for T1 PEC screening. At T1 screen, PEC risk was calculated based on maternal history, physical examination, uterine artery pulsatility (UAP), PAPP-A and PLGF levels. High risk patients (risk > 1/200, = PEC screen +ve) were recommended ASA 162mg nightly. In low risk patients 81mg ASA was optional. We compared risk strata based on ACOG and T1 PEC screening for differences in maternal characteristics, ASA utilization and outcome, including delivery for PEC < 37 weeks, using nonparametric and parametric analysis.

**Results:** Of 142 women having T1 PEC screen at 12.4 ± 0.71 weeks, 128 (90.1%) were high risk by ACOG, and 45 (31.7%) were T1 PEC screen +ve (Figure). T1 PEC screen stratified 67.2% of ACOG high-risk women as low risk. ACOG and T1 PEC screen strata had differences in maternal BMI, mean arterial pressure, UAP and biomarkers (Table). 37 (82.2%) T1 PEC screen +ve women took 162mg ASA while 67 (69.1%) low risk women continued 81mg ASA (p=<.001). ASA dosages across ACOG risk categories were similar (p=0.177). Women downgraded in ACOG risk by the T1 PEC screen did not have a higher rate of PEC < 37 weeks. The focused use of 162 mg ASA in a small group of T1 PEC screen +ve women did not increase the rate of PEC requiring preterm delivery compared to risk stratification that casts a wider net using ACOG criteria (p=0.22).

**Conclusion:** T1 PEC screening effectively stratifies women at risk for preterm PEC and refines target population that may benefit from higher dose ASA. Two thirds of women considered high risk by ACOG criteria can be appropriately reassured.

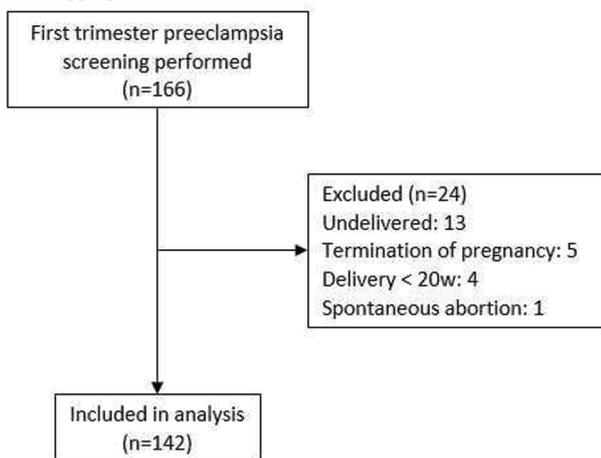


Figure. Flow diagram for the study population

Table. Maternal characteristics, therapy, and delivery outcome for the study population.

Characteristic	All N=142	T1 PEC Screening		ACOG		p
		Low (n=97)	High (n=45)	Low (n=14)	High (n=128)	
GA at screening	12.4 ± 0.7	12.5 ± 0.7	12.3 ± 0.7	12.2 ± 0.9	12.5 ± 0.7	NS
CRL at screening	64.0 ± 9.3	64.9 ± 9.1	62.8 ± 10.6	63.5 ± 6.9	64.3 ± 9.9	NS
Maternal Age (y)	32.4 ± 4.6	32.6 ± 5.0	31.7 ± 3.8	30.7 ± 3.4	32.5 ± 4.7	
Ethnicity						
Caucasian	73 (51.4)	57(58.8)	16 (35.6)	11(78.6)	62 (48.4)	0.029
AA	59 (41.5)	32(33)	27 (60)	2 (14.3)	57 (45.4)	
History						
Hypertension	53 (37.3)	32 (33)	21 (46.7)	0	53 (41.4)	0.009
Diabetes	13 (9.1)	9 (9.2)	4 (8.9)	0	13 (10.2)	NS
Prior PEC	91 (64.1)	59 (60)	32 (71.1)	7 (50)	84 (65.5)	NS
Height (cm)	164.0 ± 6.9	164.3 ± 6.4	163.5 ± 7.9	164.7 ± 6.8	164.0 ± 6.90	NS
Weight (kg)	77 [41.2-165.2]	76.9 [41.2-165.2]	78 [42.4-133.2]	62.8 [42.4-85.4]	79.5 [41.2-165.2]	0.009
BMI	27.8 [18-55.4]	27.7 [18-55.4]	28.0 [18.6-45.0]	23.5 [19.4-27.8]	29.3 [18.0-55.4]	0.002
MAP	90.7 ± 9.5	89.3 ± 8.5	93.6 ± 10.9	83.6 ± 6.4	91.4 ± 9.5	0.004
Uterine PI	1.7 [0.61-3.2]	1.5 [0.6-2.6]	2.2 [1.2-3.2]	2.0 [1.2-3.0]	1.7 [0.6-3.2]	<0.001
PAPP-A MoM	0.9 [0.1-4.3]	0.9 [0.2-4.3]	0.6 [0.1-2.7]	0.7 [0.2-1.3]	0.9 [0.1-4.3]	0.026
PLGF MoM	0.9 [0.2-4.4]	0.96 [0.2-4.4]	0.7 [0.2-1.7]	1.0 [0.2-1.8]	0.9 [0.2-4.4]	0.001
Aspirin use						
None	19 (13.4)	18 (18.6)	1 (2.2)	4 (28.6)	15 (11.7)	
81 mg daily	74 (52.1)	67 (69.1)	7 (15.6)	7 (50)	67 (52.3)	<0.0001
162 mg daily	49 (34.5)	12 (12.4)	37 (82.2)	3 (21.4)	46 (35.9)	
Delivery for preeclampsia prior to 37 weeks	16 (11.3)	7 (7.2)	9 (20)	0	16 (12.5)	0.22

Data are median [range], mean ± standard deviation, or n/N (%). AA, African American; BMI, body mass index; CRL, crown rump length; GA, gestational age; MAP, mean arterial pressure; MoM, multiples of the median; PAPP-A, pregnancy associated plasma protein A; PEC, preeclampsia; PI, pulsatility index; PLGF, placental growth factor

**W-197**

**Investigation of Premature Cellular Senescence in Pre-Eclampsia and Intrauterine Growth Restriction.** Samprikta Manna†, Fergus P McCarthy\*, Colm J Mc Elwain†, Marta Giralto Martín†, Cathal McCarthy\*. *University College Cork, Cork, Ireland.*

**Introduction:** Placental ageing is a normal physiological response in progressing pregnancy with the organ exhibiting extreme morphological and physiological senescence at term. Pre-eclampsia and intra-uterine growth restriction can cause premature placental ageing, resulting in maternal and foetal morbidity and mortality worldwide. **Aim:** To investigate senescence-associated secretory phenotype (SASP) in maternal blood and markers of cellular senescence in the placenta in pre-eclampsia and intra-uterine growth restriction.

**Methods:** Maternal plasma samples were taken at term gestation from nulliparous women with pre-eclampsia (n=9), intra-uterine growth restriction (n=10), and age-matched control uncomplicated pregnancies (n=15). SASP panel of cytokines was evaluated using a multiplex Mesoscale ELISA assay in all groups. Statistical analysis was performed using GraphPad Prism 8®. Placental samples were collected after cesarean sections from pre-eclampsia (n=6), intra-uterine growth restriction (n=11) and control uncomplicated pregnancies (n=16) for absolute telomere length analysis by RTqPCR. Multiple regression and statistical analysis was performed using IBM SPSS v26.

**Results:** SASP consists of both inflammatory cytokines and mediators including, Interferon-γ (INF-γ), Interleukins (IL-13, IL-6, IL-8), Monocyte chemoattractant protein-1 (MCP-1) and Macrophage Inflammatory Protein 1 alpha (MIP-1α) and Matrix Metalloproteinase-3 (MMP-3). There was a significant increase in IL-6 in pre-eclampsia when compared to healthy controls (0.54 pg/ml ± 0.271 v 0.3 pg/ml ± 0.102; P=0.017). In addition, there was a non-significant increase in MMP3 in both pre-eclampsia (15.049 ng/ml ± 4.98 v 8.005 ng/ml ± 1.078; P=0.095) and intra-uterine growth restriction (16.12 ng/ml ± 5.40 v 8.005 ng/ml ± 1.078; P=0.088) when compared to controls. No significant differences were seen between study groups for other cytokines in the SASP panel. The calculated absolute telomere length of the placenta showed no significant difference between controls (4.87 ± 3.74 kbp) compared to pre-eclampsia (7.45 ± 3.17 kbp) and IUGR (4.87 ± 4.07 kbp). Correlation analysis shows an inverse association between BMI in controls and intra-uterine growth restriction and a positive correlation between gestational age and pre-eclampsia (p=0.058).

**Conclusion:** Our data show increased inflammatory markers such as IL-6 in maternal plasma in pre-eclampsia only suggesting altered inflammatory signaling, which may modulate senescence. Absolute telomere length of the placenta at term may be dependent on other variable factors such as BMI and gestational age. Our results indicate that improper placentation may be associated with maternal physiologic factors, leading to advanced placental ageing and playing a pathophysiologic role in pregnancy disorders such as pre-eclampsia and IUGR.

## W-198

**Using Machine Learning Tools to Classify and Predict Preeclampsia in Asymptomatic Women.** Jianhong Zhang\*,<sup>1</sup> Melanie Audette,<sup>1,2</sup> Nir Melamed,<sup>3</sup> Stephen J. Lye,<sup>1,2</sup> John C. Kingdom,<sup>1,2</sup> Kelsey McLaughlin.<sup>1,2</sup>  
<sup>1</sup>Sinai Health System, Toronto, ON, Canada; <sup>2</sup>University of Toronto, Toronto, ON, Canada; <sup>3</sup>Sunnybrook Health Sciences, Toronto, ON, Canada.

**Introduction:** Preeclampsia (PE) has varied clinical manifestations and timing of onset, early detection for effective treatments is the key to optimizing maternal-infant outcomes. Many screening models have been proposed to predict the risk of PE using selected maternal/fetal characteristics and biomedical measurements. Although with relatively high sensitivity and specificity, these conventional methods have low positive predictive value (precision) to report the likelihood of true PE. Machine learning provides advanced analytical methods to process high dimensional data and to support PE prognosis with great accuracy and effectiveness.

**Methods:** To discriminate potential PE from low risk pregnancy, non-linear analysis (t-Distributed Stochastic Neighbor Embedding, t-SNE) was performed to cluster 856 asymptomatic women according to 37 biomedical parameters collected at early/mid pregnancy (Toronto Placenta Health Study cohort). Logistic regression was used to analyze the probability of developing PE. To improve model performance, synthetic minority oversampling technique (SMOTE) was applied to balance the minor PE class. Feature selection was conducted to rank the importance of biomarkers for possible PE. Predictive models were evaluated by train/test split or 10-fold cross validation to ensure their quality.

**Results:** Patients who are susceptible to PE can be stratified from low risk pregnant women by t-SNE clustering based on integrated maternal and clinical features. Both classification modelling and feature selection indicate that mean blood pressure (MAP), mean uterine artery Doppler pulsatility index, and serum placental growth factor are the top 3 informative biomarkers to predict the incidence of PE. MAP remains a powerful and convenient factor among all PE screening tests and should be routinely monitored. A standardized ultrasound examination at mid pregnancy is more important than blood protein test or maternal characteristics for PE prediction. The validated models have high performance indices (AUC ROC 0.9, AUC PRC 0.8, specificity 0.8, sensitivity 0.8, precision 0.8) to predict the probability of PE risk using 1<sup>st</sup>/2<sup>nd</sup> trimester data.

**Conclusion:** Using novel machine learning tools, women at-risk for developing PE can be accurately and effectively identified at early stages of pregnancy. Our improved PE modelling is cost-effective and operable for routine clinical practice. This analytic pipeline could be applied to other research and clinical application for different pregnancy complications.

## W-199

**Fast Kit for Eclampsia.** Marcello Bargione, Simona Lunardi, Pietro Alimondi, Maria Chiara Di Liberto, Walter Alio, Claudia Amato, Francesco Forlani, Federica Cusimano, Clara Ferrara, Jessica Presti, Margherita Giunta, Roberta Vaccaro, Maria Antonietta Coppola, Rosaria Amato, Daniele Francesco Lo Gerfo. *ARNAS Civico, Palermo, Italy.*

**Introduction:** In Europe, the incidence of eclampsia is estimated at 2-6 cases per 10,000 deliveries: antepartum (45%), postpartum (36%) and during labor (19%). Given the speed and unpredictability of the eclamptic event, which strikes even in the absence of premonitory signs and symptoms, it would be useful to have a portable and always available rapid assistance kit equipped in the stretchers and wheelchairs of the maternity department. This kit is an inexpensive and useful solution, especially in facilities that provide care for many pregnant women with hypertensive conditions during pregnancy. In our facility, we recorded 60 cases of preeclampsia during the year 2019. Of these, 10% developed eclampsia. In two cases, the eclamptic attack occurred outside the inpatient ward. In these two cases, the presence of the Fast-Kit in the patient's stretcher allowed prompt assistance and prevented a worsening of the clinical condition.

**Methods:** Pregnant woman PARA 0000 at 40 weeks gestation, polydramnios. During outpatient control she has blood pressure of 170/103

mmHg, so she started antihypertensive therapy with nifedipine orally. The cardiocardiographic recording from American College of Obstetricians and Gynecologists was TYPE I. Gradually appeared neurological signs: visual disturbances, headache and mental confusion. Given the worsening of the neurological symptoms, a consultation was requested, with the result of suspected Posterior Reversible Encefalopathy Syndrome with visual disturbances. CTG gets worse. Continued antihypertensive therapy and MgSO<sub>4</sub> for neuroprotection. Given the persistence of symptoms, the delivery was performed by Caesarean section and a CT scan of the brain was performed, which did not reveal any changes. On returning to the ward, during the lift ride, the woman suffered an eclamptic attack. The presence of the Fast Kit allowed the airway to be kept clear and the bolus of MgSO<sub>4</sub> to be administered promptly. Once the eclamptic attack had been resolved and the patient returned to the operating theatre, she continued her therapy and monitoring until she was stabilized. The patient was transferred to the inpatient ward and the Fast Kit was reinstated.

**Results:** Creation of a fast kit for the management of the eclamptic emergency.

**Conclusion:** In our experience, such an inexpensive and easy-to-generate fast-track kit can be of great help in unpredictable situations that could cause harm to patients.



## W-200

**Analysis of Inflammatory Signaling in Reprometabolic Syndrome Induced by Acute Hyperinsulinemia and Hyperlipidemia in Normal Weight Women.** Andrew Tannous†,<sup>1</sup> Andrew P Bradford\*,<sup>1</sup> Katherine N Kuhn,<sup>1</sup> Angela Fought,<sup>1</sup> Irene Schauer\*,<sup>2,1</sup> Nanette Santoro\*,<sup>1</sup> <sup>1</sup>University of Colorado School of Medicine, Aurora, CO, United States; <sup>2</sup>Rocky Mountain Regional Veterans Affairs Medical Center, Aurora, CO, United States.

**Introduction:** Obesity, is a state of chronic inflammation, characterized by elevated lipids, insulin resistance and relative hypogonadotropic hypogonadism. We have defined the accompanying decreased Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), ovarian steroids and reduced pituitary response to Gonadotropin-releasing Hormone as Reprometabolic syndrome, a phenotype that can be induced, in healthy normal weight women, by acute infusion of fatty acids and insulin.

**Objective:** To identify potential mediators of insulin and lipid-related reproductive endocrine dysfunction (hypogonadotropic hypogonadism) characteristic of Reprometabolic syndrome.

**Methods: Design, Setting, Participants:** Secondary analysis of crossover study of 11 eumenorrheic reproductive aged women of normal Body Mass Index (<25 kg/m<sup>2</sup>) at an academic medical center.

**Intervention:** Participants underwent 6-hour intravenous infusions of either saline/heparin or insulin plus fatty acids (Intralipid) plus heparin, in the early follicular phase of sequential menstrual cycles, in random order.

Euglycemia was maintained by glucose infusion. Frequent blood samples (q10 min) were obtained. **Main Outcome Measures:** Pooled serum from each woman was analyzed for cytokines, interleukins, chemokines, adipokines, Fibroblast Growth Factor-21 (FGF-21) and markers of endoplasmic reticulum (ER) stress (CHOP and GRP78). Wilcoxon signed-rank tests were used to compare results across experimental conditions.

#### Results:

Cytokines, Growth factors, ER-stress markers				
hsCRP	Eotaxin	Eotaxin-3	GM-CSF	IFN- $\gamma$
IL-1 $\alpha$	IL-1 $\beta$	IL-2	IL-4	IL-5
IL-6	IL-7	IL-8	IL-10	IL-12
IL-12p70	IL-13	IL-15	IL-16	IL-17A
IP-10	MCP-1	MCP-4	MDC	MIP-1 $\alpha$
MIP-1 $\beta$	TARC	TNF- $\alpha$	TNF- $\beta$	
FGF-21	VEGF		CHOP	GRP78

Table shows the serum analytes measured. Except for Macrophage Inflammatory Protein-1 $\beta$  (MIP-1 $\beta$ ; p=0.032), no significant differences were observed in serum levels of any of the inflammatory cytokines, chemokine or ER stress markers tested.

**Conclusion:** Acute infusion of lipid and insulin, to mimic the metabolic syndrome of obesity and induce a decrease in LH and FSH, was not associated with a significant increase in levels of a comprehensive panel of mediators and markers of inflammation. These results imply that the endocrine dysfunction and adverse reproductive outcomes in women with obesity are not a consequence of the ambient inflammatory environment but may be mediated by direct lipotoxic effects on the hypothalamic-pituitary-ovarian axis.

#### W-201

**Dydrogesterone Supplementation in Cycles Triggered with Lone GnRH Agonist for Final Oocyte Maturation Resulted in an Acceptable Pregnancy Rate.** Myriam Safrai $\dagger$ , Shmuel Hertsberg $\dagger$ , Assaf Ben Meir\*, Benjamin Reubinoff\*, Tal Imbar\*, Talya Mordechai-Daniel\*, Alexander Simon\*. *Hadassah Medical Center, Jerusalem, Israel.*

**Introduction:** Ovarian hyperstimulation syndrome (OHSS) is dramatically reduced when using antagonist cycle with GnRH agonist trigger before ovum pick up. This trigger induces short luteinizing hormone (LH) and follicle stimulating hormone (FSH) peaks, which are associated with reduced progesterone and estrogen levels during the luteal phase resulting in an inadequate luteal phase and a significantly reduced implantation rate. We aimed to evaluate if luteal oral Dydrogesterone (Duphaston) supplementation in an antagonist cycle after a lone GnRH agonist trigger rescue the luteal phase, allowing the possibility to peruse with fresh embryo transfer.

**Methods:** A retrospective cohort study. The study group (n=123) included antagonist cycle triggered with lone GnRH-agonist trigger due to imminent ovarian hyperstimulation syndrome (OHSS). The control group (n=374) included patients under 35 years old, who underwent an antagonist protocol with a dual trigger of a GnRH-agonist and hCG (hCG group). In the study group (Duphaston group) patients were given Duphaston in addition to standard luteal support. The outcomes were pregnancy rate and OHSS events.

**Results:** The mean number of oocytes retrieved and estradiol plasma levels were significantly higher in the Duphaston group (16.9 $\pm$ 7.7 vs. 10.8 $\pm$ 5.3 and 11658 $\pm$ 5280 pmol/L vs. 6048 $\pm$ 3059 pmol/L, respectively). Fertilization rate, the mean number of embryos transferred and the clinical pregnancy rate were comparable between groups (65.4% vs 66.3%, 1.5 $\pm$ 0.6 vs 1.5 $\pm$ 0.5 and 46.3% vs 40.9%, respectively). No OHSS was reported.

**Conclusion:** This study was the first to evaluate outcomes of Duphaston supplementation for luteal support in an antagonist cycle with lone GnRH agonist trigger and a fresh embryo transfer. We showed that the functionality of the luteal phase in those cycle could be restored resulting

in a pregnancy rate comparable to that obtained in an antagonist cycle with dual trigger. This approach was safe, and prevented the need to postpone embryo transfer in case of pending OHSS.

#### W-202

**The Correlation between Human Papillomavirus Infection on Sperm Motility and Morphology among Infertile Men.** Sareh Abdollahifard $\dagger$ \*, Iman Arbab Kazem Zadeh\*.<sup>2</sup> *Jahrom University of Medical Sciences, Jahrom, Iran, Jahrom, Iran, Islamic Republic of;* <sup>2</sup>*University of Kassel, Kassel, Germany, Kassel, Germany.*

**Introduction:** Infection of human papillomavirus infection (HPV) is present in 10-13% of sexually active men (with non-symptomatic antibodies detected in some 70-80%), it was proposed that as an active infection this might have major consequences for male fertility. However, infection with any of the several types of human papilloma virus seems considered almost exclusively as a prelude to cervical cancer in females. There are no enough studies and research on the correlation between HPV, therefore, this study was conducted to investigate the presence of human papillomavirus (HPV) in human sperm cells and to evaluate potential effects of HPV on the motility and morphology sperm functions, specially motility and morphology sperm of infertile men in patients referring at infertility clinics of Jahrom University of Medical Sciences in 2017-2019.

**Methods:** In this double-blind placebo-controlled clinical trial, Specimens of semen were collected from 176 randomly selected men patients who attended the fertility clinics at infertility clinic in Jahrom University of Medical Sciences. The presence of HPV DNA and RNA were examined by polymerase chain reaction. Semen quality and sperm cell function (motility and morphology sperm) were analyzed by computer-aided autoanalyzer.

**Results:** There was significant difference among the studied variables in terms of confounding variables. Human papillomavirus type 16 DNA and RNA were found in 30% of the sperm cells specimens, respectively. Human papillomavirus type 18 DNA and RNA were present in 22% of the same sperm cells specimens, respectively. Incidence of asthenozoospermia among patients infected with either HPV was significantly higher than in those without HPV in their sperm cells (70% versus 19%). The data were analyzed using descriptive and inferential statistics (P < 0.0001).

**Conclusion:** These results suggest that human papillomavirus can be found in human sperm cells and that certain HPV-specific genes are actively transcribed. Sperm motility and morphology parameters seem to be affected by the presence of HPV in the sperm cells, and also the incidence of asthenozoospermia may be associated with HPV infection.

#### W-203

**Anti-Mullerian Hormone and Time to Dominant Follicle in Intrauterine Insemination Cycles.** Victoria W Fitz $\dagger$ , Stylianos Vagios $\dagger$ , Irene Souter\*, Caitlin Sacha $\dagger$ , Karissa Hammer $\dagger$ , Charles Bormann, Kaitlyn James. *Massachusetts General Hospital, Boston, MA, United States.*

**Introduction:** AMH inhibits primordial follicle recruitment and reduces responsiveness of early antral follicles to FSH in mice<sup>1</sup>. In humans, AMH level predicts response to ovarian stimulation for IVF. Recently, high AMH levels (>8ng/mL) were found to be associated with longer follicular phase length among regularly cycling, fertile women attempting to conceive naturally<sup>2</sup>. Our question was whether AMH level is associated with length of time to development of a dominant follicle in ovulatory women undergoing gonadotropin stimulation for ovulation induction and intrauterine insemination.

**Methods:** Retrospective cohort study of 2,340 gonadotropin/IUI cycles performed at Massachusetts General Hospital between 11/2007 and 09/2020 were evaluated. Cycles in women with a diagnosis of PCOS or anovulation were excluded. Serum AMH levels obtained within a year of cycle start were recorded. Univariate and linear regression analysis were performed in Stata. included univariate analysis and linear regression adjusting for patient age, BMI and multiple cycles per patient.

**Results:** The most common diagnosis was idiopathic infertility (38%) followed by diminished ovarian reserve (22%) and male factor infertility (13%). Mean age was 36.8 (SD 4.0) years, median AMH was 1.5 (IQR

0.6, 3.01) ng/mL and mean time to trigger was 10.5 (SD 2.4) days. When analyzed by AMH (ng/mL) quartiles (Q1-Q4), the mean time from baseline US until trigger was 11.2 days (SD 2.3) among individuals in the highest AMH quartile (Q4: 3.05-14) compared to 10.5 (SD: 2.9), 10.0 (SD: 1.9), and 10.5 days (SD: 2.18) in AMH quartiles Q1 (0.03-0.6), Q2 (0.61-1.5), and Q3 (1.5-3.01), respectively. Higher AMH levels were significantly associated with longer length of stimulation in the unadjusted model and when adjusted for patient age, BMI, and multiple cycles per patient ( $p < 0.001$  for all comparisons). In the adjusted model, an increase of 1 ng/mL in serum AMH level was associated with a 0.21 (95% CI: 0.145, 0.271) day increase in time from baseline ultrasound to administration of HCG.

**Conclusion:** The role of AMH in the follicular phase of human folliculogenesis is difficult to characterize in vitro. These results suggest that there is an association between pre-treatment serum AMH levels and time to development of a dominant follicle in gonadotropin/IUI cycles with higher serum AMH level associated with longer duration of stimulation. Therefore, AMH may decrease follicle responsiveness to gonadotropins in regularly cycling infertile women. While the impact may not be clinically significant, this has implications for characterizing the physiologic role of AMH.

#### W-204

**The Effect of Medical Treatment Including Dienogest after Surgical Removal of Ovarian Endometrioma on In Vitro Fertilization (IVF) Outcome.** Sejin Kim, Han Soo Jin, Kim Sung Woo, Kim Hoon, Ku Seung-Yup, Suh Chang Suk, Kim Seok Hyun. *Seoul National University Hospital, Seoul, Korea, Republic of.*

**Introduction:** The potential benefits of dienogest for pain relief and lowering endometriosis recurrence after surgery are widely known. However, the effect of pre-treatment of dienogest after endometrioma surgery on IVF outcome has not been demonstrated. This study evaluated the effect of pre-treatment of dienogest in infertile women who had surgical ovarian endometrioma removal and postoperative IVF.

**Methods:** A retrospective cohort study of 142 women who underwent IVF after surgical removal of ovarian endometrioma was conducted. Patients were divided into two groups: group I (n=122) without medical treatment after surgery and group II (n=20) with medical treatment including dienogest after surgery. To adjust preexisting difference of age and AMH level of two groups, ANCOVA was used. Primary outcome is the number of retrieved oocytes and secondary outcomes are clinical pregnancy rate and live birth rate.

**Results:** There was no significant difference in mean age, basal LH, FSH, E2 level, the size of endometrioma, and the duration of controlled ovarian stimulation (COS) between two groups. Anti-mullerian hormone (AMH) level was higher in group II (2.3±1.6 vs. 1.3±1.1,  $p=0.015$ ). Bilaterality of endometrioma was also significantly higher in group II than in group I (68.4% vs. 31.0%,  $p=0.002$ ). The number of retrieved oocytes was significantly higher in group II (11.6±7.3 vs. 5.1±4.2,  $p=0.002$ ). After the adjustment of age and AMH, the number of retrieved oocytes was higher in group 2 (9.8 [95% CI, 7.8-11.8] vs. 5.6 [95% CI, 4.5-6.6],  $p=0.000$ ). Clinical pregnancy rates (group I 20.9% vs. group II 14.3%,  $p=0.566$ ) and live birth rates (group I 10.5% vs. group II 7.1%,  $p=0.701$ ) showed no statistical difference.

**Conclusion:** In infertile women who received endometrioma surgery, pre-treatment of dienogest before IVF revealed higher number of retrieved oocytes after the adjustment of age and AMH level. However, clinical pregnancy rate and live birth rate showed no significant difference regardless of the use of dienogest before IVF.

	Total (n=142)	Group I (n=122)	Group II (n=20)	p-value
Age (Yr)	34.6±3.6	34.3±4.1	33.9±2.8	0.555
AMH (ng/mL)	2.0±1.2	1.3±1.1	2.3±1.6	0.015
Bilateral endometrioma (%)	29.5	31.0	68.4	0.002
Size of endometrioma (cm)	4.6±1.9	4.9±1.9	4.7±1.6	0.550
Number of retrieved oocytes	6.0±4.3	5.1±4.2	11.6±7.3	0.002
Adjusted number of retrieved oocytes		5.6 (95% CI, 4.5-6.6)	9.8 (95% CI, 7.8-11.8)	0.000
Clinical pregnancy rate (%)	20	20.9	14.3	0.566
Live birth rate (%)	9.5	10.5	7.1	0.701

#### W-205

**Intramuscular Progesterone for Luteal Phase Support May Not Improve Live Birth Rate Following Frozen Embryo Transfer (FET) in Patients with Polycystic Ovarian Syndrome (PCOS).** Daniel Miranian†, Colby Foster†, Emily Kobernik, Erin Inman†, Micaela Stevenson†, Samantha B Schon, Molly B Moravek\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** Recent interim analysis from a randomized controlled trial demonstrated that intramuscular (IM) progesterone for luteal phase support during FET cycles leads to higher ongoing pregnancy rates compared to vaginal (PV) progesterone. We hypothesized that there may be certain patient characteristics that convey a greater benefit of IM progesterone compared to PV progesterone.

**Methods:** The Current Procedural Terminology code 89352, which represents frozen embryo thaw, was used to identify all patients undergoing FET from a single infertility clinic from 1/2015-7/2019 (n=865). Retrospective data on patient demographics, comorbidities, baseline laboratory parameters, route of progesterone supplementation, and pregnancy outcomes were collected in a RedCap database using chart review. Bivariate analysis was used to compare pregnancy outcomes between FET cycles that received IM progesterone versus FET cycles that received PV progesterone supplementation alone. After controlling for age, race, body mass index, insurance status, gravidity, and parity, multivariable logistic regression was used to produce adjusted odds ratios (aOR) of live birth stratified by route of progesterone supplementation.

**Results:** 428 patients (49.5%) received IM progesterone compared to 437 (51.5%) that received PV progesterone. The total live birth rate (LBR) was 41.6%; however, the LBR amongst patients that received IM progesterone was 47.6% compared to 35.5% for those who received PV ( $p=0.0003$ ). Patients that received PV progesterone had a biochemical pregnancy rate of 19.6% compared to 10.5% ( $p=0.0002$ ) amongst those that received IM, whereas the rate of no documented pregnancy, pregnancy of unknown location, ectopic pregnancy, and miscarriage were similar between the two groups. Among patients with a diagnosis of PCOS, PV progesterone was associated with an increased odds of live birth (aOR=1.89,  $p=0.02$ ) as opposed to a decreased odds of live birth (aOR=0.52,  $p=0.005$ ) when they received IM progesterone. The following factors were not found to be statistically different between patients receiving PV versus IM progesterone and had a live birth: age at oocyte retrieval, BMI, or embryo grade.

**Conclusion:** As expected, we found a significantly higher LBR when analyzing all patients that received IM compared to PV progesterone for luteal support during FET cycles, which seems to be driven by a lower biochemical pregnancy rate. Conversely, patients with PCOS may actually have better outcomes with PV progesterone following FET, and prospective studies are warranted to determine the best route of progesterone for luteal support in this population

W-206

**Development and Validation of a Noninvasive High-Resolution Imaging System for Uterine Peristalsis in Nonpregnant Women.** Sicheng Wang†, Zichao Wen, Stephanie Pizzella, Kelsey Anderson, Yiqi Lin, Wenjie Wu, Qing Wang, Valerie Ratts, Yong Wang. *Washington University in St. Louis, St. Louis, MO, United States.*

**Introduction:** Uterine Peristalsis Imaging (UPI) is a three-dimensional electrophysiology imaging system to noninvasively image and quantify nonpregnant uterine peristalsis during the menstrual cycle. We developed UPI by integrating novel magnetic resonance imaging (MRI) and body surface electrical mapping. We validated the accuracy and resolution of this advanced imaging system by comparing the UPI-derived uterine propagation patterns with transvaginal ultrasound (US) measurements.

**Methods:** UPI system consists of two major components: MRI scan of patient-specific uterus-abdomen geometry and body surface electrical imaging (Fig. 1). Firstly, patients underwent an MRI scan to generate accurate uterus geometry. Body surface electrodes' locations were also imaged using MRI markers. Secondly, electrodes were applied to abdomen surface to record the electrical peristalsis signals. Thirdly, a transvaginal ultrasound was conducted during the electrical measurements to record the peristalsis in two dimensional B-mode. Finally, UPI integrated patient's geometry to image the uterine peristalsis waves (red bands) on the uterus (blue). We tracked the real-time endometrium movements of uterine peristalsis from the ultrasound video and compared with the UPI findings of uterine peristalsis for validation. Seven independent UPI validation sessions were conducted in this pilot study.

**Results:** A representative UPI result from a 26 year old, G0 female during the ovulatory phase (Day 13) is shown in Fig. 2. Two complete uterine peristalsis cycles are shown: (A) a cycle initiating at the cervix and propagating to the fundus over 18 secs, and (B) a subsequent cycle initiating at the fundus and propagating to the cervix over 19 secs. Blue regions represent inactive areas, and red regions represent the areas experiencing peristalsis. In the corresponding transvaginal ultrasound videos, the same directional uterine peristalsis patterns were visualized by observing the contour of the endometrial canal, which confirmed the UPI findings.

**Conclusion:** The developed UPI system provides rich spatial-temporal information of uterine peristalsis in a noninvasive and quantitative way. The initiation and propagation patterns can be imaged and quantified. Future work will compare uterine peristalsis patterns between patients with normal and abnormal menstrual cycles, with the goal of aiding in clinical diagnosis and treatment of female infertility.

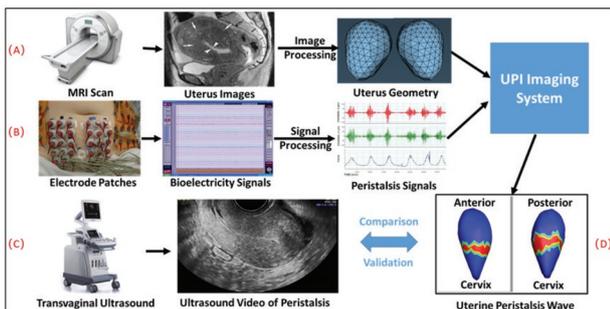


Fig1. Uterine Peristalsis Imaging Methodology and Validation.

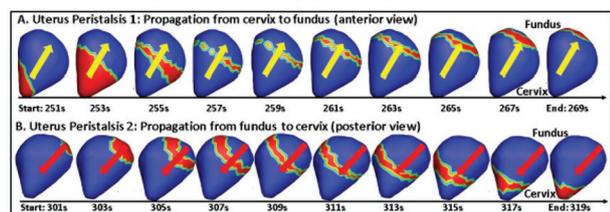


Fig 2. UPI representative results (A) Cervix to fundus peristalsis. (b) Fundus to cervix peristalsis.

W-207

**Estradiol Level during Ovulation Induction and Intrauterine Insemination with Letrozole Does Not Correlate with Pregnancy Outcome.** Erika P New†,<sup>1</sup> Shayne Plosker\*,<sup>2,1</sup> Kate Devine,<sup>3</sup> Samad Jahandideh,<sup>3</sup> Anthony N Imudia.<sup>2,1</sup> *University of South Florida, Tampa, FL, United States; <sup>2</sup>Shady Grove Fertility Center Tampa Bay, Tampa, FL, United States; <sup>3</sup>Shady Grove Fertility Reproductive Science Center, Rockville, MD, United States.*

**Introduction:** The aromatase inhibitor letrozole is used for ovulation induction (OI) in women with unexplained infertility and Polycystic Ovarian syndrome. Aromatase is an enzyme that converts androgens to estradiol (E2). Inhibiting aromatase results in transient low E2 levels. Frequently in letrozole OI cycles, a mature follicle is noted in the late follicular phase, concurrently with lower than anticipated E2 levels per dominant follicle. A low E2 level is characteristic of patients with poor ovarian response, but the significance of low E2 in patients on letrozole is unknown. We hypothesize that low peri-ovulatory E2 will not be associated with decreased clinical pregnancy rates after ovulation induction and intrauterine insemination (OI-IUI) with letrozole, suspecting that the low E2 reflects inhibition of aromatase and not a poor ovarian response.

**Methods:** A retrospective medical record review was performed at a large, multi-center private practice. Women with infertility having autologous OI-IUI cycles using letrozole from 2004 to July 31, 2019 were included. Patient age, BMI, E2 level on day of hCG trigger or LH surge, the largest ovarian follicle diameter, and cycle outcome was collected. Data were compared by a student *t*-test and chi square as appropriate.

**Results:** 2,051 cycles met inclusion criteria. As shown in Table 1, cycles were categorized by E2 level  $\leq 50$  pg/ml (n=1320, mean  $E2 \pm SD = 35.37 \pm 9.7$ ) vs  $> 50$  pg/ml (n=731,  $E2 = 82.48 \pm 66.99$ ). Patient age and BMI were comparable between groups. Clinical pregnancy rate was similar in both groups ( $E2 \leq 50: 14.5\%$  vs  $E2 > 50: 14\%$ ). The mean dominant follicle diameter (mm) at time of ovulation was  $21.02 \pm 2.34$  in  $E2 \leq 50$  vs  $21.31 \pm 2.30$  in  $E2 > 50$  ( $p=0.035$ ). The mean number of follicles  $\geq 14$  mm was similar in both groups ( $E2 \leq 50: 2.08 \pm 0.79$  vs  $E2 > 50: 2.09 \pm 0.82$ ,  $p=0.643$ ). We further analyzed outcomes at the E2 cutoff values of 100, 150, and 200 pg/ml, and still there were no differences in pregnancy outcome (Table 1). Stratification based on peri-ovulatory follicle diameter  $< 20$  mm or  $\geq 20$  mm revealed no difference in mean E2 level ( $52.39 \pm 44.49$  vs  $52.85 \pm 43.09$ ,  $p=0.832$ ) or in clinical pregnancy rates (14.7% vs 13.8%).

**Conclusion:** Clinical pregnancy rate achieved with OI-IUI with letrozole was not affected by peri-ovulatory E2 level and was similar whether the lead follicle was  $<$  or  $\geq 20$  mm.

Outcome variable	Estradiol threshold comparisons											
	$\leq 50$	$> 50$	p	$\leq 100$	$> 100$	p	$\leq 150$	$> 150$	p	$\leq 200$	$> 200$	p
n	1320	731		1971	80		2011	40		2025	26	
E2 (pg/ml) = mean (SD)	35.37 (9.7)	82.48 (67.0)	<0.001	45.59 (18.3)	214 (142.5)	<0.001	47.09 (21.0)	307.33 (151.8)	<0.001	48.00 (23.7)	376.49 (147.1)	<0.001
Largest follicle = mean diameter (mm) (SD)	21.02 (2.3)	21.31 (2.3)	0.035	21.13 (2.3)	21.11 (2.6)	0.956	21.14 (2.3)	20.83 (2.2)	0.495	21.13 (2.3)	20.94 (1.9)	0.73
Number of follicles $\geq 14$ mm (SD)	2.08 (0.79)	2.09 (0.82)	0.643	2.08 (0.79)	2.12 (1.0)	0.635	2.08 (0.79)	2.15 (1.19)	0.595	2.08 (0.80)	2.04 (0.77)	0.773
Clinical intrauterine gestation = n (%)	191 (14.5)	102 (14)	0.799	277 (14.1)	16 (20)	0.185	283 (14.1)	10 (25)	0.085	288 (14.2)	5 (19.2)	0.658
Biochemical pregnancy loss = n (%)	48 (3.6)	26 (3.6)	1	71 (3.6)	3 (3.8)	1	73 (3.6)	1 (2.5)	1	74 (3.7)	0 (0.0)	0.643

W-208

**Drug-Drug Interactions In-Silico Analysis in Female Treatments Reveals Drugs with a High Number of Unconsidered Interactions and Potential Side Effects.** Ismael Henarejos-Castillo†,<sup>1,2</sup> Pablo Garcia-Acero†,<sup>1,2</sup> Patricia Sebastian-Leon,<sup>1</sup> Alejandro Aleman,<sup>1</sup> Antonio Parraga-Leo†,<sup>1,2</sup> Patricia Diaz-Gimeno\*. *<sup>1</sup>IVI Foundation / IIS La Fe, Valencia, Spain; <sup>2</sup>Universidad de Valencia, Valencia, Spain.*

**Introduction:** Reproductive medicine includes a variety of conditions that need to be treated with the administration of drugs of different properties. Moreover, some patients are on medication to treat other unrelated conditions. As a result, unexpected drug interactions can occur when undergoing assisted reproductive treatments (ARTs) leading to side effects and decreased effectiveness of ARTs. However, few potential drug-drug interactions (DDIs) are considered in clinical practice. Here, we analyze

current drugs used in ARTs, covering all life-stages of female reproduction including contraception, In Vitro Fertilization (IVF) or pregnancy, and reproductive diseases to highlight potential DDIs for safer ARTs.

**Methods:** To extract approved drugs used in ARTs for women, seven guidelines from the ESHRE were consulted (2013-2019) together with DrugBank database, ClinicalTrials.gov (Phase IV, 2013-2020) and a literature review. Drugs were classified according to their Anatomical Therapeutic Classification (ATC) code. DDIs previously reported for all drugs were obtained from DrugBank. A Fisher's Test (normalized by the number of drugs in each ATC group) was performed to compare the number of DDIs between ARTs drugs and the rest of approved drugs in Drugbank (4,014) to highlight unexpected effects in IVF treatments, pregnancy, labor, contraception, and reproductive diseases. Odds Ratios (OR) with a statistical significance of  $FDR < 0.05$  were analyzed.

**Results:** 193 drugs related to 54 female reproductive conditions were obtained. Amitriptyline, used in endometriosis, was on the top with 1,574 DDIs with approved drugs, and commonly used drugs in IVF treatments such as Progesterone, Estradiol or Estradiol valerate, used in hormone replacement therapy, had also a great number of DDIs (952, 1,356 and 1,381 respectively). Genito-urinary and sex hormones group, where ART drugs were included, had significantly more interactions (Fisher's Test) with 11 drug groups such as alimentary tract and metabolism ( $OR = 2.7$ ,  $FDR = 9.2e-136$ ), nervous system ( $OR = 3.1$ ,  $FDR = 8.0e-118$ ), blood and blood forming organs ( $OR = 3.4$ ,  $FDR = 2.9e-59$ ) cardiovascular systems ( $OR = 2.2$ ,  $FDR = 8.1E-55$ ).

**Conclusion:** Commonly used drugs in female reproduction, included in the Genito-urinary group, had a great number of significant interactions with other drug groups. Therefore, patients undergoing ARTs should be carefully monitored to avoid risk factors associated with these DDIs, providing an opportunity to improve ARTs. We encourage the scientific community to cover the gaps between DDIs studies and their clinical consequences to achieve a personalized medicine in ARTs.

## W-209

**A Call to Action: The Need for Genetic Carrier Screening Guidelines.** Sonia Patel, Lauren Grimm, Caroline Peschansky, Sarah Dynia, Safina Usmani, Jawaria Amir, Kayla Vitale, Royi Lynn, Erica Loudon, Angeline Beltsos, Roohi Jeelani. *Vios Fertility Institute, Chicago, IL, United States.*

**Introduction:** In Apr. 2020, the American College of Obstetric and Gynecology (ACOG) reaffirmed their recommendations that all pregnant women should be offered genetic carrier screening (GCS), ideally prior to conception. However, these guidances are vague and only mention a small number of tests on the market. Current panel discrepancies among genetic testing companies, allelic variations screened, and large expanded screening panels make clinical application of such recommendations burdensome on both the patient and practice. This is especially true for couples seeking assisted reproductive technology (ART) therapy, as reciprocal positive couples have the option of preimplantation genetic testing of their embryos. No streamlined recommendations currently exist to guide both genetic testing companies and clinicians on best practices regarding expanded screening and subsequent genetic counseling. Thus, we wanted to understand the clinical impact of GCS prior to the use of ART therapy.

**Methods:** A retrospective chart review was performed on couples who were offered GCS over the last 6 months. Patients were divided into groups: those who accepted and those who declined screening. If testing was accepted and patient and/or partner results were positive, genetic counseling was required. If testing was accepted and both partners were negative, or testing was denied, genetic counseling was optional; if couples declined genetic counseling, a declination waiver was required. Patients who used donor gametes, gestational carriers, or underwent egg freezing were excluded. Results were analyzed using SPSS (SPSS Inc., Chicago, IL, USA).

**Results:** 674 couples were offered GCS over the last 6 months with 577 couples accepting and 97 declining (85.6% vs 14.4%). 5 different GCS labs were used with test panels, screening from 18 to 283 diseases. 47 couples out of the 577 that accepted had originally declined testing. 375 females of the 577 couples tested positive as a carrier for at least 1 disease. Of the

202 females who tested negative, 77 of them had partners test positive for at least 1 disease. 241 couples had both the patient and partner test positive for at least 1 disease. 125 couples had both the patient and partner test negative. Almost 20% of couples who had at least 1 spouse test positive declined to receive counseling with a signed waiver.

**Conclusion:** Given these results, there is variation in screening outcomes between the patient and partner that make specific GCS guidelines necessary. As GCS continues to evolve, expanded carrier screening will inevitably become more complicated, raising confusion and increasing the burden on clinicians to reevaluate patient and practice protocols. Thus, more comprehensive clinical guidelines are necessary to limit practice liability and increase the number of informed patients regarding their genetic health.

## W-210

**Parenterally-Delivered Human Mesenchymal Stem Cells Are Effective in Restoring Fertility in a Chemotherapy Induced Premature Ovarian Insufficiency Mouse Model.** Hang-soo Park,<sup>1</sup> Rishi Man Chugh,<sup>2</sup> Esra Cetin,<sup>1</sup> Hiba Siblini,<sup>1</sup> Amro Elsharoud,<sup>3</sup> Mara Ulin,<sup>3</sup> Sahar Esfandiyari,<sup>3</sup> Ayman Al-Hendy.<sup>1</sup> <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>University of Kansas Medical Center, Kansas City, KS, United States; <sup>3</sup>University of Illinois at Chicago, Chicago, IL, United States.

**Introduction:** Primary ovarian insufficiency (POI) refers to the ovarian loss of function under the age of 40 years and lead those patients to amenorrhea and infertility. Our previous study showed that transplantation of human bone marrow-derived mesenchymal stem cells (hBM-MSC) in chemotherapy-induced POI mouse ovary can reverse POI phenotype and achieve pregnancy without other significant side effects. In this previous study, we used direct intraovarian injection via surgical access. For reliable and probably repetitive application of hBM-MSC treatment for POI patients, we must establish alternative simple administration routes such as intravenous injection. Intravenous injection of hBM-MSC may require different dosage and injection intervals to treat POI condition. Comparing the therapeutic effect of intravenous injection using hBM-MSC with several different dosage in POI mouse model will reveal the optimal treatment protocol.

**Methods:** In this study, we induced POI in C57/BL6 mice by chemotherapy (CXT) using a standard protocol. We then injected four different dose levels of hBM-MSCs by retro-orbital injection (6 mice/group) at day 7 and day 14 post CXT. After hBM-MSC treatment, we performed breeding experiment to screening optimal dosage of hBM-MSC for intravenous injection to restore fertility in this preclinical POI model. Mice in separate parallel experiments were sacrificed without breeding 2 weeks after MSC treatment and we collected serum, ovary, and other major organs (5 animals/group). We also analyzed the transmission of human DNA with fetus genomic DNA (8 fetuses/group) to confirm safety of intravenous injection.

**Results:** The experimental group receiving 2,000,000 cells injection were all dead immediately likely due to embolic accident secondary to high viscosity of the inoculum. The pregnancy rate in the three other treated groups were significantly higher ( $70 \pm 23\%$  to  $90 \pm 10\%$ ) that untreated negative control ( $25 \pm 21\%$ ). We noticed that a single time injection was sufficient to restore fertility ( $88 \pm 13\%$ ) in POI mouse model. human genes representing engrafted hBM-MSC were not detected in mouse blood nor in neonatal pup livers. We also found that pups delivered by the hBM-MSC treated mice had normal morphology and has similar weight gain to healthy controls.

**Conclusion:** Our data confirm the feasibility of delivering mesenchymal stem cell treatment through Intravenous injection to restore fertility in chemotherapy induced POI mouse model. Our study support the feasibility of hBM-MSC as potentially effective therapy for POI. Pilot clinical trials are warranted to validate the utility of such approach in POI patients.

## W-211

**Testosterone Independent Biomarkers May Improve Prediction Models of Polycystic Ovary Syndrome.** Ky'era V. Actkins<sup>†</sup>,<sup>1</sup> Lea K. Davis\*,<sup>2</sup> Meharry Medical College, Nashville, TN, United States; <sup>2</sup>Vanderbilt University Medical Center, Nashville, TN, United States.

**Introduction:** Up to 75% of women with polycystic ovary syndrome (PCOS) remain undiagnosed. PCOS is a highly heterogeneous endocrine disorder that is characterized by both reproductive and metabolic features. The symptom heterogeneity of PCOS is a leading cause of its lengthy and difficult diagnosis process, which typically requires a combination of clinical evaluation and biochemical assessment for diagnosis. This can include clinical tests for androgens, sex hormones, or other hormones to exclude the possibility of non-PCOS conditions.

**Methods:** In this study, we utilized health data and laboratory measurements recorded in electronic health records (EHRs) to understand which frequently ordered laboratory measurements best explained PCOS disease risk. To do this, we evaluated the biomarker profiles of 8,292 PCOS cases and 34,771 controls identified from a validated EHR phenotyping algorithm in a multivariable logistic regression model.

**Results:** In our combined multivariable logistic regression analysis using inverted normalized age-adjusted labs covaried for median age and race, free testosterone demonstrated the strongest association with PCOS (OR = 2.44,  $p < 2.2e-16$ ). This association was consistent among White (OR = 3.17,  $p = 1.35e-06$ ) and Black (OR = 2.33,  $p = 1.05e-18$ ) female patients in a race stratified analysis. Given the magnitude of the association with testosterone, we accounted for testosterone levels in the analysis of other biomarkers. After accounting for free testosterone, we observed that six labs remained associated ( $p < 0.05$ ) with a PCOS diagnosis, including triglycerides (OR = 1.32,  $p = 7.35e-03$ ) and sex hormone-binding globulin (OR = 1.37,  $p = 6.20e-03$ ). However, when total testosterone was accounted for, only four labs remained significant and progesterone explained the most variance in the model at 5% (OR = 1.98,  $p = 0.01$ ).

**Conclusion:** High testosterone is a salient feature of PCOS, but its strong effects may mask significant, but smaller effects of other biomarkers. These findings present insight into androgen-independent biomarkers for PCOS that may be useful in prediction models, which warrants further investigation.

## W-212

**Impact of Gestational Metformin-Exposure on Fetal Anthropometric Measures in PCOS.** Carol Nader<sup>†</sup>,<sup>1</sup> Rachel Isaacs<sup>†</sup>,<sup>1</sup> Vasantha Padmanabhan,<sup>2</sup> Jean Claude Veille,<sup>1</sup> Arpita Vyas\*,<sup>1</sup> <sup>1</sup>California Northstate University, Elk Grove, CA, United States; <sup>2</sup>University of Michigan, Ann Arbor, MI, United States.

**Introduction:** Polycystic ovarian syndrome (PCOS) is the most common endocrine disease in women of reproductive age and is associated with a 40-80% obesity rate. PCOS is associated with an increased risk of pregnancy complications including gestational diabetes mellitus (GDM) and premature deliveries. While metformin has been shown to be beneficial in reducing early pregnancy loss and preterm delivery in PCOS, its impact on fetal growth is not clear. Since metformin can cross the placenta and expose the developing fetus to persistently high concentrations, we hypothesized that gestational metformin exposure would alter fetal anthropometric measures in PCOS pregnancies

**Methods:** This retrospective study included 127 women with PCOS receiving prenatal care at Valley Children's Hospital in Madera, CA over the last 10 years. Past medical history, family history, current medications, BMI, age, gravity and parity, and fetal anthropometric measurement (CI, FL/BPD, FL/AC, estimated fetal weights at 20 weeks, and rate of weight gain) were retrieved from their medical charts. Fetal growth measures from obese and non-obese women were compared between metformin and no metformin groups. Data were analyzed using a 2-tailed independent t-test for parametric data and Wilcoxon signed-rank test for non-parametric data with significance set at  $\alpha = 0.05$ .

**Results:** 21 patients were excluded from data analysis as they presented for pre-pregnancy counseling. Of the remaining 106 patients, 27.4% were on metformin, 20.8% had GDM, 71.3% were obese, and 21.7% were advanced maternal age. Metformin had no impact on fetal morphometric

measurements in the non-obese PCOS group. In contrast, FL/AC differed significantly ( $p = 0.02$ ) between Obese PCOS-metformin and Obese PCOS-no metformin group (20.9 +/- 0.15 n=15 vs 21.5 +/- 0.13 n=54, respectively). The average FL/BPD also tended to be different ( $p = 0.07$ ) between the Obese PCOS-metformin and Obese PCOS-no metformin groups (71.5 +/- 1.01 n=16 vs 73.8 +/- 0.65 n=54, respectively). Sub analysis of Obese PCOS with GDM revealed no effect of metformin on fetal measures. There were no differences in rate of weight gain or estimated fetal weight around 20 weeks gestation in any of the groups.

**Conclusion:** Metformin had a BMI-specific effect on fetal growth measures with obese PCOS but not nonobese PCOS pregnancies showing lower FL/AC, a characteristic associated with intrauterine growth restriction - a risk factor for adult onset diseases. Further studies are required to assess the significance of differences in fetal measures with metformin exposure on postnatal outcome. **Abbreviations:** AC = abdominal circumference, BPD = biparietal diameter, BMI = body mass index, CI = cephalic index, FL = femur length, GDM = gestational diabetes

## W-213

**Increased Prevalence of Positive Depression Screen during COVID19 in PCOS Patients at an Academically-Affiliated Institution.** Elizabeth Kravitz<sup>†</sup>, Liubin Yang<sup>†</sup>, Janet Bruno-Gaston<sup>†</sup>, Amy Schutt\*, Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Polycystic ovarian syndrome (PCOS) is associated with an increased prevalence of depression as compared to the general population. PCOS is also associated with physical disturbances and infertility, thus depression and anxiety are often overlooked and under-treated. Given these risks, early identification and treatment is crucial. This project investigates the differences in depression prevalence, as determined by the Edinburgh Postpartum Depression Scale (EPDS), among PCOS and non-PCOS patients presenting to a fertility clinic, as well as changes in prevalence of depression during the COVID19 pandemic.

**Methods:** A retrospective case-control study was performed of all new patients seen during the period of March 2018 to December 2020 at a large, academic-affiliated, urban fertility clinic. Descriptive and demographic variables were gathered. Inclusion criteria was new patients aged greater than 18, who had completed the EPDS questionnaire. Patients who had refused completing the EPDS or had missing data were excluded. The pre-COVID period was defined as before March 2020. Operational definition for a positive depression screen was determined by either a previous diagnosis of depression, or by an EPDS greater than 8. Chi-square tests, t-tests, Wilcoxon ranked tests, and multivariate logistic regression were used for statistical analyses.

**Results:** At our academic center, 785 new patients met criteria, of which 185 were diagnosed with PCOS (4.2%). The rate of positive screen for depression in the PCOS population was higher during the COVID pandemic (30.56%, n=22/72) compared to before (16.81% n=19/113) ( $p = .03$ ) [OR=2.18, (1.08, 4.40)]. After controlling for primary infertility status, recurrent pregnancy loss, and desire for procreative management, the difference in positivity rate of depression screen was still significant ( $p < .001$ ). However, among the concurrent non-PCOS new patient population (n=600), the rate of positive depression screening did not increase during COVID pandemic compared to before (18.29% vs 19.69%), after controlling for desire for procreative management. There was no difference in positive depression screening between the PCOS and non-PCOS population, however, the PCOS population had higher rates of primary infertility and decreased rates of recurrent pregnancy loss compared to the non-PCOS new patient population.

**Conclusion:** It is crucial that health care providers are aware of the possible increase in depression prevalence among PCOS patients, heightened in the context of COVID19. Utilizing an EPDS in clinic to screen for depression allows providers to quickly and efficiently identify patients who may require additional mental health care intervention.

**W-214**

**Effect of Caloric Restriction on Reproductive Parameters on the Aged Wistar Rats.** Pablo López de Jesús<sup>†,1,2</sup> Israel Enrique Crisanto López<sup>†,3</sup> Saúl Rodríguez Flores<sup>†,4</sup> Edith Arenas Ríos<sup>\*,1</sup> Isabel Arrieta Cruz<sup>\*,5</sup> Marcela Arteaga Silva<sup>\*,1</sup> Juan Carlos Flores Alonso<sup>\*,6</sup> <sup>1</sup>Universidad Autónoma Metropolitana, Mexico, Mexico; <sup>2</sup>Instituto Mexicano del Seguro Social, Atlixco, Puebla, Mexico; <sup>3</sup>Benemérita Universidad Autónoma de Puebla, Puebla, Mexico; <sup>4</sup>Benemérita Universidad Autónoma de Puebla, Mexico, Mexico; <sup>5</sup>Instituto Nacional de Geriatria, Mexico, Mexico; <sup>6</sup>Instituto Mexicano del Seguro Social, Mexico, Mexico.

**Introduction:** Aging is a gradual process that affects to all individuals and it is associated with chronic degenerative diseases, cardiovascular diseases and a decrease in reproductive processes. It can be influenced by different factors like diets. Thus, the control of the diet can be an important factor over longevity and reproductive function, like the Male Sexual Behavior (MSB), the fertilizing capacity of the spermatozoa and metabolic health. Some studies shown the reduction of 10 to 40 % in the nutritional regimen (Caloric Restriction "CR") is the most effective physiological manipulation to extend the maximum life expectancy. In addition, the CR can maintain the MSB and can even achieve fertility in aged wistar rats. However, the sperm quality parameters as well as the MSB and metabolic parameters have not been detailed. **OBJECTIVE:** Evaluate the effects of CR on the reproductive and metabolic parameters in the aged Wistar rats.

**Methods:** Wistar male rats (12 months old) were housed individually into acrylic cages. The food intake, the MSB tests and quality sperm were evaluated for 15 days before at CR. After, the rats were separated in different groups accord its diets: 15, 35% of CR and a control group with *ad libitum* food. MSB, sperm quality and hormonal test were performed at 6 and 12 months of CR.

**Results:** The CR after 6 and 12 months in animals of 18 and 24 months age improve the sperm quality compared to control. A progressive decrease in sperm quality is observed for the control group, it was significative at 24 months of age, not there for the RC groups, the MSB decreased for the control group since the 18 months of age, while RC groups maintained the MSB at 24 months of age, thus, the metabolic parameters show homogeneity for the groups CR while the control presented alterations in testosterone, Estradiol, Insulin and leptin.

**Conclusion:** We can conclude that the CR improves the sperm quality, the MSB and metabolic parameters during aging.

**W-215**

**Outcomes of IVF Cycles Using Testicular and Zymot Obtained Sperm from Men with High Sperm DNA Fragmentation.** Nicole D Ulrich<sup>†</sup>, Min Xu, James Dupree, Samantha Schon\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** Elevated DNA fragmentation in sperm has been linked to poor IVF outcomes and miscarriage. Previous research recommended the use of testicular sperm for fertilization with ICSI (T-ICSI) to improve live birth rates. However, there are concerns associated with T-ICSI including decreased fertilization rates and increased embryo aneuploidy. Recently, the Zymot sperm sorting device has been approved to sort out sperm with low DNA damage, which may provide another treatment option for couples with elevated DNA fragmentation.

**Methods:** We conducted a retrospective chart review of men who underwent DNA fragmentation testing and their partners from an academic fertility clinic from April 1, 2017 - January 31, 2021. All DNA fragmentation testing performed at a commercial company using SCSA. We included men with high-DNA fragmentation ( $\geq 25\%$ ) who did either sequential IVF cycles first with T-ICSI then with ejaculated sperm prepared with Zymot sperm sporting device (Z-ICSI) or one cycle with split fertilization using both T-ICSI and Z-ICSI. Data abstracted included sperm retrieval method, sperm preparation, fertilization method, fertilization rate, embryo development, and pregnancy outcomes. Embryo morphology was evaluated using the Gardner grading system. Additional variables assessed included infertility diagnosis, number of oocytes

retrieved, fertilization rate, rate of good quality day 3 embryos (A or B grade), pregnancy (serum hCG), ongoing pregnancy (fetal cardiac activity), and pregnancy outcome.

**Results:** Five couples met our criteria with mean DNA fragmentation index (DFI) of 45%, (range 28 - 70) and underwent 9 total IVF cycles. Four of these couples underwent 8 total cycles, using T-ICSI in the first cycle followed by Z-ICSI in the second. The fifth couple underwent one IVF cycle with split fertilization using both a frozen sample for T-ICSI and a fresh sample for Z-ICSI. The fertilization rate in the T-ICSI cycles was 48% vs 83% in Z-ICSI cycles. Rate of good quality day 3 embryos was 40% in T-ICSI cycles and 47% in Z-ICSI cycles. Of the 5 cycles with T-ICSI sperm, 4 did not achieve pregnancy and one resulted in frozen euploid embryo not yet transferred. Of the 5 cycles with Z-ICSI, 2 did not achieve pregnancy, one resulted in frozen euploid embryo not yet transferred, one resulted in pregnancy but has not yet had an OB ultrasound, and one resulted in live birth.

**Conclusion:** We present a case series of 5 couples using both T-ICSI and Z-ICSI during their IVF cycles. The Z-ICSI cycles had higher fertilization rates and two positive pregnancies, one of which has resulted in a live birth. In comparison, the T-ICSI cycles from the same patients did not achieve pregnancy. Use of a device like the Zymot in patients with elevated DNA fragmentation may provide a less-invasive treatment option with higher fertilization rates. Larger studies are needed to further investigate these outcomes.

**W-216**

**Caloric Restriction Effects on Female Wistar Rats Reproductive Health.** Israel Enrique Crisanto López<sup>\*,1</sup> Pablo López de Jesús<sup>\*,2</sup> Isabel Arrieta Cruz<sup>\*,3</sup> Juan Carlos Flores Alonso<sup>\*,4</sup> <sup>1</sup>Benemérita Universidad Autónoma de Puebla, Puebla, Mexico; <sup>2</sup>Universidad Autónoma Metropolitana, Iztapalapa, Ciudad de México, Mexico; <sup>3</sup>Instituto Nacional de Geriatria, Ciudad de México, Mexico; <sup>4</sup>Laboratorio de Biología de la Reproducción, Centro de Investigación Biomédica de Oriente-IMSS, Puebla, Mexico.

**Introduction:** The caloric restriction (CR) refers to the restricted intake of food from *ad libitum* feeding. CR has been proposed as increases longevity factor. It has been observed so conclusively in yeasts. However, CR in mammals can improve the state of health of many organisms also humans. Thus, it is know that the reduction in the diet that ranges from 10 to 40 % in young organisms can improve the quality of life (Health General) and can even improve neuroendocrine signals of the hypothalamic-pituitary-gonadal axis (HHG) by modifying the hormonal reals patter that can be reflected in the alteration of metabolic homeostasis. On the other hand, it is known that as individual beings the aging, the ovarian function declines like the metabolic homeostasis. However, CR effets on female wistar rat during th adult-elderly step are unknown. The **objective** of this work was to determine if CR improves the reproductive health parameters in the adult Wistar female rat.

**Methods:** Eighteen female Wistar rats of 3 months of age were used and it was divided into three experimental groups: Control or *Ad libitum* food intake, CR 15% and CR 25%. All subjects were placed into individual cages and kept in inverted light cycles 12:12 hours, the total food intake was evaluated for 15 days before to CR. During CR, the body weight and the estrus cycle of the rats were monitored, after 3 months they were euthanized by decapitation for blood serum extraction; the metabolic and hormonal parameters were quantified.

**Results:** The results obtained show that the CR keeps the females in constant cyclicity, which suggests normal ovarian physiology, but there is an increase in the concentrations of fatty acids in the metabolism of the subjects of the control group. The hormonal data with metabolic activity conclude that CR is a strategy that favors the health of experimental groups still in the young stage.

**Conclusion:** We can conclude that the CR is a propitious rate to improve the health of the Wistar female rats.

## W-217

**Exposure to Synthetic Glucocorticoids Modifies the Sperm MicroRNA Profile: Implications for Intergenerational Transmission.** Christopher Casciaro<sup>†</sup>,<sup>1</sup> Hirotaka Hamada<sup>†</sup>,<sup>1</sup> Alisa Kostaki,<sup>1</sup> Stephen Matthews\*,<sup>1,2,3</sup> <sup>1</sup>University of Toronto, Toronto, ON, Canada; <sup>2</sup>University of Toronto, Toronto, ON, Canada; <sup>3</sup>Sinai Health System, Toronto, ON, Canada.

**Introduction:** 1-3% of the adult population use synthetic glucocorticoids (sGCs) for a variety of conditions, from autoimmune disorders to cancer. sGCs provide acute relief; however, chronic exposure has been shown to increase vulnerability to diabetes, hypertension and various neuropsychiatric disorders. Studies have shown that stress in males prior to conception can lead to abnormal neuroendocrine function and behaviours in offspring. Recent evidence indicates that epigenetic factors including microRNA (miRNA) within sperm may offer an explanation as to how the effects of paternal chronic stress are transferred intergenerationally. It remains unknown if chronic exposure to sGCs leads to changes in sperm that could result in intergenerational transmission. We hypothesized that sGC exposure in adult male guinea pigs will modify the miRNA profile in epididymal sperm.

**Methods:** Adult male guinea pigs were exposed to the sGC dexamethasone (Dex) in drinking water (equating to a dose of 3mg/kg) or normal water (Ctrl) (N=6/group) every other day for 48 days. Water, food intake and weight were monitored. Sperm collected from epididymal seminal fluid in the caput and cauda regions were counted and visually assessed for motility, morphology and vitality. Sperm were isolated and total RNA extracted. miRNA was assessed by miRNA 4.0 microarray and data were processed by TAC 4.0.1. miRNA probes were selected from human, mouse and rat miRNA IDs. miRNA expression was evaluated using a one-way ANOVA and p-values were FDR adjusted by the Benjamini-Hochberg algorithm. Log2Fold-Change (FC) >+/-1.5, and FDR corrected p-value < 0.05 was considered significant.

**Results:** Dex-treatment did not affect water and food intake or weight. In addition, sperm did not differ in morphology, vitality, sperm count, or motility. miRNA expression analysis revealed 42 miRNA that were significantly down-regulated, and 45 up-regulated by sGC in the caput. In the cauda, 112 miRNA were down-regulated while 10 were up-regulated. Examples of miRNA that were significantly (FDR p-value<0.05) downregulated by Dex included miR-30a (FC= -8.25) and miR-30c (FC=-6.92).

**Conclusion:** We have shown that chronic sGC exposure alters the miRNA profile of sperm in adult male guinea pigs. This is highly clinically relevant given the widespread usage of acute and chronic glucocorticoids treatment in humans. Importantly, the miR-30 family has been shown to be of central importance for embryo development. While the potential effects of dex-induced alterations in sperm miRNA in offspring remains to be determined, this study provides new insight into the possible molecular mechanisms underpinning transmission of paternal experiences.

## W-218

**Developing a Genetically Modified Marmoset Model of Neurodevelopmental Disease.** Joshua T Brennan,<sup>1</sup> Jessica Izzi,<sup>1</sup> Yan-Ling Feng,<sup>1</sup> Ping Xia,<sup>1</sup> Xindong Song,<sup>1</sup> Jacqueline Maher,<sup>1</sup> Bhuchitra Singh,<sup>1</sup> Shanshan Zhu,<sup>1</sup> Christopher Ross,<sup>1</sup> John Davis,<sup>2</sup> James C Harris,<sup>1</sup> Xiaoqin Wang,<sup>1</sup> James H Segars\*,<sup>1</sup> <sup>1</sup>Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>2</sup>University of Illinois, Chicago, IL, United States.

**Introduction:** The common marmoset (*Callithrix jacchus*) provides significant advantages over murine models to assess neurobiological mechanisms related to autism, intellectual disability, and schizophrenia. As a first step to establish a marmoset neurodevelopmental model by gene-editing, the present study sought to optimize ovarian stimulation and *in vitro* fertilization in marmosets.

**Methods:** Several stimulation regimens were tested. After estrumate (0.1 ml of 8 µg/ml), ovarian stimulation started on day 1 with 25IU of follicle stimulating hormone (FSH). FSH was increased to 50IU on days 2 and 6 of stimulation to simulate the 2 FSH peaks of a marmoset ovarian cycle. Cetrotide (EMD Serono) (0.01mg/100g body weight) was administered on days 5 and 7 to prevent a premature LH surge. Ovarian follicles were

monitored via ultrasound. hCG was administered on day 9 of stimulation and oocytes were surgically collected 30-32hr post hCG trigger. Mature eggs were inseminated with sperm and immature oocytes were incubated overnight at 38C (P-1 medium, Irvine Scientific, 5% CO<sub>2</sub>, 5% O<sub>2</sub>). Fertilization was assessed on Day 1 post oocyte retrieval, zones were removed, and embryos were incubated with a lentiviral vector (LVV) with GFP driven by an EF1α or CAG promoter. Embryos were incubated for 2 days at 38C (MultiBlast medium, Irvine Scientific, 5% CO<sub>2</sub>, 5% O<sub>2</sub>). Fluorescence was evaluated on Day 4 using a THORLABS Quantalux sCMOS camera. Plasma progesterone was measured via ELISA kit (Cayman Chemical Company).

**Results:** In a successful stimulation, progesterone levels of two females fell from 57.06 ng/ml to 3.37 ng/ml and from >100 ng/ml to 8.23 ng/ml, respectively after estrumate. Oocytes were collected from donors and inseminated on Day 0, or if immature, on Day 1. Embryos fertilized on Day 1 and all inseminated eggs were transferred to 30 µl droplets containing 1 µl LVV or PBS control. Three of five developing embryos in the EF1α-GFP treatment had evidence of expressed GFP on Day 4, while none of the CAG-GFP-treated embryos showed fluorescence greater than controls.

**Conclusion:** This is the first reported use of Cetrotide in marmoset ovarian stimulation. Without Cetrotide and increased FSH doses, fewer oocytes were collected and all were immature. Compared to CAG, the EF1α promoter appeared to be a superior driver for GFP in marmoset embryos. These results represent the first step toward developing a genetically modified marmoset model accessible to the neuroscience community to study neurodevelopmental disease.

## W-219

**Effects of Road Salt Components on Zebrafish Embryo Development and Viability.** Denis Aydin Seli<sup>†</sup>, Hugh Taylor\*. Yale School of Medicine, New Haven, CT, United States.

**Introduction:** During winter, many roads have salt spread on them to prevent them from freezing. Salt can wash into adjacent ecosystems and increase salinity exposure in plants and animals. It has been shown that a drastic increase in salinity can be harmful for most types of plants that are not naturally found in saline environments. In this study, we aimed to investigate if added salinity can also be harmful for animals.

**Methods:** The impact of salts on zebrafish development was investigated in two stages: 1) from the 2-cell embryo stage (0.5 h after fertilization) to the blastocyst stage (2.5 h after fertilization, containing 256 cells); 2) from the blastula stage to a larval stage (6-day-old, freely swimming fish). Control zebrafish embryos were cultured in E3 medium containing 5 mM NaCl, 0.17 mM KCL, 0.33 mM CaCl<sub>2</sub>, and 0.33 mM MgSO<sub>4</sub>. Experiments were conducted using increasing concentrations of each individual salt at 5X, 10X, 50X, and 100X of the concentration found in E3 medium. For each salt and each concentration, 30-50 embryos were assessed. Experiments were performed at Zebrafish Phenotyping Core for Precision Medicine at Yale University. Rates of normal development were measured, and Kaplan-Meier survival analyses were performed.

**Results:** When we assessed development from the 2-cell to blastocyst stage, 5X and 10X NaCl concentrations did not affect embryo development, while 50X and 100X NaCl caused embryonic death (p<0.01). KCL, CaCl<sub>2</sub> and MgSO<sub>4</sub> did not affect early embryo growth at any of the concentrations tested. When we assessed development from blastula to larval stage, NaCl at 5X and 10X concentration resulted in uninflated swim bladder in 12% and 65% of zebrafish, respectively, compared to 4.2% of controls (p<0.01). This indicated a serious developmental abnormality, as the gas-filled swim bladder is necessary to provide the lift that allows fish to attain neutral buoyancy and swim. Higher concentrations of NaCl of 50X and 100X were not compatible with larval development. The other salts tested (KCL, CaCl<sub>2</sub> and MgSO<sub>4</sub>) did not result in uninflated swim bladder or lethal abnormalities in any of the concentrations tested.

**Conclusion:** Increased exposure to certain salts correlated with worsened embryo development in zebrafish. Although further testing is needed, both on zebrafish and other aquatic animals increased salinity can have a negative impact on the development of animals. Our findings indicate that high concentrations of certain types of salt are an environmental

developmental toxicant affecting animals found in roadside ecosystems. Further studies are necessary to characterize molecular changes that may occur in response to increasing concentrations of salt in the environment.

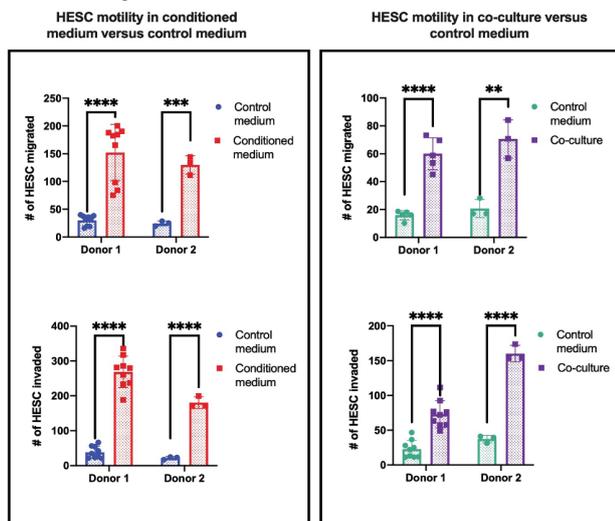
## W-220

**Umbilical Cord-Derived Mesenchymal Stem Cells Modulate Endometrial Stromal Cell Migration and Invasion.** Anat Chemerinski†, Qingshi Zhao, Daniel Cho, Natak Douglas\*, Yahaira Naaldijk, Pranela Rameshwar\*, Sara Morelli\*. *Rutgers New Jersey Medical School, Newark, NJ, United States.*

**Introduction:** The migratory and invasive properties of human endometrial stromal cells (HESC) are critical for cyclic endometrial regeneration, as well as embryo implantation and trophoblast invasion during conception cycles. Mesenchymal stem cells (MSC) in the endometrium have been implicated in facilitating endometrial regeneration and repair, but these cells can only be obtained from biopsy or surgical samples. Human umbilical cord is a rich and accessible source of MSC. The secretome of umbilical cord (UC)-MSC promotes cellular motility and wound healing in nonreproductive tissues, but its effect on HESC function is not well studied. We hypothesized that the UC-MSC secretome upregulates HESC migration and invasion.

**Methods:** confirmed by flow cytometry and multilineage differentiation assays. A commercially available telomerase-immortalized HESC (T-HESC) line was used for motility assays. Transwell migration and invasion assays were performed to determine effect of the UC-MSC secretome on T-HESC motility. For invasion assays, transwell inserts were coated with 0.3 mg/ml Matrigel. T-HESC were seeded in the upper chamber and exposed to UC-MSC conditioned medium (or DMEM + 10% FBS as control) in the lower chamber for 24h. In co-culture experiments, UC-MSC were cultured in the lower chamber for 24 hours (or DMEM + 10% FBS as control), followed by seeding the upper chamber with T-HESC. After 24h, migrated or invaded T-HESC were H&E stained, imaged and quantitated using ImageJ software.

**Results:** Flow cytometry demonstrated that UC-MSC express MSC markers CD44, CD90, CD73 and CD29 and lack expression of CD45 (a hematopoietic marker). UC-MSC exhibited multilineage differentiation into osteocytes and adipocytes. Exposure of T-HESC to the UC-MSC secretome for 24 hours, in both conditioned medium and co-culture experiments, significantly increased T-HESC transwell migration and invasion. Student t-test was used for statistical analysis with  $p < 0.05$  considered significant.



Control medium = DMEM (Dulbecco's Modified Eagle Medium) + 10% FBS  
Donor = UC-MSC donor  
\*\*\*\* =  $p < 0.0001$ ; \*\*\* =  $p < 0.0005$ ; \*\* =  $p < 0.005$

**Conclusion:** Our findings demonstrate that the UC-MSC secretome upregulates HESC migration and invasion, two cellular processes that are required for cyclic remodeling and repair of the endometrium. Given these

findings, and the ability to access UC-MSC without invasive procedures, these studies raise the possibility of a therapeutic application for UC-MSC in disorders of endometrial regeneration.

## W-221

**Transcriptional Regulation Analysis from a Systems Biology Perspective Reveals New Ways in Menstrual Cycle Regulation and Disease.** Antonio Parraga-Leo†, Patricia Sebastian-Leon, Almudena Devesa-Peiro†, José Remohí, Patricia Diaz-Gimeno\*. <sup>1</sup>University of Valencia, Valencia, Spain; <sup>2</sup>IVI Foundation - Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain; <sup>3</sup>IVI-RMA IVI Valencia, Valencia, Spain.

**Introduction:** Prioritized genes in endometrial receptivity are inconsistent between studies and their transcriptional regulators remain poorly known. Our aim was to discover consensus key regulators of endometrial receptivity from previous reported genes to understand its complexity from a systems biology perspective and to deepen in the hormonal and non-hormonal basis of implantation dysfunction.

**Methods:** Endometrial receptivity gene lists were retrieved from transcriptomic datasets searched at Gene Expression Omnibus. Regulators for these gene lists - transcription factors (TFs), miRNAs and progesterone and/or estrogens - were consulted in KEGG, Gene Ontology, Tarbase and Dorothea databases. Relative contributions of each regulation type (Hormonal or non-hormonal, progesterone or estrogens, TFs or miRNAs) were evaluated with Fisher's exact tests for comparing proportions. Consensus TFs and miRNAs regulating most gene lists were validated in independent datasets of 109 and 20 healthy patients biopsied across the menstrual cycle, prioritizing those with significant endometrial gene expression changes in mid-secretory phase (ANOVA and post hoc test). **Results:** Nineteen gene lists from endometrial receptivity studies were collected including 3,608 unique genes (50-818 genes/list). Most of these gene lists (n=17) were regulated in a higher proportion by TFs and showed a high hormonal regulation, 8 of them being significantly more regulated by progesterone than estrogens (FDR < 0.05). The remaining 2 gene lists, previously associated to implantation failure dysfunction, exhibited a lower hormone-dependent behaviour and tended to be more regulated by estrogens and miRNAs (FDR < 0.05). Genes targeted by hormonal regulation were higher than non-hormonal (odds ratio = 91.94, FDR < 0.05). However, we identified a 43.96% of TFs and a 77.27% of miRNAs not associated to ovarian hormones showing a strong non-hormonal regulation in receptivity acquisition. Transcription factor CTCF and 10 miRNAs were validated in an independent patient cohort for regulating a higher proportion of gene lists and for showing a significant expression change in mid-secretory phase (FDR < 0.05).

**Conclusion:** CTCF was newly proposed as the most consensus TF involved in endometrial receptivity. While menstrual cycle progression seemed to be triggered mainly by TFs, gene lists associated previously to implantation failure dysfunction showed less influenced by TFs and ovarian hormones. An appreciable proportion of hormone-independent TFs and miRNAs were identified revealing new ways in menstrual cycle regulation and disease.

## W-222

**Spontaneous Early Embryonic Loss in Wild-Type Mice Is Associated with Dysregulated Uterine and Serum Lipid Profiles.** Sydney L Lane†, William B Schoolcraft, Mandy G Katz-Jaffe\*. *Colorado Center for Reproductive Medicine, Lone Tree, CO, United States.*

**Introduction:** Biochemical pregnancy loss occurs in 12-15% of IVF pregnancies and is an emotional burden for infertility patients. Early pregnancy maintenance relies on proper lipid regulation in the uterus for placental growth and differentiation, steroid hormone and prostaglandin production, and immune factors that contribute to local immunosuppression. We hypothesized that early pregnancy loss correlates with a dysregulated uterine lipidome. We utilized a mouse model to investigate viable pregnancy vs. spontaneous embryo resorption.

**Methods:** Wild-type CF-1 mice were bred and embryonic day (E) 10.5 uterus samples were collected from implantation sites containing either viable embryos (n=5) or embryos that were in the early stage of resorption

and not yet hemorrhagic (n=5). Serum samples were collected from E10.5 dams with 0-1 (n=5) or 5-7 resorptions (n=5). Untargeted lipid profiling was performed on methanol extracts using ultra-high performance liquid chromatography mass spectrometry (MS) in positive and negative ion modes with electrospray ionization. Raw MS data were mined using LipidSearch (Thermo). Significance was determined by Student's t-test and defined as  $P < 0.05$ . Data are presented as fold change (FC) in resorption vs. control groups.

**Results:** Palmitic acid (PA) was elevated in resorptive implantation sites (FC 1.28,  $P=0.023$ ). Elevated PA could hinder pregnancy maintenance, as it induces ER stress in cultured blastocysts, inhibits *in vitro* trophoblast invasion, and activates placental inflammation. A low PA to oleic acid (OA) ratio is important for embryo development and preventing ER stress, but uterine OA levels were unchanged (FC 0.99,  $P=0.96$ ) such that this ratio increased. The SM lipid class, which is pro-apoptotic in the endometrium, was elevated (FC 1.11,  $P=0.008$ ). Serum PG lipids were lower in mice with a high number of resorptions (FC 0.52,  $P=0.008$ ); low PG levels have been associated with repeat implantation failure. Serum bile acids taurocholic acid and taurochenodeoxycholic acid were also lower or trended lower in mice with a high number of resorptions (FC 0.46,  $P=0.030$  and FC 0.35,  $P=0.061$ , respectively). Reduced serum bile acids could result in a loss of protection against detrimental processes that reduce embryo viability.

**Conclusion:** Little is known regarding the molecular mechanisms leading to early pregnancy loss. Metabolomics revealed dysregulated uterine and serum lipidomes associated with spontaneous pregnancy loss in mice. The lipid molecules and classes identified suggest that ER stress, hindered cell-cell communication, and impaired placentation may contribute to early fetal loss in wild-type mice. Ongoing studies will identify changes in upstream lipid regulation and downstream lipid modifications and effectors associated with loss of early pregnancy maintenance.

#### W-223

**Structural and Functional Reorganization of Secretory Organelles in Decidualized Endometrial Stromal Cells.** Marco Dalla Torre, Tiziana Anelli, Paola Panina-Bordignon. *Vita-Salute San Raffaele University, Milano, Italy.*

**Introduction:** Protein secretion is a complex task for a cell: therefore, to be efficient, it requires adaptation of both the Endoplasmic Reticulum (ER) and downstream secretory organelles. This occurs in endometrial stromal cells (EnSC) that after decidualization become *professional secretors*: in the midluteal phase of the menstrual cycle, EnSC change their morphology and start secreting a wide array of proteins required for correct blastocyst implantation. Clarifying the mechanisms of decidualization is essential to get a better insight of human reproduction and associated diseases. Additionally, this inducible model of secretory differentiation offers the opportunity to study transcriptionally regulated and cargo-induced modifications of the secretory organelles.

**Methods:** We studied *in vitro* decidualization of the cell line T-HESC (human endometrial stromal cells immortalized with telomerase) by high resolution microscopy to visualize the morphological changes of the organelles, and by proteomic and transcriptomic analysis. We also studied decidualization in a condition of para-physiological stress: accumulation of unfolded collagen in the ER due to ascorbic acid deprivation.

**Results:** Our data show that decidualized T-HESC recapitulate well the secretory phase of primary EnSC in terms of morphological changes, secretion of soluble mediators and induction of ER chaperones and enzymes, and Golgi complex proteins. Interestingly - and differently from most secretory differentiation models - not only the ER but also the Golgi compartment is significantly enlarged. The transcriptomic analysis revealed distinct clusters of secretion-related genes with a similar expression pattern, likely representing a co-regulation. Decidualization requires a mild activation of the unfolded protein response (PERK pathway), likely to increase the capacity of the secretory organelles. Transcriptomic and proteomic analysis suggest that specific pathways besides the UPR are likely induced to control the enlargement of the Golgi

compartment. Interestingly, ascorbic acid deprivation does not induce a further activation of UPR, suggesting that these cells are programmed to endure high ER stress.

**Conclusion:** Our results indicate that proliferative endometrial stromal cells are already well equipped with folding enzymes and chaperones, likely anticipating their secretory differentiation. Decidualization entails a further upregulation of these proteins, and the massive expansion of secretory organelles, to achieve efficient secretion without full UPR induction.

A full understanding of these molecular mechanisms, that regulate protein secretion in the decidualizing stroma will be clinically relevant to target spontaneous decidualization in a variety of reproductive disorders, including implantation defects, recurrent pregnancy loss, and pregnancy disorders.

#### W-224

**Endometrium >13mm Negatively Impacts Pregnancy Rates Following Transfer of a Single Euploid Embryo.** Tia Y Brodeur<sup>1,2</sup>, Katelyn Tessier,<sup>1</sup> April Batcheller\*.<sup>3</sup> <sup>1</sup>University of Minnesota, Minneapolis, MN, United States; <sup>2</sup>University of Vermont, Burlington, VT, United States; <sup>3</sup>Colorado Center for Reproductive Medicine, Minneapolis, MN, United States.

**Introduction:** Optimization of maternal factors to improve *in-vitro* fertilization (IVF) outcomes has been widely studied. Endometrial thickness has been implicated as a prognostic factor in IVF success, however this has largely been studied in the context of minimum endometrial thickness requirements. Whether there is a threshold at which a thick endometrium becomes detrimental to pregnancy rates has been unclear. Thin endometrium has been associated with lower pregnancy rates following IVF, however, data has been conflicting, and no clear guidelines exist on when embryo transfer may be futile due to thin endometrium. The impact of atypically thick endometrium is even less clear and results have been similarly conflicting.

**Methods:** A retrospective review of IVF cycles performed at a single private institution between 2015-2017 was performed. Patients with normal uterine anatomy who underwent frozen eSET of a euploid embryo as determined by comprehensive chromosome screening were included. Endometrial thickness was measured by transvaginal ultrasound following 10-14 days of estradiol exposure. A total of 235 cycles met inclusion criteria. Pregnancy outcomes were summarized using frequencies and percentages, and compared using Chi-square or Fisher's Exact tests, when appropriate. These were done including and excluding patients with PCOS or endometriosis. Statistical analyses were performed using R 3.4.2. All reported p-values are two-sided and a significance level of 0.05 was used.

**Results:** Specific infertility diagnoses did not significantly impact endometrial lining thickness. Endometrial thickness was grouped into four categories: <8mm, 8-10mm, 10-13mm, and >13mm. Clinical pregnancy rates were 71.4%, 75.3%, 73.2%, and 54.8%, respectively ( $p = 0.172$ ). Patients with an endometrial lining >13mm had statistically higher rates of negative pregnancy tests, 38.7% for >13mm vs 28.6%, 17.6%, and 15.2% for <8mm, 8-10mm, 10-13mm, respectively ( $p=0.029$ ). All four groups had similar biochemical pregnancy rates.

**Conclusion:** In euploid embryos an endometrial lining >13mm negatively impacts rates of positive pregnancy tests. Given differences in patient population and protocols for individual practices, it is possible that a specific measurement (e.g. >13mm) may be less clinically useful than a percentile cutoff (e.g. 95th percentile). Future prospective studies and histological analysis are needed to better understand this association.

#### W-225

**Risk Factors for the Delayed Diagnosis of Interstitial Ectopic Pregnancies.** Christana O Ajewole<sup>†</sup>, Ann Doherty<sup>†</sup>, Joseph Politch, Alexis Gadson<sup>†</sup>, Yeon Woo Lee<sup>†</sup>, Neha Khemani<sup>†</sup>, Christina LeBedis, Wendy Kuohung\*. *Boston University School of Medicine, Boston, MA, United States.*

**Introduction:** Interstitial ectopic pregnancy comprises 5% of ectopic pregnancies but are dangerous given the risk of catastrophic hemorrhage compared to other ectopic pregnancies. The diagnosis of interstitial ectopic

pregnancy is difficult and relies on radiological imaging. We aimed to identify factors associated with delayed or misdiagnosis of interstitial ectopic pregnancies.

**Methods:** All patients who presented to Boston Medical Center (BMC) between January 1, 2012 and April 30, 2019 with an admission or intraoperative diagnosis of interstitial ectopic pregnancy were retrospectively identified by ICD-9/ICD-10 codes for “other ectopic pregnancy” and the BMC Clinical Data Warehouse using the keywords “interstitial ectopic pregnancy” and “cornual ectopic pregnancy.” Data collected included age, gravidity, parity, BMI, last menstrual period (LMP), date of presentation, estimated gestational age (EGA), major medical conditions, history of sexually transmitted infection or pelvic inflammatory disease, prior pelvic surgery, presence of uterine structural abnormalities, adnexal lesions, and smoking status. Continuous variables were analyzed using analysis of variance with post hoc pairwise comparisons and unpaired t-tests, and discrete variables were analyzed using chi squared and Fisher’s exact tests.

**Results:** We identified 53 patients with suspected or verified interstitial ectopic pregnancy. Of these, 15 (28%) were diagnosed correctly by initial ultrasound imaging, 20 (38%) were suspected to have an interstitial ectopic pregnancy on initial imaging but did not have it on final diagnosis, and 18 (34%) were not diagnosed by initial imaging and found to have an interstitial ectopic on subsequent imaging or at surgery. Patient variables that significantly impacted diagnosis accuracy were age and gravidity. The mean age of those with a correct diagnosis was 35 years, while the mean age of patients incorrectly diagnosed was 30 ( $p=0.0045$ , unpaired t-test). Additionally, those with lower gravidity were more likely to have an incorrect diagnosis. The mean gravidity of patients accurately diagnosed was 4.27, while those with an incorrect diagnosis had a mean gravidity of 2.62 ( $p=0.005$ , unpaired t-test). Parity demonstrated a trend similar to gravidity but did not reach significance. No other patient variables analyzed impacted the accuracy of diagnosis including ultrasonographer work shift (day or night).

**Conclusion:** Younger women who have had fewer pregnancies are more likely to have an incorrect diagnosis of interstitial ectopic pregnancy. There may be lower tolerance and resultant lower accuracy of transvaginal ultrasound in those with less reproductive experience. Future studies should examine the level of patient discomfort during radiological imaging and its relation to the diagnostic accuracy of the transvaginal scan.

## W-226

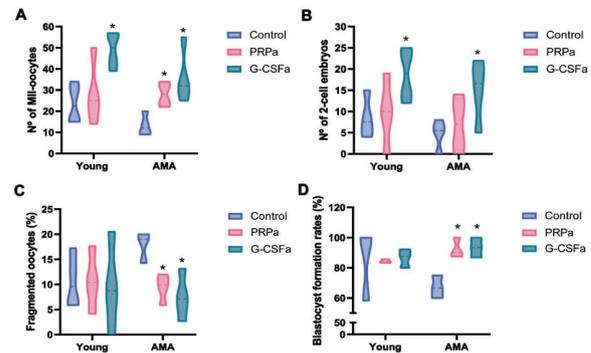
**Plasma Enriched in Stem Cell Secreted Factors Improves Ovarian Function in a Mouse Model of Advanced Maternal Aged.** Maria Marchante†, Anna Buigues†, Jessica Martinez, Antonio Pellicer\*, Sonia Herraiz\*. <sup>1</sup>IVI Foundation-University of Valencia, Valencia, Spain; <sup>2</sup>IVI Foundation-IIS la Fe, Valencia, Spain; <sup>3</sup>IVIRMA, Rome, Italy.

**Introduction:** Tissue regeneration and repair in aged organisms have been described after the exogenous administration of platelet-rich plasma (PRP) and plasma-specific proteins. Adult stem cells produce and secrete a broad variety of growth factors that could have regenerative properties, acting in a paracrine manner. Thus, we aimed to analyze if the administration of plasma enriched in both stem cell secreted factors and platelet enclosed growth factors could improve the ovarian function in a mouse model of advanced maternal age (AMA).

**Methods:** Young (8 weeks old,  $n=12$ ) and AMA (28 weeks old,  $n=12$ ) NOD/SCID females were randomized to receive intraovarian injection (10  $\mu$ L/ovary) of: saline solution (control group,  $n=4$ ), standard activated PRP (PRPa group,  $n=4$ ), or activated plasma from aphaeresis previously enriched in bone marrow derived stem cell secreted factors by a granulocyte colony stimulating factor (G-CSF) mobilization treatment (G-CSFa group,  $n=4$ ). Plasmas were obtained from poor responder women and activated with 5% calcium chloride 0.1M to release additional platelet enclosed factors. A week after treatment, animals underwent ovarian stimulation and mated with males. Mice were sacrificed 36h after mating, to collect Metaphase-II (MII) oocytes and embryos for further culture to the blastocyst stage.

**Results:** G-CSFa increased the number of MII oocytes and 2-cell embryos in both young and AMA model (Fig. 1A-B) when compared to controls.

Moreover, G-CSFa injection was also able to reduce the percentage of oocyte fragmentation in the AMA model to those levels observed in the young control mice (Fig. 1C), and increase the blastocyst formation rate to 94% compared to 66% in the AMA controls (Fig. 1D). Furthermore, embryos from the AMA G-CSFa-treated mice reached the hatched blastocyst stage in a higher percentage (Control:  $46\pm 6\%$ , PRPa:  $50\pm 11\%$ , G-CSFa:  $63\pm 1\%$ ; Control vs. G-CSFa  $p=0.01$ ). Positive effects were also induced by PRPa injection in the AMA model for the total amount of MII-oocytes (Control:  $13\pm 5$  vs. PRPa:  $28\pm 5$ ,  $p=0.01$ ), percentage of oocyte fragmentation (Control:  $18\pm 3\%$  vs. PRPa:  $9\pm 3\%$ ,  $p=0.02$ ) and blastocyst formation rate (Control: 66%, PRPa: 92%), although were not as relevant as those described for the G-CSFa.



**Figure 1.** Effects of human plasma enriched in stem cell secreted factors (G-CSFa). Number of MII-oocytes (A), 2-cell embryos (B) and percentage of fragmented oocytes (C) recovered from young and AMA mice after plasma treatments. Blastocyst formation rates (D) registered after in vitro culture of 2-cell embryos from different plasma treated-group. \*  $p < 0.05$  vs. control group.

**Conclusion:** G-CSFa plasma ovarian injection improves ovarian function in old mice, increasing the number and quality of MII oocytes, as well as embryo development. Support: PROMETEO/2018/137

## W-227

**Generation of a Maternal Haploinsufficient *Akap13* Allele in Mice via Oocyte-Specific Cre-Lox Recombination Does Not Impair Fertility.** Joshua T Brennan,<sup>1</sup> Jia Huang,<sup>2</sup> Kamaria C Cayton Vaught,<sup>1</sup> Carter M Owen,<sup>1</sup> Kimberlyn M Baig-Ward,<sup>1</sup> Hisashi Koide,<sup>3</sup> James H Segars\*. <sup>1</sup>Johns Hopkins University School of Medicine, Baltimore, MD, United States; <sup>2</sup>The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China; <sup>3</sup>Chiba University Graduate School of Medicine, Chiba, Japan.

**Introduction:** Folliculogenesis requires coordination of maturation between the oocyte and surrounding granulosa cells. We found that a homozygous deletion of A-kinase anchoring protein 13 (*Akap13*) resulted in an embryonic lethal phenotype, but female mice with a heterozygous deletion of the *Akap13* gene had smaller sized litters, suggesting an impairment of fertility. Additionally, when siRNA directed against *Akap13* was injected into oocytes, results suggested a release of meiotic arrest resulting in premature oocyte activation. Since *Akap13* was expressed in both granulosa cells and oocytes, we sought to determine the mechanism of fertility impairment in the mice by creating a mouse model with conditional knockout of *Akap13* solely in oocytes.

**Methods:** A Cre-lox recombination strategy was utilized where LoxP sites were inserted into the *Akap13* gene, flanking exons 24 and 25, to create *Akap13*<sup>fllox/fllox</sup> mice in a C57BL/6J background. Mice with floxed alleles were mated with *Gdf9-Cre*-expressing mice. Genotypes were determined by sequencing ear or tail tissue samples. Female mice containing either *Gdf9-Cre+ / Akap13*<sup>fllox/fllox</sup> or *Akap13*<sup>fllox/fllox</sup> were mated with wild-type male mice. Outcomes included litter sizes, sex of offspring, and time between litters. Up to six litters were analyzed in a minimum of five individual mating pairs. Cre excision of the *Akap13* gene was confirmed by PCR. Statistical significance was determined by Mann-Whitney or two-tailed, unpaired t-test with  $p < 0.05$ .

**Results:** Analysis of offspring revealed that Cre excision of the *Akap13* allele was 100% efficient and resulted in 157 offspring with an allele lacking exons 24 to 25 in the *Akap13* gene, consistent with a maternal

haploinsufficient allele. Litter sizes varied slightly among mating pairs but averaged 7-8 pups per litter. There were no differences in litter sizes between females with genotypes of *Gdf9-Cre+/Akap13<sup>lox/lox</sup>* or *Akap13<sup>lox/lox</sup>* mice lacking *Gdf9-Cre* (n=5; p=0.973). Similarly, the sex of male and female offspring did not differ between the 21 *Gdf9-Cre+/Akap13<sup>lox/lox</sup>* and 24 *Akap13<sup>lox/lox</sup>* matings. Time between litters was slightly reduced (n=5; p=0.059) at the second litter in the *Gdf9-Cre+/Akap13<sup>lox/lox</sup>* mice, suggesting a possible reduction in cycle length.

**Conclusion:** In this murine model with oocyte-specific targeted mutation of the *Akap13* gene, we observed no decrease in litter size indicating the excised GEF region of *Akap13* may not be required for oocyte competence or fertility. We did observe a subtle reduction in times between litters, suggesting the possibility of altered follicle dynamics in these mice that begs further study.

## W-228

**Anti Mullerian Hormone (AMH) Is Not Increased in Women with Spontaneous Conceptions at Highly Advanced Reproductive Age 43-47y.** Keren Rotshenker-Olshinka, Jennia Michaeli, Naama Srebnik, Arnon Samueloff, Sophie Magen, Rivka Farkash, Talia Eldar-Geva. *Shaare Zedek Medical Center, Jerusalem, Israel.*

**Introduction:** Fertility decreases with advanced maternal age, and spontaneous conceptions (SC) are uncommon  $\geq 43$ y. Ovarian aging and decrease in oocytes number and quality is the suggested underlying etiology. AMH, the main biomarker for ovarian reserve is decreased during pregnancy, gradually recovering postpartum (PP), but time to full recovery is unknown. We aimed to determine if greater fertility potential in women  $\geq 43$ y with SC is related to increased AMH levels. We also assessed AMH recovery PP.

**Methods:** We conducted a Prospective cohort study, 2015-2018, IRB approved. Informed consent was obtained. Women 43-47y with SC were tested for AMH 1-4d and 3-11 months PP. AMH 3-11m PP was compared with AMH in healthy age-matched women who last gave birth  $\leq 42$ y. Excluded -women with current use of hormonal contraceptives (CHC), ovarian insult, and PCOS. Peripheral blood AMH was measured via Elecsys AMH fully automated assay (Roche; Elecsys). The sample size was supported by power analysis. Data was analyzed by Student's t-test, Mann-Whitney U test, Chi-square test and Spearman's correlation as appropriate.

**Results:** 98 women included, 40 case, 58 control, mean age 44.8 ( $\pm 1.3$ ) y. Median AMH was similar in both groups (0.5 vs 0.45ng/ml p=0.51). This remained when analyzing by age ( $\geq < 45$ y) (Table). No correlation was found between age and AMH within the cases (r=0.017 p=0.92); a weak negative correlation was found within the controls (r=-0.23 p=0.08). Maternal age, age at menarche, past CHC use or history of fertility concern were similar between the groups. Parity differed but showed no significant effect on AMH (linear model). AMH was significantly higher 3-11mPP (0.5 [0.21-1.23]) than 1-4dPP (0.18 [0.06-0.4]) p<.001. The two results were highly correlated (R= 0.82; p<0.001).

**Conclusion:** Ovarian reserve measured by AMH is not higher in women with SC at highly advanced reproductive age and is not a hallmark for their reproductive potential; AMH measures oocytes quantity but may not reflects their quality. AMH suppression associated with pregnancy resides 1-4dPP, showing significant recovery 3-11m PP. This should be considered when timing AMH sampling.

Demographics and results, Mean (SD) Median (IQR) n(%) as appropriate			
	Case	Control	P value
Age y	44.75 $\pm$ 1.3	44.86 $\pm$ 1.3	.67
AMH ng/ml	0.5 (0.21-1.23)	0.45 (0.16-0.89)	.5
<45	0.44 (0.2-1.1)	0.63 (0.26-1.6)	.5
$\geq 45$	0.56 (0.2-1.54)	0.34 (0.13-0.66)	.18
Gravidity	11.7 $\pm$ 5.2	6.3 $\pm$ 3.6	<.001
Parity	9.25 $\pm$ 4	4.9 $\pm$ 2.7	<.001
Miscarriage rate	17%	14%	.23
Caesarian	0.87 $\pm$ 1.3	0.3 $\pm$ 0.8	.004
Living Children	9.2 $\pm$ 3.9	5 $\pm$ 2.8	<.001
Smoking	0	2 (3.5%)	.34
Age at menarche	13.4 $\pm$ 1	13.1 $\pm$ 1.3	.13
Past CHC	15 (38.5%)	29 (50%)	.3
Past Fertility concern	8 (20.5%)	13 (22.8%)	.79

## W-229

**Dysfunctional Multidrug Resistance Transporter-1 (MDR1) Associated with Premature Diminished Ovarian Reserve and Metabolic Syndrome.** Dalileh Nabi<sup>†</sup>, Zijing Zhang<sup>†</sup>, Lynae Maria Brayboy<sup>\*,3,4,5</sup> <sup>†</sup>*Technische Universität Dresden, Dresden, Germany, Germany;* <sup>2</sup>*University of Arkansas for Medical Sciences, Little Rock, AR, United States;* <sup>3</sup>*Clue by Biowink, Berlin, Germany, Germany;* <sup>4</sup>*Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany;* <sup>5</sup>*Alpert Medical School of Brown University, Providence, RI, United States.*

**Introduction:** Mitochondrial dysfunction has been implicated in several pathophysiological processes such as aging, diabetes, obesity, and infertility (Chappel 2013). Mitochondria are essential for oocyte physiology. Our group has shown that multidrug resistance transporter protein-1 (MDR1) is expressed on the mitochondrial membrane and is important for oocyte mitochondrial homeostasis.

**Methods:** To assess the link between oocyte mitochondrial physiology and MDR1 we isolated oocytes from oviducts of *mdr1a* mutant mice at estrus and their internal organs.

**Results:** *Mdr1a* mutant mice have a higher yield of ovulated oocytes per cycle at 2 months (Clark et al 2019). However, at 7 months the oocytes were significantly reduced by 50% (wild type n=11 vs *mdr1a* mutant n=6, p<0.05). Previously we reported that *mdr1a* mutant pups weight is higher at PND 21 & 42 compared to wild types (Clark et al 2019). Now, at 2 months the wild type and *mdr1a* mutant mice present a difference in internal organ weights. The weights were the following (wild type vs mutant): liver weight= 1.98 mg vs 2.36 mg, p<0.05, heart weight= 1.3 mg vs 1.6 mg, p<0.05 and spleen weight= 1.3 vs 1.9 mg, p<0.001. However, after aging the mutants perpetually had higher liver weights at 6 months (1.3 mg vs 2.1 mg, p<0.001). Moreover, *mdr1a* mutant ovaries contain higher free cholesterol levels compared to wild type (0.13 ug/ul and 0.12 ug/ul, p<0.01, respectively).

**Conclusion:** Altered metabolism is associated with reactive oxygen species (ROS) (Forrester et al 2019). Oocytes are exposed and vulnerable to excessive ROS as they age (Sasaki et al 2019) and *mdr1a* mutants have high ROS in GV oocytes (Clark et al 2019). Mitochondria DNA (mtDNA) is susceptible to damage because it lacks DNA repair genes (Garcia-Lepe et al 2019) and it has been established that the mutated mtDNA is associated with abnormal metabolism. We speculate that MDR1 function is important for mitigating premature aging by efflux of toxic metabolites/ROS. These data show a role of MDR-1 in oocyte mitochondrial physiology and ovarian reserve maintenance. The future directions will be to analyze mtDNA in mutants and to assess metabolic dysfunction such as diabetes, dyslipidemia and cardiovascular disease via phenotype testing. The translational next step is to survey MDR1 mutation

in a genomic database and associated fertility outcomes. In conclusion, our mouse model is well-suited for studying ovarian reserve in the context of chronic diseases due to aberrant metabolism.

### W-230

**Single Cell RNAseq Analysis Identifies Previously Unacknowledged Populations and Across-Cycle Transcriptomic Changes in Immune Cells in Healthy Human Endometrium.** Wanxin Wang<sup>†</sup>, Juan Irwin, Linda Giudice\*. *University of California, San Francisco, San Francisco, CA, United States.*

**Introduction:** Resident and infiltrating immune cells play essential roles in cycling human endometrium tissue homeostasis and as sentinels of local infection. Recent single cell (sc) RNAseq analysis has provided across-cycle transcriptomic profiles of diverse endometrial cell types, including those from both lymphoid (L) and myeloid (M) lineages. Unbiased analysis on endometrial immune hierarchy beyond the L-M dichotomy, however, has yet to be performed systematically. Herein, we provide single cell level definition of immune cellular hierarchy and across-cycle transcriptomic changes in healthy human endometrium.

**Methods:** Analyses were conducted using custom R scripts and the Seurat package on a published dataset where scRNAseq via Chromium 10x was performed on endometrial biopsies from ten donors (2 proliferative, 8 secretory) with no endometrial pathology in natural menstrual cycles. Cell groups supported by at least four donors were subject to marker discovery. Top cell group defining markers were identified as those with  $p < 0.01$  (Wilcoxon Rank Sum test),  $\log_{10}$  fold change (FC)  $> 0.25$  and were ranked by decreasing  $\log_{10}$  FC. Markers reported in results were the highest ranked for each subtype.

**Results:** We observed four major immune populations that are transcriptomically distinct and consistently present in all samples across the menstrual cycle, including KIT upregulated (KIT<sup>+</sup>), CD3<sup>+</sup>, NCAM1<sup>+</sup> lymphocytes, and AIF1<sup>+</sup> macrophages. CD3<sup>+</sup> and NCAM1<sup>+</sup> lymphocytes were further categorized into three (FGFBP2<sup>+</sup>, CD8<sup>+</sup>, IL7R<sup>+</sup>) and two (CD160<sup>+</sup>, COX6A2<sup>+</sup>) subtypes, each found in all samples with statistically significant markers. Two macrophage subtypes were observed: one (IL1B<sup>+</sup>) was present in all samples; whereas, the other (IL1B downregulated) had dropouts. Intriguingly, we identified two cell groups that were transcriptomically similar to macrophages but with upregulated immunoglobulin machinery - both found in multiple samples, yet with dropouts in at least one menstrual cycle phase. Within-subtype analyses revealed notable across-cycle transcriptomic changes in CD3<sup>+</sup> and NCAM1<sup>+</sup>CD160<sup>+</sup> lymphocytes. Cell cycling activity was observed in CD3<sup>+</sup>CD8<sup>+</sup> lymphocytes and AIF1<sup>+</sup>IL1B<sup>+</sup> macrophages and demonstrated phase-dependent changes. Notably, cross-cycle changes were less pronounced in the previously unacknowledged lymphocytes expressing KIT, the receptor for stem cell factor SCF and whose interactions play essential roles in stem-cell maintenance.

**Conclusion:** Our analyses reveal a more complex immune hierarchy in healthy and cycling human endometrium than previously described, including a previously unacknowledged KIT<sup>+</sup> lymphocyte population. Across-cycle transcriptomic changes observed in immune subsets revealed additional contributors to cyclic endometrial transformation. Support: NIH P50HD055764

### W-231

**Fibroblasts of the Bovine Corpus Luteum Release Prostaglandin F2 $\alpha$  into the Microenvironment in Response to Pro-Inflammatory Cytokines.** Corrine F Monaco<sup>†</sup>, John S. Davis\*. *University of Nebraska Medical Center, Omaha, NE, United States.*

**Introduction:** The ovarian corpus luteum (CL) is a transient endocrine gland that produces the progesterone required for embryo development, implantation, and maintenance of pregnancy. In the absence of an embryo, luteal progesterone secretion is disrupted and the CL regresses to form a fibrotic corpus albicans. Prostaglandin F2 $\alpha$  (PGF2 $\alpha$ ) has been implicated as a major luteolytic hormone in many species, including domestic farm animals, primates, and humans. PGF2 $\alpha$ -induced regression in cows rapidly elevates the expression of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF) and interleukin 1 $\beta$  (IL1B). In addition to

steroidogenic cells, the CL contains vascular endothelial cells, pericytes, and fibroblasts. While the exact role of luteal fibroblasts is unknown, previous studies show that luteal fibroblasts produce components of a fibrotic matrix. We hypothesize that luteal fibroblasts respond to pro-inflammatory cytokines and alter the microenvironment by producing additional luteolytic factors that contribute to successful luteal regression.

**Methods:** Fibroblasts were isolated from bovine CL tissue punches, passaged 2-3 times, and treated with 10 ng/mL of TNF or IL1B to examine early intracellular signaling responses and production of PGF2 $\alpha$ . **Results:** Western blot analysis showed significant increases in NF $\kappa$ B (p65) (8-fold,  $p < 0.05$ ,  $n = 6$ ), and p38 MAPK (5.6-fold,  $p < 0.001$ ,  $n = 6$ ) phosphorylation by 10 minutes of TNF treatment. TNF increased phospholipase A2 phosphorylation nearly 2-fold ( $p < 0.05$ ,  $n = 4$ ) after 4 hours of treatment and prostaglandin-endoperoxide synthase 2 (PTGS2) protein expression 2-fold after 12 and 24 hours ( $p < 0.05$ ,  $n = 4$ ). TNF $\alpha$  also induced a 42-fold increase in PGF2 $\alpha$  production ( $n = 2$ ). Responses to IL1B were not as robust as TNF, but p65 phosphorylation was increased 2-fold after 10 minutes ( $n = 6$ ,  $p < 0.05$ ) and p38 phosphorylation increased 2.4-fold after 30 minutes ( $p < 0.05$ ,  $n = 6$ ). IL1B also increased PTGS2 expression (3-fold,  $p < 0.05$ ,  $n = 4$ ) and PGF2 $\alpha$  production 9-fold by 4 hours and 28-fold by 24 hours ( $n = 2$ ) of treatment.

**Conclusion:** These results indicate that cytokines implicated in CL regression activate signaling pathways in fibroblasts to stimulate PGF2 $\alpha$  release into the luteal microenvironment, potentially exacerbating regression of the CL.

### W-232

**Physical Activity Throughout Gestation Differentially Regulates CD206 Expression in Term Placenta.** Alexandra D Goudreau<sup>†</sup>, Catherine Everest<sup>†</sup>, Velislava Tzaneva\*, Kristi B Adamo\*. *University of Ottawa, Ottawa, ON, Canada.*

**Introduction:** Physical activity (PA) during pregnancy is associated with health benefits for both mother and fetus, including healthy placental development and reduced risk of prenatal complications. However, the mechanisms through which these benefits arise are yet to be fully understood. In the non-pregnant population, habitual PA induces a phenotypic switch in a range of tissue-resident macrophages, from an M1 state (predominantly secretes pro-inflammatory cytokines) to an M2 state (predominantly secretes anti-inflammatory cytokines). Placenta-resident macrophages, known as Hofbauer cells (HC), reflect the ability of other macrophage subtypes to polarize between M1 and M2 states. While HCs exist in a predominantly M2 state, dysregulations have been associated with adverse health outcomes such as pre-eclampsia, gestational diabetes, and pre-term birth. The effects of PA on HCs have not yet been investigated. This study aimed to examine the relationship between habitual PA throughout gestation and key protein markers of HCs polarization in term placenta.

**Methods:** Pregnant individuals ( $n=22$ ) were recruited through the PLACENTA study in Ottawa, ON, before 28 weeks gestation. Participants wore an accelerometer in both their second (24-28 weeks) and third (34-38 weeks) trimesters for a minimum of four days to objectively assess PA status. Placental tissue and full-thickness histology sections were taken within an hour of delivery from term placenta ( $>37$  weeks gestation). The 11 most and least physically active participants were identified for further analysis. Western blotting was used to examine the protein expression of HC markers CD68, a pan-macrophage marker, and CD206, an M2 macrophage marker. Immunofluorescence was used to quantify the number of CD206<sup>+</sup> cells, indicative of the number of M2 macrophages. **Results:** While CD68 protein levels ( $P = 0.401$ ) and CD206<sup>+</sup> cells ( $P = 0.521$ ) were not significantly different between active and inactive participants, CD206 protein levels were significantly decreased in the physically active group ( $P = 0.0297$ ). Additionally, CD206 levels were negatively correlated with second ( $R^2 = 0.272$ ;  $P = 0.013$ ) and third ( $R^2 = 0.247$ ;  $P = 0.019$ ) trimester moderate to vigorous PA (MVPA).

**Conclusion:** Increased expression of CD206 has been associated with a wide range of detrimental conditions, including chronic obstructive pulmonary disease, myeloma, and cirrhosis, as well as exposure to pathogens and toxins. The results of this study suggests that although

the number of M2 HCs remains consistent between physically active and inactive individuals, PA may initiate a mechanism downregulating CD206 expression within HCs while maintaining the number of M2 HCs present in term placenta. This mechanism and its significance in fetal outcomes should be investigated in future research.

### W-233

**Vascular-Tissue Trafficking of Decidual Innate Lymphoid Cells.** Jessica Vazquez†, Payton Lindner, Yan Li, Aleksandar K Stanic\*. *University of Wisconsin-Madison, Madison, WI, United States.*

**Introduction:** Innate lymphoid cells (ILCs), including decidual NK cells, have emerged as important players in mucosal defense, tissue homeostasis and decidual remodeling. Recent discovery of significant ILCs lineage complexity at the maternal-fetal interface, lead to a pressing question - which ILC subsets traffic between systemic vasculature and decidual stroma? To address this question we used sequential, sub-saturating intravascular labeling of ILCs, and addressed lineage diversity by genetic Tbet fate map.

**Methods:** Timed mating of female C57BL/6J (wild-type) and B6.Rosa-TdTomato/Tbet<sup>Cre</sup> (Tbet fate mapper) mice (6-13 weeks) were performed (vaginal plug as gestational day ~ GD 0.5). Mice were tail-vein injected with 6.7 µg each of A488-conjugated (at 24h) and A647-conjugated anti-CD45 antibody (at 5 minutes) before sacrifice (Figure 1). Virgin uteri and mouse decidua (GD 12-17) mononuclear cells (MCs) were isolated by mechanical (GentleMACS) and enzymatic (Collagenase, DNase) disruption. MCs were labeled with fluorescent antibodies to CD3e, 11c, 19, 45 (PE-Cy5), Ly6G, and TCRβ. Data was acquired using BD Fortessa flow cytometer and analysis was performed using FlowJo 10.2. Vascular status amongst all CD45-PE-Cy5+ ILCs was determined as: A488+A647+ (always vascular), A488+A647- (vascular only at 24-hours), A488-A647+ (vascular only at 5-min), and A488-A647- (non-vascular). Statistical significance was determined by Likelihood Ratio Chi-square tests followed by Wilcoxon multiple comparisons tests.

**Results:** First, non-vascular ILCs decreased, while exclusively vascular ILCs proportionally increased with gestational age ( $p < 0.0001$ ). ILCs that exited the tissue at or after the 5-min injection remained at a constant proportion, while cells that entered tissues after the 24-hour injection decreased upon pregnancy. Furthermore, a greater proportion of non-vascular ILCs were Tbet fate mapped in both the decidua and the virgin uterus.

**Conclusion:** Assessing immune cell trafficking across gestation is an understudied area of decidual immunology. Our data indicates increasing vascular-tissue traffic of ILCs with advancing gestation. Furthermore, Tbet expression history is implicated in determination of tissue entry/residency. Future studies will determine whether dynamic Tbet regulation is necessary for acquisition of permanent tissue residency.

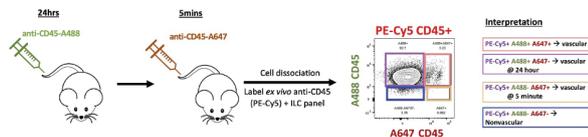


Figure 1 Experimental workflow for intravascular labeling of Innate Lymphoid Cells (ILCs) in murine virgin uterus and decidua (GD 12-17).

### W-234

**One-Sided Chronic Intervillositis of Unknown Etiology in Dizygotic Twins: A Description of 3 Cases.** Manon Bos,<sup>1</sup> Lotte van der Meeren,<sup>1</sup> Juliette Krop,<sup>1</sup> Kyra Dijkstra,<sup>1</sup> Kitty Bloemenkamp,<sup>2</sup> Emily Cornish,<sup>3</sup> Peter Nikkels,<sup>2</sup> Marie-Louise van der Hoorn.<sup>1</sup> *<sup>1</sup>Leiden University Medical Center, Leiden, Netherlands; <sup>2</sup>University Medical Center Utrecht, Utrecht, Netherlands; <sup>3</sup>University College London, London, United Kingdom.*

**Introduction:** Chronic intervillositis of unknown etiology (CIUE) is a rare, poorly understood, histopathological diagnosis of the placenta that is frequently accompanied by adverse pregnancy outcomes including miscarriage, fetal growth restriction and intrauterine fetal death. CIUE is thought to have an immunologically driven pathophysiology and may be

related to human leukocyte antigen mismatches between the mother and the fetus. Dizygotic twins with one-sided CIUE provide an interesting context to study the influence of immunogenetic differences in such cases.

**Methods:** Dizygotic twin cases were selected from the pathology registry between 2000 and 2015 of a tertiary hospital (University Medical Center Utrecht). CIUE was defined as an infiltrate occupying at least 5% of the intervillous space with approximately 80% of CD68-positive mononuclear cells, in the absence of infection.

**Results:** We identified 3 dizygotic twin pregnancies in which CIUE was present in only 1 of the 2 placentas. Two of the pregnancies ended in term delivery and 1 ended in preterm delivery. Presence of CIUE was correlated with lower placental weight and lower birthweight when compared with the co-twin whose placenta was unaffected by CIUE.

**Conclusion:** The presence of one-sided CIUE in dizygotic twin pregnancy is associated with selective growth restriction in the affected twin. This suggests a unique fetal immunogenetic contribution to the pathogenesis of CIUE. Further study of dizygotic and monozygotic placentas affected by CIUE could identify new insights into its pathophysiology and into the field of reproductive immunology.

### W-235

**Inhibition of MLL1 and HDAC Activity Reverses Reprogrammed Inflammatory Components Induced by Developmental Exposure to an Endocrine Disruptor (Diethylstilbesterol) in Myometrial Stem Cells.** Mohamed Ali†,<sup>1</sup> Hoda ElKafas†,<sup>2</sup> Nahed Ismail\*,<sup>2</sup> Ayman Al-Hendy\*,<sup>3</sup> Qiwei Yang\*,<sup>3</sup> *<sup>1</sup>Ain Shams University, Cairo, Egypt; <sup>2</sup>University of Illinois at Chicago, Chicago, IL, United States; <sup>3</sup>University of Chicago, Chicago, IL, United States.*

**Introduction:** Inflammation is linked to development of many diseases including tumorigenesis. Previously, we showed that adverse developmental environmental exposures to endocrine disruptor diethylstilbesterol (DES) epigenetically program myometrial stem cell (MMSCs) towards a pro-inflammatory profile and thus create a chronic inflammatory milieu in myometrium (MM), eventually leading to uterine fibroid (UF) development. However, the mechanism responsible for initiation of this persistent (DES)-induced epigenetic alteration is unknown

**Methods:** Female newborn *Tsc2*-mutant Eker rats were treated S.C. with vehicle (VEH) or 10 µg/kg of DES, on postnatal days 10-12 (key period of uterine development). SCs were isolated from MM tissue (N=5/group) using Stro-1 and CD44 surface markers at adult age (5 months). Whole genome RNA-sequencing and ChIP-sequencing (with anti-H3K4me3 antibody) were performed in DES- and VEH-MMSCs. qRT-PCR was performed to confirm the differential gene expression. knockdown (KD) of *Tasp1* enzyme responsible for activation of MLL1 and thus methylation of H3K4 was performed using 3 lentiviral particles. DES-MMSCs were treated with histone decetylase (HDAC) inhibitor HDACiVIII at 2.5-5 µg/ml for 24&48 hr. Differentiated MM cells were exposed to secretome from VEH/DES-MMSCs. Western blot analysis was performed

**Results:** Our previous RNA-seq data showed that 28.3% of inflammatory responsive genes (IRGs) were upregulated in DES- vs VEH-MMSCs including key cytokine genes that contribute to recruitment of macrophage as *Ccl2*, *Ccl7*, *lpar1* and *pdpn*. By ChIP-seq, we identified 66 of 123 IRGs with greater enrichment of H3K4me3 at their promoters in DES- vs VEH-MMSCs. the higher expression of IRGs was positively correlated with the elevated H3K4me3 mark ( $p < 0.05$ ). In this study, using qPCR, several cytokines including interleukins (ILs) 1a, 1b, 6, 17 and TNF- $\alpha$  showed significant upregulation in DES- vs VEH-MMSCs. ( $p < 0.05$ ). DES-MMSCs secretome significantly increased expression of aforementioned ILs and reprogrammed IRGs in the surrounding differentiated MM cells ( $p < 0.05$ ). HDACi significantly reversed the expression of reprogrammed genes *Ccl2*, *Ccl-7*, *lpar1* and *pdpn* while increasing the protein expression of IL-1 receptor antagonist by qPCR and WB respectively. *TASP1* KD resulted in similar effects of HDACi treatment.

**Conclusion:** These data suggest that developmental exposure to DES alters the inflammatory microenvironment in the MM and increases the risk of adult onset of UFs by reprogramming pro-inflammatory genes in MMSCs. Intervention by HDACi and *TASP1* KD are capable of reversing the DES-induced activated pro-inflammatory pathways in MMSCs.

**W-236**

**Using Live Imaging and Cell Cycle Indicator Embryonic Stem Cells to Predict Dose-Dependent Suppression of Leading Indicators of Growth by PFOA and DEP.** Daniel A Rappolee\*, Mohammed Abdulhasan\*, Ximena Ruden\*, Douglas M Ruden\*, Elizabeth E Puscheck\*. *Wayne State University, Detroit, MI, United States.*

**Introduction:** Environmental toxicants affect pregnancy and pre- and post-natal health effects occur tens of billions of health care costs in the US every year. A means to test single and mixtures of many toxicants and to predict toxic levels is needed. We report here a high throughput screen (HTS) using transgenic embryonic stem cells (ESC) cultured in a live imager that records time lapse growth and cell cycle progression. These ESC predict future growth deficits as well as past decreased cell accumulation.

**Methods:** Mouse fluorescence ubiquitinated cell cycle indicator (FUCCI) ESC were tested with zero stress in normal stemness (NS) media, or with increasing levels of two environmental toxicant families: PFAS (PFOA/perfluorooctanoic acid) and phthalates (DEP/diethyl phthalate). ESC were cultured for 72hr with daily media change in a Sartorius Incucyte zoom or a Biotek Cytation 5 live imager and assayed in time lapse every 2hr for confluence and green fluorescence. Green fluorescence is an indicator that the cells are past G1 (red) and into S-G2-M-phase (green) parts of the cell cycle. Cyclicity of G1 delay before media change and release into S-G2-M-phase was established. The suppression of the rapid green peak after media change by stress was validated for quantitation of toxicant levels that suppress cell cycle progression.

**Results:** It was observed that the fraction of FUCCI ESC in green decrease over time from 12-24hr after the previous feeding and then rapidly enter S-G2-Mphase of cell cycle after media change as a leading indicator that predicts future cell division. The fed/unfed fold change increase in green cell cycle advancement ranged from 4.5 on day 1 to 6.75 on day 2 during NS ESC culture. However, when ESC were cultured with NS media and 100uM PFOA these fold change green peaks were suppressed to 1.3 and 4.52, respectively. DEP also significantly decrease fed/unfed increase in green, but only on day 1 and only from 4.5 to 3.0.

**Conclusion:** Suppression of entry into S-G2-M-phase strongly by PFOA and weakly by DEP after media change was a predictor of stressful levels of environmental toxicant that can be used to screen single and mixed environmental and hormonal stressors in high throughput. Human FUCCI pluripotent stem cells should be validated for HTS use.

**W-237**

**Towards the Development of the Bioengineered Ovary: Comparative Proteomic Analysis of Different Extracellular Matrix Hydrogels.** Hannes Campo†,1,2 Emilio Francés-Herrero†,2,3 Sara López-Martínez†,2 Amparo Faus,2 Antonio Pellicer\*,4 Irene Cervelló\*.2 <sup>1</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States; <sup>2</sup>IVI Foundation-IIS La Fe, Valencia, Spain; <sup>3</sup>Universitat de València, Valencia, Spain; <sup>4</sup>IVIRMA-Rome, Rome, Italy.

**Introduction:** Research aimed at preserving female fertility is increasingly using tissue engineering techniques to develop new biomimetic platforms capable of supporting ovarian cell function *in vitro* and *in vivo*. In the particular case of *in vitro* folliculogenesis, leading edge research highlights the important role of the native extracellular environment. For this study, we did an extensive proteomic analysis and compared three different tissue-specific bovine ovarian extracellular matrix (ECM) hydrogels with the human ovarian matrisome. Establishing a suitable xenogeneic ECM hydrogel would provide a cheap, valuable and versatile biomaterial for translational research.

**Methods:** Ovarian cortex hydrogels made from acellular tissues previously characterized by our group (n=9) and obtained using three different detergents-based protocols (Sodium dodecyl sulfate (SDS), P1; Sodium deoxycholate (SDC), P2 and Tri-n-butyl phosphate (TnBP), P3) were subjected to a mass spectrometry analysis (LC-MS/MS). In a subsequent bioinformatics study, the protein profiles of these hydrogels were compared with each other, a non-decellularized hydrogel and with published data of the human ovarian proteome.

**Results:** Data showed an important enrichment in extracellular proteins after P1 and P2 (83.3% and 76.6%, respectively), which was lower in P3 (56.97%) (P<0.05). When compared against the Matrisome Project database, we were able to identify 23, 17 and 37 matrisomal proteins after P1, P2 and P3, respectively, with 69.5%, 70.5% and 75% of them found in human ovaries. While P2 had a milder effect on basement membrane members COL4A1 and COL4A2, P1 maintained ECM glycoproteins with crucial roles in ovarian function (EMILIN-1, LAMA5, LAMB2) and ECM-Regulators (TGM2).

**Conclusion:** The proteomic analysis of different ovarian extracellular matrix hydrogels places SDS and SDC-based protocols as good candidates to obtain functional supports that could be used for *in vitro* folliculogenesis. P3 was excluded, as it did not efficiently decellularize the ovarian cortex. Specifically, P1 seems to have a minor effect on ovarian matrisomal proteins, possibly providing the best native extracellular environment for future translational research. Interestingly, previous histological and molecular characterizations performed by our group support these findings. Therefore, we propose that buffered solutions with low concentrations of SDS as the best option to generate decellularized tissues and hydrogels to be used for follicle culture.

HC and EF contributed equally.

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**W-238**

**Microenvironment or Microstructure: Potential Role of Acellular Lesion's in the Pathogenesis of Endometriosis.** Masoumeh Majidi Zolbin†,1 Ashkan Azimzadeh Fardkhatoni,1 Roxana Sahmani,1 Ameneh Haghgoo,2 Ali Mohebbi,1 Abdol-Mohammad Kajbafzadeh.1 <sup>1</sup>Tehran University of Medical Sciences, Tehran, Iran, Islamic Republic of; <sup>2</sup>Nikan Hospital, Tehran, Iran, Islamic Republic of.

**Introduction:** The endometriosis (EMS) pathogenesis and its outcome appears to be associated with multiple factors, less attention was focused toward the extracellular matrix (ECM) of endometriosis lesions and their potential role in the progression of disease moreover, there are well-documented confirmations represent the key role menstrual blood stem cell (MenSCs) in the pathogenesis of endometriosis. Using the matrices of decellularized (DEC) lesion as a 3D-organotypic approach, from native human ECM source, with MenSCs transplantation, will provide new insights into the complex crosstalk established at the lesion microenvironment.

**Methods:** Specimens were collected from the patients in the fertility age and DEC based on the established protocol. EMS model induced by implantation of DEC scaffold of ectopic lesion and transplantation of MenSCs in the mouse model. H&E, Trichrome, and DAPI staining, as well as scanning electron microscopy (SEM), conducted to examine the preservation of ECM structure and the presence of a nucleus. Cell viability and scaffolds toxicity measured by MTT assay. The tensile strength conducted to measure the biomechanical properties of each group. Cell proliferation potential, immune regulation, MenSCs engraftment, and angiogenesis on the ECM of implanted scaffold determined by immunofluorescence staining.

**Results:** Microscopic evaluation of specimens revealed that decellularization was successfully removed from the cells. SEM confirmed the ECM structure preservation following the decellularization process. MTT assay findings revealed that the percentage of cell survival was greater than 85%. It was presented that decellular lesions bio-scaffold is a collagen-rich matrix with significant mechanical properties. Among mice implanted with the DC-ECM EMS+ MenSCs versus DC-ECM of non -EMS+ MenSCs there was a significant difference in the amount of stem cell engraftment (p<0.004). There was no significant engraftment in animals with no stem cell treatment in both groups. Stem cell treatment with DC-ECM EMS+ MenSCs led to significant increases in angiogenesis markers CD31 and CD34 and proliferation factor (PCNA) compared to the DC-ECM of non -EMS+ MenSCs group (p<0.02 and p<0.003, respectively). This project is still ongoing and additional results will be updated in near future.

**Conclusion:** Based on the findings of this study, we would be able to understand the crosstalk of ECM and stem cells in the trafficking toward the lesion and the role of matrix in the development of disease based on that we can improve the therapy to block those factors recruitment or at least a therapy that can cease the inflammation.

#### W-239

##### **On Prognosis after Unexplained Recurrent Pregnancy Losses (RPL); a Systematic Review and External Validation of Clinical Prediction Models.** *Angelos Youssef†. LUMC, Leiden, Netherlands.*

**Introduction:** RPL remains unexplained in 50-75% of couples. For these couples, there is no effective treatment option and clinical management rests on supportive care. Essential part of supportive care consists of counselling on the prognosis of subsequent pregnancies. Indeed, multiple prediction models exist, however the quality and validity of these models varies. In addition, the prediction model developed by Brigham et al. is the most widely used model, but has never been externally validated. The aim for this study is therefore to identify prediction models for pregnancy outcome in couples with unexplained RPL, to evaluate those prediction models and to identify the performance of the most used prediction model.

**Methods:** A systematic search was performed in December 2020 in Pubmed, Embase, Web of Science and Cochrane library to identify prediction models for pregnancy outcome after unexplained RPL. Eligible studies were selected and assessed according to the TRIPOD (Transparent Reporting of a multivariable prediction model for Individual Prognosis Or Diagnosis) statement, covering topics on model performance and validation statement. In addition, we performed an external validation of the Brigham model in a retrospective cohort, consisting of 668 couples with unexplained RPL that visited the RPL clinic of the Leiden University Medical Centre (LUMC) between 2004 and 2019. The performance of predicting live birth in the Brigham model was evaluated through calibration and discrimination, in which the observed pregnancy rates were compared to the predicted pregnancy rates.

**Results:** Seven models were compared and assessed according to the TRIPOD statement. This resulted in two studies of low, three of moderate and two of above average reporting quality. These studies did not follow the recommended steps for model development and did not calculate a sample size. Furthermore, the predictive performance of neither of these models was internally- nor externally validated. We performed an external validation of Brigham model. Calibration showed overestimation of the model and too extreme predictions, with a negative calibration intercept of -0.52 (CI 95% -0.68 - -0.36), with a calibration slope of 0.39 (CI 95% 0.07 - 0.71). The discriminative ability of the model was very low with a concordance statistic of 0.55 (CI 95% 0.50 - 0.59).

**Conclusion:** We identified seven prediction models; none followed the recommended prediction model development steps. Currently, there are no suitable models that predict on pregnancy outcome after RPL. Moreover, the most used model showed poor predictive performance. Thus, clinical practice in RPL couples is in need of a model with several variables such that prognosis is individualized, and factors from both the female and the male to enable a couple specific prognosis.

#### W-240

**Placental Gross Pathology in a Population Based Case-Control Study of Autism Spectrum Disorder.** *Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Mehrin Jan,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Jillamika Pongsachai,<sup>1,2</sup> Michael Joyce,<sup>1</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*,<sup>1,6,2</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute of Basic Research, Staten Island, NY, United States; <sup>6</sup>Institute for Basic Research, Staten Island, NY, United States.*

**Introduction:** We have shown subtle differences in gross placental pathology in children with an older sibling with autism (and at increased genetic risk of autism) compared to controls. We here explore whether placental gross features can distinguish the at-risk child in a low-risk community-based population.

**Methods:** A community hospital based sample with universal placental examination was searched for those births followed to at least age 2 years at our institution. Billing codes were searched for diagnoses related to autism spectrum disorder (ASD) among the patient population of the Department of Pediatrics Developmental Pediatrics group. At least 2 diagnoses related to ASD as per Newschaffer et al were required to be considered an ASD case. Controls were selected from the next infant born of same gender, gestational age +/-2 weeks, and season of birth +/- 2 weeks. Gross placental examination was performed according to a protocol that recorded trimmed placental weight (PW), major and minor disk axes, minimum and maximum disk thickness and cord eccentricity. 165 ASD and 617 controls were included in the current analysis. Due to nonnormal distribution of growth variables, non-parametric tests were used.

**Results:** While placental weights did not differ between ASD cases and controls, ASD cases had significantly smaller major disk axis (p=0.011), reduced chorionic plate area (p=0.044), reduced average thickness (p<0.0001) and reduced thickness variability (p<0.0001). Both fetalplacental weight ratios and beta, the nonlinear representation of the fetal to placental relationship (p=0.034, p=0.011, respectively). The significant associations persisted after stratification for sex in males with only average disk thickness (p=0.05) and disk thickness variability (p=0.003) retaining significance in the small set of 10 ASD females and 40 matched controls.

**Conclusion:** We have demonstrated significant differences in gross placental features in ASD cases in a population based sample with universal placental examination. This complements our previous reports of similar gross differences in ASD cases diagnosed in the 1990's in a British convenience sample, and also from a high-ASD risk cohort of infants with an older sibling with autism. The present sample represents sporadic ASD. Future studies should be directed to identify factors that can reduced placental expansion and arborization in fetuses without genetic predisposition.

#### W-241

**Gestational Diabetes Mellitus and Placental DNA Methylation in the Rhode Island Child and Health Study.** *Corina Lesseur†,<sup>1</sup> Amber Burt†,<sup>2</sup> Jia Chen\*,<sup>1</sup> Carmen J Marsit\*,<sup>2</sup> <sup>1</sup>Icahn School of Medicine at Mount Sinai, New York, NY, United States; <sup>2</sup>Emory University, Atlanta, GA, United States.*

**Introduction:** Gestational diabetes mellitus (GDM) is a common obstetric complication that affects approximately 10% of pregnancies worldwide. Epidemiologic studies have shown that GDM can negatively impact maternal health after pregnancy, but also influence offspring risk of metabolic disease later in life (fetal metabolic programming). The placenta is the crucial fetal organ linking the maternal-fetal unit; placental epigenetic marks, in particular DNA methylation, have been increasingly recognized as plausible molecular mediators of the maternal intrauterine environment effects (i.e. GDM-exposure) on metabolic programming. In this study, we aimed to investigate relationships between GDM and DNA methylation in placentas from term pregnancies.

**Methods:** We profiled the placental DNA methylome using the Infinium MethylationEPIC BeadChip Array of 222 term newborns, 22 from GDM-complicated pregnancies and 200 from pregnancies without complications, GDM status was obtained from medical chart review. DNA methylation array data was preprocessed using functional normalization. After quality control, we performed an epigenome-wide association study (EWAS) to assess possible effects of GDM on term placenta DNA methylation at more than 700,00 CpG sites. We used robust linear models adjusted for maternal and infant confounders including in-silico estimates of placenta cell subtypes. False discovery rate (FDR) used to adjust for multiple comparisons. RNAseq data was used to evaluate the relation between expression and DNA methylation in genes near to GDM-related CpG sites.

**Results:** GDM status was significantly associated with placental DNA methylation at multiple CpG sites (n=15, FDR 5%). Some of these loci mapped to regions associated to GDM in previous placental EWAS (e.g., 6p21.33). Other GDM-linked CpGs mapped to novel genomics regions.

One of these mapped to 12p22.11 near the *TMTCl* gene, methylation at this site was also associated with gene expression. Genes near significant CpGs were enriched for carbohydrate and glycolipid related pathways.

**Conclusion:** In utero GDM exposure influences the newborn placental DNA methylome and may be one mechanism through which an adverse hyperglycemic environment can program offspring health later in life. Future studies should explore the influence of GDM on placental DNA methylation patterns in larger samples and in diverse populations.

#### W-242

**Fetal Growth Restriction and Longer Term Outcomes. Are We Causing Harm?** Roshan Selvaratnam<sup>†,1,2</sup> Euan M Wallace<sup>\*,1,2</sup> Rory Wolfe<sup>\*,3</sup> Peter J Anderson<sup>\*,4</sup> Mary-Ann Davey<sup>\*,1,2</sup> <sup>1</sup>The Ritchie Centre, Monash University, Melbourne, Australia; <sup>2</sup>Safer Care Victoria, Melbourne, Australia; <sup>3</sup>Monash University, School of Public Health and Preventative Medicine, Australia; <sup>4</sup>Monash University, Melbourne, Australia.

**Introduction:** Fetal growth restriction (FGR) is a major risk factor for stillbirth. Timely delivery of infants suspected of having fetal growth restriction (FGR) is a balance between preventing stillbirth and minimizing prematurity. This balance is particularly important because half of infants suspected of FGR are normally grown. We sought to explore the association between birthweight centile and childhood school outcomes, and between appropriate and inappropriate iatrogenic delivery for suspected FGR and childhood school outcomes.

**Methods:** A retrospective whole-of-population cohort study linking perinatal data from 1 January 2003 to 31 December 2013 to developmental and educational test scores at prep, Grades 3, 5, and 7 in Victoria, Australia. Births  $\geq 32$  weeks' gestation were linked to child developmental and educational results. The primary outcome was being in the bottom 10<sup>th</sup> centile on 2 of 5 developmental domains at school entry and being below the national minimum standard on 2 of 5 educational domains in grades 3, 5, or 7. To explore the association between the iatrogenic delivery for suspected FGR and childhood outcome, severe SGA babies (birthweight  $< 3^{\text{rd}}$  centile) iatrogenically delivered for suspected FGR were compared to severe SGA babies not suspected of FGR. To explore potential harm, babies with birthweight  $\geq 10^{\text{th}}$  centile who were delivered for suspected FGR were compared with babies  $\geq 10^{\text{th}}$  centile not suspected FGR.

**Results:** There were 181,902 children with developmental results and 425,717 children with educational results. With decreasing birthweight centile, a stronger association with poor school outcomes was observed. Severe SGA infants (birthweight  $< 3^{\text{rd}}$  centile) delivered for suspected FGR were born earlier (mean (SD) gestation: 37.9 (1.6) weeks' vs. 39.4 (1.3) weeks',  $P < 0.001$ ) and had increased odds of poor educational outcome across grades 3, 5, and 7 (aOR=1.38, 95% CI:1.08-1.77) than undetected severe FGR infants. There was no difference in educational outcome across grades 3, 5, and 7 (aOR=1.01, 95% CI:0.77-1.32) between infants with birthweight  $\geq 10^{\text{th}}$  centile delivered for suspected FGR and those not suspected of FGR despite being born earlier (38.0 (1.7) weeks' vs. 39.1 (1.5) weeks',  $P < 0.001$ ).

**Conclusion:** FGR carries significant risks for a baby's developmental and academic trajectory. Moreover, appropriate iatrogenic delivery of severe SGA infants due to suspected FGR was associated with poorer school outcomes. Inappropriate iatrogenic delivery of normally grown infants due to suspected FGR was not associated with poorer school outcomes.

#### W-243

**Association of Phthalate and DINCH Urinary Metabolite Concentrations during Pregnancy with Gestational Age at Birth and Birth Weight.** Victoria Fruh,<sup>1</sup> Emma V Preston,<sup>1</sup> Marlee R Quinn,<sup>1</sup> Michele R Hacker,<sup>2</sup> Blair J Wylie,<sup>2</sup> Karen O'Brien,<sup>2</sup> Tamarra James-Todd<sup>\*,1</sup> Shruthi Mahalingaiah<sup>\*,1</sup> <sup>1</sup>Harvard University, Boston, MA, United States; <sup>2</sup>Beth Israel Deaconess Medical Center, Boston, MA, United States.

**Introduction:** Exposure to phthalates is ubiquitous. As phthalates are reproductive and developmental toxicants, perinatal exposure is of particular concern for parents and their offspring. Therefore, we aimed to assess associations of prenatal urinary phthalate metabolites with gestational age at birth and birth weight.

**Methods:** We conducted a pilot study on associations of phthalate and DINCH (Di(isononyl) cyclohexane-1,2-dicarboxylate) metabolite concentrations during pregnancy with birth outcomes in 123 pregnant participants in Boston, Massachusetts. We measured 14 phthalate and 2 DINCH metabolite concentrations (ng/mL) in urine samples collected at a mean gestational age of 35 weeks. Gestational age at birth and birth weight were abstracted from medical records. We evaluated individual metabolites, the molar sum of di-(2-ethylhexyl) phthalate metabolites ( $\Sigma$ DEHP), and the sum of available butyl ( $\Sigma$ butyl) phthalate metabolites (mono-n-butyl phthalate, mono-isobutyl phthalate, monobenzyl phthalate). We estimated associations of tertiles for specific gravity-corrected metabolite concentrations with gestational age at birth and birth weight using individual linear regression models adjusted for maternal age.

**Results:** Mean  $\pm$  SD for gestational age at birth and birth weight was  $39.4 \pm 1.2$  weeks and  $3.3 \pm 0.5$  kg, respectively. Most urinary metabolites were detected for  $>90\%$  of participants. In regression models, mean gestational age at birth was 4.3 days shorter (95% confidence interval (CI): -7.9, -0.7) among participants with the highest versus lowest tertile concentrations of mono-carboxyisononyl phthalate (MCNP).  $\Sigma$ DEHP and  $\Sigma$ butyl phthalate metabolite results suggested inverse or positive associations, respectively, with gestational age at birth ( $\Sigma$ DEHP  $\beta$ : -2.6 days, 95% CI: -5.9, 1.4;  $\Sigma$ butyl  $\beta$ : 2.8 days, 95% CI: -0.9, 6.4) and birth weight ( $\Sigma$ DEHP  $\beta$ : -0.7 kg, 95% CI: -2.2, 0.8;  $\Sigma$ butyl  $\beta$ : 0.7 kg, 95% CI: -0.8, 2.2). We saw little evidence of associations for other metabolite concentrations with gestational age at birth or birth weight.

**Conclusion:** Results suggest that prenatal exposure to MCNP in late pregnancy may be associated with decreased gestational age at birth. However, other phthalate metabolites, phthalate summary measures, and DINCH metabolite concentrations appeared not to be associated with gestational age at birth or birth weight in this study population. Results may be limited by sample size.

#### W-244

**Placental Histology of Acute and Chronic Inflammation in a Population Based Case Control Study of Autism.** Jillamika Pongsachai,<sup>1</sup> Mehri Jan,<sup>1,2</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury<sup>†,1,2,3</sup> Adwoa Nantwi<sup>†,4</sup> Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Michael Joyce,<sup>1</sup> Jennifer S Feng<sup>†,1,2,3</sup> Serena Chen,<sup>1,2</sup> Beata Dygulska,<sup>2</sup> Christine Chen,<sup>1,2</sup> Carolyn Salafia<sup>\*,1,2,5</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute of Basic Research, Staten Island, NY, United States.

**Introduction:** A smaller study in this unique population with universal placental examination suggested that specific patterns of placental acute and chronic inflammation carry significant odds of autism case status. We have tripled our autism case numbers and seek to confirm these findings.

**Methods:** A community hospital based sample with universal placental examination was searched for those births followed to at least age 2 years at our institution. Billing codes were searched for diagnoses related to autism spectrum disorder (ASD) among the patient population of the Department of Pediatrics Developmental Pediatrics group. At least 2 diagnoses related to ASD as per Newschaffer et al were required to be considered an ASD case. Controls were selected from the next infant born of same gender, gestational age  $\pm 2$  weeks, and season of birth  $\pm 2$  weeks. Maternal and fetal acute inflammation (MAIR, FAIR) was scored as 0-4 based on the number of affected sites (membranes, chorionic plate- maternal and umbilical cord and chorionic plate vessels-fetal) and semi-quantitative estimation of neutrophil infiltrate density. Chronic placental inflammation (CPI) was scored as "confined to basal plate and maternal-placental interface", and "villous infiltrates/chronic villitis", and subjectively scored as to extent of mononuclear lymphocyte infiltrate. Contingency tables considered  $p < 0.05$  significant.

**Results:** Placental coding was completed for 134 ASD cases and 418 controls. Any MAIR was seen in 31 (23%) of ASD and 58 (13.9%) of controls, and in both membranes and chorionic plate in 8 (6%) and 18 (4%) of controls (linear-by-linear association  $p = 0.06$ ). 22 (16.4%) ASD cases and 52 (12.4%) controls had any FAIR. 10 (7%) ASD had both umbilical cord and chorionic plate vessel acute inflammation, compared

to 20 (4.8%) of controls ( $p=0.16$ ). (CPI was seen in the basal plate only in 13 (9.7%) of ASD cases and 16 (3.8%) of controls, and CPI involving at least two of chorionic plate, fetal stem and/or terminal villi in 14 (11%) of ASD and 39 (9%) of controls (overall  $p=0.009$ , with linear by linear association  $p=0.076$ )

**Conclusion:** These data suggest a dose response relationship of both MAIR and CPI with sporadic/population based ASD. Maternal immune activation of either form (acute or chronic) is associated with increased risk of ASD.

#### W-245

**Maternal Microchimeric Cells Predict Early Life Respiratory Infections in Children.** Christopher Urbschat†, Steven Schepanski, Kristin Thiele, Agnes Wieczorek, Marie Albrecht, Boris Fehse, Anke Diemert, Petra Arck\*. *University Medical Center Hamburg-Eppendorf, Hamburg, Germany.*

**Introduction:** Upon formation of the hemochorial placenta during mammalian pregnancies, maternal immune cells are transferred from the mother to the fetus. Since these cells occur at very low frequencies in fetal tissues, they are referred to as maternal microchimeric (MMc) cells. To date, insights into the functional role of MMc cells are ambiguous. Pilot data arising from observational studies with low numbers of participants point toward advantageous effects such as replacement of diseased cell subsets like  $\beta$ -islet cells in diabetes type 1. Conversely, some studies also suggest disadvantageous consequences, such as graft-versus-host reactions. We here sought to disentangle the function of MMc cells on early life immunity in humans in a large, prospectively designed pregnancy cohort.

**Methods:** We assessed MMc cells at birth upon screening for deletion-insertion-polymorphisms (DIP) specific for the mother among the neonatal cells in cord blood samples ( $n=142$ ), MMc-specific sequences were then amplified by DIP-specific duplex digital PCR. We linked the number of MMc cells in cord blood to other features of vertical transfer, i.e., the transplacental transfer rate (TPTR) of maternal pathogen-specific antibodies. Lastly, we associated the number of MMc to the incidence of early life infections of the neonate.

**Results:** When evaluating the association between MMc cells at birth and subsequent parent-reported infections during the first year of life, we could identify a significant prediction in boys, revealing a higher number of infections during early life when MMc cells were low at birth. No such association was present in females. This sex-specific risk for infections in boys was not due to lower MMc cell numbers or higher numbers of infections in boys in general, as the number of infections was similar in boys and girls. Further, we observed that the transfer of MMc via the placenta occurs independently from maternal antibody transfer.

**Conclusion:** Taken together, our data strongly support that MMc cells mitigate offspring's immunity, which reduces the risk for early life infections, at least in male infants.

#### W-246

**Does Placental Acute or Chronic Inflammation Impact Placental Efficiency in Term Newborns in a Low Risk Community Setting?**

Sadia F Chowdhury†, <sup>1,2,3</sup> Ruchit Shah, <sup>4,1</sup> Michael Joyce†, <sup>1</sup> Mehrin Jan, <sup>1,2</sup> Serena Chen, <sup>1,2</sup> Christine Chen, <sup>1,2</sup> Jillamika Pongsachai, <sup>1,2</sup> Jennifer S Feng†, <sup>1,2,3</sup> Joan Krickellas, <sup>1,2</sup> Hannah Bromberg, <sup>1,2</sup> Adwoa Nantwi†, <sup>5</sup> Sylvia Dygulski, <sup>1,2</sup> Beata Dygulska, <sup>2</sup> Carolyn Salafia\*. <sup>1,2,4</sup> *Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>Institute for Basic Research, Staten Island, NY, United States; <sup>5</sup>NYU CGPH, New York, NY, United States.*

**Introduction:** Placental acute or chronic inflammation is very commonly identified as subclinical even in low risk newborns. We explore whether placental inflammation impacts early childhood growth of term well newborns via effects on placental efficiency.

**Methods:** A university affiliated community hospital in an ethnically diverse urban setting instituted a protocol of universal placental examination between January 2010 and March 2015. Gross and histologic examinations were performed according to a standard protocol. Inclusion

criteria were limited to term singleton liveborns who had follow up in resident or faculty pediatric practices to at least 2 years of age, and whose placentas had been fully evaluated. In 232 children, maternal and fetal acute inflammation was scored as 0-4 based on the number of affected sites (membranes, chorionic plate- maternal and umbilical cord and chorionic plate vessels-fetal) and semi-quantitative estimation of neutrophil infiltrate density. Chronic placental inflammation was scored as “confined to basal plate and maternal-placental interface”, and “villous infiltrates/chronic villitis”, and subjectively scored as to extent of mononuclear lymphocyte infiltrate. Analyses used nonparametric tests due to nonnormal distribution of growth variables.

**Results:** In this term population, no acute or chronic inflammatory pathology was associated with altered placental efficiency, whether calculated as a linear fetal placental weight ratio or as the nonlinear “beta”, that relates placental and birth weight by a power law. No differences were observed between sexes.

**Conclusion:** We have previously found associations with fetal acute and chronic placental inflammation and conditions such as autism; prenatal inflammatory exposures have also been linked to increased risk of obesity. The current data do not support a strong relationship of placental acute or chronic inflammation with placental efficiency which might be a pathway for abnormal metabolism that could predispose to obesity. Negative results may reflect the current small sample size, which precluded analysis of sex-specific effects or more quantitative differentiation among acute and chronic inflammatory infiltrates.

#### W-247

**Does Placental Histopathology Impact Fetal Placental Weight Ratio in Term Well Newborns?**

Adwoa Nantwi, <sup>1</sup> Sylvia Dygulski, <sup>2,3</sup> Hannah Bromberg, <sup>2,3</sup> Ruchit Shah, <sup>2,4</sup> Michael Joyce, <sup>2</sup> Mehrin Jan, <sup>2,3</sup> Serena Chen, <sup>2,3</sup> Christine Chen, <sup>2,3</sup> Jillamika Pongsachai, <sup>2,3</sup> Jennifer S Feng†, <sup>2,3,5</sup> Joan Krickellas, <sup>2,3</sup> Sadia F Chowdhury†, <sup>2,3,5</sup> Beata Dygulska, <sup>3</sup> Carolyn Salafia\*. <sup>2,3,6</sup> *NYU CGPH, New York, NY, United States; <sup>2</sup>Placental Analytics, New Rochelle, NY, United States; <sup>3</sup>NYPBMH, Brooklyn, NY, United States; <sup>4</sup>Institute for Basic Research, Staten Island, NY, United States; <sup>5</sup>CUNY Hunter College, New York, NY, United States; <sup>6</sup>Institute of Basic Research, Staten Island, NY, United States.*

**Introduction:** Placental acute and/or chronic inflammation and maternal and fetal vascular pathology have been shown to impact placental growth and, by extension, birthweight. We test whether these associations are mediated by effects of histopathology processes on the fetal placental weight ratio (FPR).

**Methods:** In a community hospital in an ethnically diverse urban setting, a mandate for universal placental examination led to gross and histologic examinations performed according to a standard protocol. 232 term singleton liveborns followed to at least 2 years of age had pathology report data extracted for diagnoses made by a single observer at birth. Maternal and fetal acute inflammation (MAIR, FAIR) was scored as 0-4 based on number of affected sites (membranes, chorionic plate- maternal and umbilical cord and chorionic plate vessels-fetal) and semi-quantitative estimation of neutrophil infiltrate density. Chronic placental inflammation (CPI) was scored as “decidual-placental interface only”, and “chronic villitis”, and subjectively scored as to extent of mononuclear lymphocyte infiltrate. Maternal uteroplacental vascular pathology (UVP) considered both villous fibrosis and hypovascularity and lesions such as infarct and thrombosis; fetal vascular pathology (FVP) was coded to distinguish terminal villous lesions (avascular villi, stromal karyorrhesis and erythrocyte fragmentation) and mural thrombi embedded in the walls of muscular placental vessels. Analyses used nonparametric tests due to nonnormal distribution of FPR.

**Results:** In this population of term newborns, rates of high grade histopathology lesions were low, with 11.8% of MAIR in both membranes or chorionic plate, 3% with FAIR in umbilical and chorionic vessels, 11.8% with CPI, and 13.7% with UVP. 10.8% of FVP confined to terminal villi only, and a 5.3% rate of mural thrombus in chorionic or fetal stem vessels (FVP). UVP demonstrated negative associations with birth weight

centile and placental weight ( $r = -0.27$ ,  $r = -0.24$ , respectively) but not FPR. A trend to lower birth weight centile ( $p = 0.07$ ) but no impact on FPR was found with FVP lesions.

**Conclusion:** CPI and maternal UVP have been linked to fetal growth restriction. Lack of association in our population sample may reflect current sample size, but our data provide no support for an hypothesis that placental histopathology, apart from UVP with gross infarcts and/or thrombosis, has any fetal impact via effects on the FPR.

## W-248

**The Association between Vitamin D Levels and Insulin Resistance.** Roxana Guerra,<sup>1</sup> Cassandra Charles,<sup>2</sup> Fadi Yacoub,<sup>2</sup> Daniella Alviar,<sup>2</sup> Courtney Chiu,<sup>2</sup> Serin Seckin,<sup>2</sup> Ozgul Muneyyirci-Delale.<sup>2</sup> <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>SUNY Downstate Health Sciences University, Brooklyn, NY, United States.

**Introduction:** Low vitamin D levels have been associated with increased risk for numerous health outcomes including cardiovascular disease, autoimmune disease, dementia, and glucose intolerance amongst others. The aim of the study is to investigate the association of vitamin D levels with insulin resistance in predominantly Afro-Caribbean population and provide insight into other medical health issues associated with vitamin D levels.

**Methods:** An IRB-approved retrospective chart review was conducted using the electronic medical records of women aged 18 years and above seen at the State University of New York (SUNY) Downstate Health Sciences University's gynecology clinic from 2012 to 2017. Pertinent information extracted from the electronic medical record included: age, race, history of Diabetes mellitus, history of hypertension, vitamin D 25-OH (Vit D) level, hemoglobin A1C level, body mass index (BMI), and signs of insulin resistance on physical examination, specifically the presence/absence of acne, hirsutism, skin tags and/or acanthosis nigricans. Women were stratified into two groups based on their Vit D levels: normal (Vit D  $\geq 30$  ng/mL) vs. deficient (Vit D  $< 30$  ng/mL). Subsequently, the Chi-square and Fisher's exact tests were utilized to assess if there was a correlation between Vit D, hemoglobin A1C levels, medical history, demographics, and physical exam findings of insulin resistance. Significance was set at 0.05.

**Results:** Women ( $n = 917$ ) were predominantly of Black descent (86.5%) with mean age of  $39.4 \pm 11.5$  years and mean BMI of  $31.3 \pm 7.4$  kg/m<sup>2</sup>. Based on Vit D, 73.5% ( $n = 674$ ) of women were categorized as deficient and the remaining 26.5% ( $n = 243$ ) were normal. Deficient women were found to be significantly older than those with normal levels ( $p < 0.0001$ ). They were also more likely to be morbidly obese when compared to the normal group ( $p = 0.0003$ ). Women with a history of hypertension were more likely to have normal Vit D levels ( $p = 0.043$ ), while women with a history of smoking were more likely to have low Vit D levels ( $p = 0.0191$ ). No correlation was noted between Vit D levels and race ( $p = 0.1202$ ), history of drinking ( $p = 0.529$ ), history of diabetes ( $p = 0.6027$ ), as well as hemoglobin A1C levels ( $p = 0.9063$ ). Furthermore, women with acanthosis nigricans were more likely to have low Vit D levels than those without that finding on physical exam ( $p = 0.001$ ). There was no correlation noted between Vit D levels and the other signs of insulin resistance such as acne ( $p = 0.1398$ ), hirsutism ( $p = 0.5327$ ) and skin tags ( $p = 0.3354$ ).

**Conclusion:** There was no association found between vitamin D 25-OH levels and hemoglobin A1c levels in our study population. However, there appears to be an association between low vitamin D levels and smoking as well as acanthosis nigricans, a physical sign of insulin resistance.

## W-249

**Interpregnancy Interval and Adverse Pregnancy Outcome-Analysis on US Birth Data.** Vanessa Grafft,<sup>1</sup> Khadija Haleem†,<sup>1</sup> W Spencer McClelland,<sup>2</sup> Teresa Cheon,<sup>2</sup> M. Teresa Benedetto,<sup>2</sup> Yuzuru Anzai\*.<sup>2</sup> <sup>1</sup>University of Chicago, Chicago, IL, United States; <sup>2</sup>Northwell Health, New York, NY, United States.

**Introduction:** Inter-pregnancy interval (IPI) is defined as the spacing between live birth and the beginning of the next pregnancy. Past research has found that IPIs shorter than 18 months (M) are associated with adverse pregnancy outcomes. However, it is unclear whether IPI is an independent

risk factor or a marker of other confounding risk factors, such as race, socioeconomic status, and age. This makes it difficult to accurately counsel patients as to their individual risk based on IPI, especially for the older patients whose fertility is in decline.

**Methods:** CDC WONDER is a public access, open database which contains all US birth data. Using this database for 2016-2017 (the first years to contain more expanded information including IPI), we analyzed IPI in relation to adverse pregnancy outcomes—preterm birth (PTB), gestational hypertension (gHTN), low birth weight (LBW), and gestational diabetes (GDM)—accounting for race, pre-pregnancy BMI, education, marital status, and maternal age. We focused our analysis to singleton pregnancies.

**Results:** Short IPI (less than 18M) increased the rates of PTB and LBW, with stronger association with PTB rates. The highest PTB risk was seen among patients with IPI less than 12 M, modest increase was seen between 12-17 M, and rates plateaued at 18-23 M. This pattern was consistent in subgroup analysis by risk factor, indicating that IPI is an independent risk factor for PTB. While short IPI increased the risk of PTB in all races, the highest rate of PTB was seen in the non-Hispanic African-American population with short IPI, likely due to a high background PTB rate and a higher rate of IPI less than 12 M. Short IPI was an independent risk factor for LBW in all ethnic groups, with non-Hispanic African-American patients having the highest rate of LBW, followed by Asians, and then Whites. Low BMI and unmarried status were positively correlated with both PTB and LBW, while higher education (bachelor's degree or higher) was negatively correlated. In all subgroups, short IPI increased the risk independently, with the exception of LBW in the higher education group where there was no association observed. IPI less than 12 M was associated with increased PTB risk in women aged 20 years or younger, but there was no association with age otherwise. IPI was not associated with gHTN or GDM. Non-Hispanic African-American race and high BMI were associated with higher rates of gHTN. Asian race was associated with a higher rate of GDM.

**Conclusion:** In this analysis of a nationwide birth database, short IPI, particularly less than 12 M, is an independent risk factor for PTB and LBW, whereas no association was seen between IPI and gHTN or GDM. It is important to educate patients about this modifiable risk factor of pregnancy complications. It may also be reasonable for the older patients to consider pregnancy after 12M of IPI based on our observation.

## W-250

**Preliminary Results of the Shen Zhen Gong in Pregnancy Study: A Randomized Control Trial to Evaluate Maternal and Fetal Responses to Exercise.** Clarissa L Velayo\*,<sup>1</sup> Sherri Ann L Suplido\*,<sup>2</sup> Alvin R Sy\*,<sup>2</sup> Kiyoe Funamoto\*,<sup>3</sup> Yoshitaka Kimura\*.<sup>3</sup> <sup>1</sup>University of the Philippines, Manila, Philippines; <sup>2</sup>Philippine General Hospital, Manila, Philippines; <sup>3</sup>Tohoku University Graduate School of Medicine, Sendai, Japan.

**Introduction:** This study assessed chronic and acute maternal and fetal responses to a low impact form of exercise called Sheng Zhen Gong (SZG) and aimed to evaluate this as an alternative form of therapy to improve maternal physical, social and emotional well-being.

**Methods:** This was a single-blinded randomized controlled trial among low risk pregnant Filipino women. To evaluate chronic exercise effects, women were recruited from the first trimester and submitted to a physical examination and Pregnancy Physical Activity Questionnaire (PPAQ). They were then randomized into either of two groups: exercise (E) or no exercise (NoE). The E group was encouraged to exercise at least twice a week, in class or at home. In class, pre- and post-exercise evaluation using the Warwick-Edinburgh Mental Well-Being Scale (WEMWBS) questionnaire and other antenatal surveillance techniques were performed. To evaluate acute exercise effects, third trimester parturients were recruited and randomized into No Exposure (NE), Multiple Exposures (ME) and Single Exposure (SE) to exercise and also subjected to antenatal surveillance.

**Results:** For the Chronic Exercise arm of the study, there was a total of 68 pregnant women included: 35 (51.5%) in the E group and 33 (48.5%) in the NoE group. All of these patients were entered into the Acute Exercise arm as the ME and NE group. Additionally, there were 21 women in the SE

group. The demographic characteristics of the study participants showed a homogenous population with no differences in terms of sonographic characteristics. There were notable improvements in the capillary blood glucose of women in the Chronic group (F[1,54]: 4.47, p: 0.04) and there was a significant increase in the mean (F[1,267]: 8.45, p<0.01) and median (z: -12.81, p<0.01) WEMWBS score before and after exercise. It can also be noted that increasing the number of sessions, particularly more than 15 sessions, was associated with even greater scores in the well-being scale post-exercise (F[1, 267]: 5.18, p: 0.02).

**Conclusion:** The study introduced a sustainable exercise program among low to middle income pregnant women. The most immediate benefit was the improvement of blood sugar control in the chronic exercise group with no evident adverse effects of SZG to the mother or fetus. Also, SZG had positive emotional and mental well-being effects on pregnant women and with routine exercise, this further increased. For healthcare and social workers, SZG may be used in maintaining maternal health and its practice may serve as a well-rounded form of alternative therapy. In developing countries with limited tertiary center access, SZG is a preventive medicine tool already available to local community healthcare workers.

**W-251**

**Emergency Department Utilization for Miscarriage Management: Trends from the Nationwide Emergency Department Sample (NEDS) from 2006 - 2018.** Amanda R Schwartz†, Jiang Li, Min Xu, Duyhoang Dinh, Daniel Miranian, Leah Mitchell Solomon, Erica E Marsh\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** With more than 15% of pregnancies resulting in a miscarriage in the United States, women frequently seek care for diagnosis and management of pregnancy loss. Few studies have assessed trends in where this care is most often sought. The objective of this studies is to characterize the demographics, clinical features and institutional factors associated with utilization of emergency departments (ED) for women presenting with a primary diagnosis of miscarriage.

**Methods:** A retrospective longitudinal analysis was performed using the NEDS database. All ED visits from 2006 - 2018 for which ICD-9 or ICD-10 codes for miscarriage were listed as the principal diagnosis were examined. Women of reproductive age 15-44 were included for analysis. Parameters assessed included hospital region, urban vs rural setting, hospital teaching status, primary payer, income quartile and hospital charges. T-tests and Chi-square tests were performed to assess differences between groups. A regression analysis was performed to identify demographic factors associated with admission.

**Results:** From 2006 - 2018 there were 2,900,298 ED visits among reproductive age women with a primary diagnosis of miscarriage. Over this 13-year period the percentage of ED visits for pregnancy loss was stable (0.59%-0.77%). Women were more likely to utilize the ED in the setting of a miscarriage if they were 20 - 29 years of age (50.9% in 2006, 49% in 2018), lived in the South (37.1% in 2006, 45.8% in 2018) or resided in large urban areas (59.3% in 2006, 53.8% in 2018). Women in the lowest income quartile (31.7% in 2006, 38.2% in 2018) and with Medicaid (36.8% in 2006, 47.4% in 2018) consistently represented the highest proportion of ED visits. There was a significant decrease in the admission rate from 9.16% in 2006 to 3.83% in 2018. Women were more likely to be admitted if they presented to an ED in the Northeast or South, large metropolitan area, were older in age, in the lowest income quartile, or if they had Medicare or Medicaid. Mean charges per visit increased over the study period from \$3,366 in 2006 to \$6,430 in 2018 with total charges increasing from \$773,932,483 to \$1,417,357,036.

**Conclusion:** Despite a decrease in rates of admission, significant increases in average charges and total charges were observed for the management of miscarriage from 2006 to 2018 in the US. Women were more likely to utilize ED services for miscarriage management if they lived in lower income zip codes, had Medicaid or presented to large metropolitan hospitals. These findings identify potential disparities in prenatal and gynecologic care with the need for improved access to healthcare and outpatient services for early pregnancy loss.

**W-252**

**Comparing Vaginal Birth after Cesarean Section Success Rate Calculators with and without Race and Ethnicity at Mount Sinai Hospital.** Ayisha Brielle Buckley†, Tonia Ogundipe†, Stephanie Sestito†, Jacqueline Roig†, Mitchell Rosenberg†, Chelsea DeBolt†, Rachel Meislin†, Angela Bianco\*, Vieira Luciana\*. *Icahn School of Medicine at Mount Sinai Hospital, Manhattan, NY, United States.*

**Introduction:** Reaching a decision about whether or not to undergo a trial of labor after cesarean section (TOLAC) should be based on a discussion of the risks, benefits and alternatives for the patient. The decision should be based on the best available clinical evidence, and incorporate multiple factors including the probability of a successful vaginal birth after cesarean section (VBAC), risk of morbidity and mortality, personal values, preferences, past birthing experiences, and future pregnancy plans. The MFMU VBAC calculator is a commonly used resource used to predict a woman's chances of having a VBAC. The calculator bases its prediction on six variables, which includes race and ethnicity. In this calculator, Hispanic ethnicity and African American race have a subtractive effect. This study aims to compare 2 VBAC prediction models utilizing and excluding race/ethnicity.

**Methods:** One thousand five hundred ninety-nine records were collected between 2016 and 2019. Records were excluded if they were missing, incomplete, or were duplicates, as well as women who had more than one prior c-section. A total of 1275 participant records were used in our analysis. A result was considered statistically significant at the p<0.05 level of significance. We compare two models that utilize Grobman et al's variables in predicting VBAC success; Model 1 includes race categorized as non-Hispanic Black, Hispanic/LatinX, White or Asian/other and Model 2 excludes race and ethnicity parameters. The area under the curve (AUC) was used to assess the predictive ability of each model. AUC was based on 5 repeats of 10-fold cross-validation samples. All analyses were performed using the SAS version 9.4.

**Results:** Table 1 depicts variables associated with VBAC success in multivariable logistic regression, utilizing Grobman's et al variables. In Model 1, there was not enough evidence to conclude that race/ethnicity was associated with the probability of successful TOLAC (p=0.065). AUCs for models 1 and 2 were 0.77 and 0.78, respectively. There was not enough evidence to conclude that AUC differed between model 1 and model 2 (p=0.40).

**Conclusion:** In our population, using race and ethnicity does not appear to significantly improve prediction of VBAC success. Given potential for reinforcing inequities in TOLAC, calculators or models utilizing race and ethnicity should be used with caution.

**Table 1:** Variables associated with vaginal birth after cesarean delivery in multivariable logistic regression

Predictor Variable	Model 1: Grobman's Equation with Race and Ethnicity		Model 2: Grobman's Equation without Race or Ethnicity	
	OR (95% CI)	P value	OR (95% CI)	P value
Age	0.96 (0.93, 0.98)	0.0028	0.96 (0.93, 0.98)	0.0017
Prepregnancy BMI	0.91 (0.89, 0.94)	<0.0001	0.91 (0.87, 0.93)	<0.0001
Race		0.0650	--	--
Asian or Other	1.01 (0.53, 1.94)	0.9548	--	--
Hispanic/LatinX	1.55 (0.98, 2.47)	0.0614	--	--
White/Caucasian	1.61 (1.06, 2.46)	0.0267	--	--
Non-Hispanic Black	Ref	--	--	--
Any prior vaginal delivery	2.76 (1.83, 4.17)	<0.0001	2.83 (1.88, 4.26)	<0.0001
Vaginal Delivery after Prior Cesarean	7.05 (4.38, 11.34)	<0.0001	7.39 (4.62, 11.83)	<0.0001
Recurring indication for cesarean	0.48 (0.35, 0.66)	<0.0001	0.48 (0.35, 0.65)	<0.0001
AUC*	0.774		0.781	

\* AUCs were based on 5 repeats of 10-fold cross-validation. The p-value for comparing AUC between model 1 and model 2 was p=0.40, based on a paired t-test of AUC values drawn from validation samples.

## W-253

**Comorbidities in Pregnancy, Refugee Status, and Their Effects on Birth Outcome.** Swathi Somisetty†,<sup>1</sup> Ralph Mendez†,<sup>1</sup> Pooja Doehrmann\*,<sup>1,2</sup> *Creighton School of Medicine, Phoenix, AZ, United States;* <sup>2</sup>*Department of Obstetrics & Gynecology, Phoenix, AZ, United States.*

**Introduction:** Hypertension (HTN), diabetes (DM), mental illness, and substance abuse are comorbid conditions associated with adverse health outcomes in pregnancy. While prevention is one way to avoid these adverse outcomes, refugees often face barriers to this type of health care, such as language barriers, impoverishment, and lack of familiarity/access to care, which may disproportionately affect birth outcomes in refugees compared to the native population.

**Methods:** We collected data from a population of 2,673 high-risk women, defined as having HTN, DM, and/or obesity who delivered between the years of 2015-2019 in Maricopa County in Phoenix, AZ. Then, we determined rates of comorbid conditions (e.g., DM, HTN, psych/substance use disorder, infection, smoking) in our sample of refugee and non refugee women. SPSS statistical software was used to perform independent T tests to compare birth weight between groups. A one-way ANOVA test was used to determine the relationship of diabetes (T1DM, T2DM, GDM) on birth weight. Additionally, we conducted a univariate ANOVA to examine the interactions between comorbidities and their combined effect on birthweight.

**Results:** Hypertensive disorders during pregnancy had a significant impact on lower mean birth weight of 3118g vs. 3333.48g ( $p < .05$ ). Refugee status was associated with greater mean birth weight of 3307.63g vs. 3190.16g ( $p < .05$ ). Women with mental illness and substance abuse had a significantly lower mean birth weight of 3071g vs 3222.51g ( $p < .05$ ). We found T1DM significantly impacted birth outcomes where mean birth weight was 3182g for those who did not have diabetes, 3325.3g for women with GDM, 3325.29g for women with T2DM, and 3079g for those with T1DM ( $p < .05$ ). We did not find a disproportionate impact of pregnancy complications of HTN, mental health and substance abuse, or DM on birth outcomes for non refugee women compared to refugee women.

**Conclusion:** Women with mental illness, T1DM, and HTN had significantly lower birth weight than those who did not. Refugees status was associated with improvement of outcomes, despite the impact of language, social, and economic barriers faced by this vulnerable segment of the population. Refugee women in our cohort did not disproportionately experience worse birth outcomes when faced with medical comorbidities compared to the native population despite potential barriers to healthcare. These results suggest greater resiliency among refugee women with past literature identifying theories to explain this finding. The “healthy immigrant effect” is the phenomenon that first generation immigrants have significant health advantages relative to comparable native born populations due to a multitude of components. This study illustrates the potential transgenerational effect of this phenomenon, given the impact of birth weight on long term health outcomes.

## W-254

**Nudging Women with a Vulnerable Health Status to Encourage Adequate Pregnancy Preparation.** Sharissa M Smith†,<sup>1</sup> Rianne M.J.J. Van der Kleij,<sup>2</sup> Babette Bais†,<sup>1</sup> Maartje H.N. Schermer\*,<sup>1</sup> Régine P.M. Steegers-Theunissen\*,<sup>1</sup> Hafez Ismaili M'hamdi†,<sup>1</sup> *Erasmus Medical Center, Rotterdam, Netherlands;* <sup>2</sup>*Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** In general, women with a vulnerable health status determined by a low socioeconomic status and poor lifestyle behaviors, have a higher risk of adverse pregnancy outcomes. Offering preconception lifestyle care, tailored to the needs of these women, can significantly help to reduce these adverse pregnancy outcomes. We hypothesize that so-called ‘nudges’ can be a successful way of increasing the uptake of preconception lifestyle care. A nudge is a behavioural intervention that supports healthy choices by making them easier. Nudging, however, raises many moral questions. Effectiveness and respect for autonomy are, among other criteria, required for a nudge to be morally permissible. As the target group knows best what they find permissible and what would motivate them to change their lifestyle, this study aimed to examine,

in women with a vulnerable health status, the preferences regarding a nudge, provided via a web application that aims to help them improve their lifestyle by offering rewards.

**Methods:** We conducted semi-structured interviews of women with a low socioeconomic status, based upon their level of education and neighbourhood of residence. After transcription, a thematic content analysis was applied.

**Results:** Twelve women were interviewed. We identified five themes: (I) “Usefulness of an app as integral information source”, (II) “Permissibility and effects of offering rewards”, (III) “Preferences regarding content”, (IV) “Preferences regarding type of rewards and system of allocation”, and (V) Barriers. None of the participants objected to being rewarded for healthy behaviour; they mentioned that being rewarded could tip the scales towards healthy behaviour. Furthermore, participants stated that the freedom to choose when and with what they would be rewarded, would increase the chance of behavioural change.

**Conclusion:** The acquired insights into the preferences regarding a nudge of women with a vulnerable health status will support the design of an effective nudge to help them adequately prepare for pregnancy and ultimately prevent adverse pregnancy outcomes.

## W-255

**Association between Maternal SARS-CoV-2 Infection during Pregnancy and Adverse Pregnancy Outcomes.** Darios Getahun\*,<sup>1</sup>

Lurvey D Lawrence,<sup>2</sup> David Braun,<sup>3</sup> Sacks A David,<sup>4</sup> Alex Fong,<sup>5</sup> Neha Trivedi,<sup>6</sup> Jiaxiao Shi,<sup>1</sup> Vicki Y Chiu,<sup>1</sup> Morgan R Peltier,<sup>7</sup> Michael J Fassett,<sup>8</sup> *Kaiser Permanente Southern California, Pasadena, CA, United States;* <sup>2</sup>*Kaiser Permanente West Los Angeles, Los Angeles, CA, United States;* <sup>3</sup>*Kaiser Permanente, Los Angeles, CA, United States;* <sup>4</sup>*Kaiser Permanente Southern California; Keck School of Medicine, University of Southern California, Pasadena, CA, United States;* <sup>5</sup>*Kaiser Permanente Irvine Medical Center, Irvine, CA, United States;* <sup>6</sup>*Kaiser Permanente San Diego Medical Center, San Diego, CA, United States;* <sup>7</sup>*NYU-Long Island School of Medicine, Mineola, NY, United States;* <sup>8</sup>*Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States.*

**Introduction:** Recently, we reported pregnant women with evidence of exposure to SARS-CoV-2 virus. To better understand the impact of this, we examined the association between prenatal SARS-CoV-2 infection and adverse pre- and postnatal outcomes.

**Methods:** This retrospective cohort study utilized data extracted from the Kaiser Permanente Southern California electronic health records (EHR) of 29,323 singleton pregnancies between April 6, 2020 through December 31, 2020. Prenatal exposure to SARS-CoV-2 infection was ascertained from PCR-based test results. The test was universally offered to all pregnant women. Baseline characteristics and outcomes (using ICD-10-CM and procedure codes) were ascertained from the EHRs. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CI)s before and after adjustment for several potential confounders.

**Results:** The incidence of COVID-19 diagnosis during the study period was 4% (1,185/29,323 singleton pregnancies). Of women who tested positive, 328 (27.7%) tested positive during the 1<sup>st</sup>/2<sup>nd</sup> trimester and 857 (72.3%) during 3<sup>rd</sup> trimester. The mean age of studied women was 31.0 (standard deviation [SD]: 5.31) with significant difference between those who tested positive (29.5 [5.41] years) and negative (31.1 [5.29] years) ( $p$ -value  $< .0001$ ). Women who tested positive during pregnancy were more likely to develop preeclampsia (adj.OR: 1.24, 95% CI: 1.02, 1.51) and to be admitted to ICU at delivery (adj.OR: 6.20, 95% CI: 2.77, 13.90). Vertical transmission was observed in 13 (1.1%) of exposed neonates.

**Conclusion:** The findings suggest that prenatal SARS-CoV-2 infection increases the risk of preeclampsia and ICU admission and can put the mother and her neonate at increased risk. Further studies are needed to determine if the higher risk of preeclampsia associated with SARS-CoV-2 infection involves placental microvascular dysfunction. The rate of vertical transmission from the mother to the baby is low, suggesting that pregnancy complications resulting from maternal SARS-CoV-2 exposure may pose a greater risk to the baby than transplacental viral transmission.

## W-256

**Progesterone Suppresses SARS-CoV-2 Viral Load, Cytotoxicity and Inflammatory Response in a Lung Cell Line.** Matthew B Dacanay†, Miranda Li†, H Huang\*, Tsung-Yen Wu, T-Y Hsiang, M Gale, Jr.\*, K Adams Waldorf\*. *University of Washington, Seattle, WA, United States.*

**Introduction:** The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes COVID-19, a respiratory infection that induces lung inflammation and pathology that in the worst cases can be fatal. Although the number of cases do not differ between men and women, men have about twice the risk of mortality as women. The basis for this sex differential is unknown. Our research objective was to model the impact of sex steroids on innate immune and inflammatory responses induced by SARS-CoV-2 infection in a highly permissible lung cell line. We specifically chose to investigate the effect of progesterone (P4) as it is elevated in the second half of the menstrual cycle, as well as in pregnancy.

**Methods:** We infected a lung adenocarcinoma epithelial cell line (Calu-3) with SARS-CoV-2 USA-WA1/2020 with or without P4 (100 nM) at varying multiplicity of infection (MOI; 0, 0.5, 3). Cells were observed for cytopathic effect (CPE) and harvested at 24 or 48 hours (h) post-infection (p.i.) for evaluation of viral RNA replication (RT-qPCR), cell lysis (LDH assay) and activation of antiviral innate immunity (RT-qPCR) via measuring biomarker gene expression of innate immune activation (*ifit1*, *ifnb*), inflammation (*il6*) and interferon signaling (*mxr*). All experiments were performed four times and samples tested in triplicate. Statistical analyses were conducted using Wilcoxon rank sum.

**Results:** Calu-3 cells are highly permissible to SARS-CoV-2 infection with active viral replication confirmed by viral RNA abundance and plaque assay. CPE was visible by 24 h.p.i. and cytotoxicity was significantly higher at 48h than in uninfected cells (MOI 3,  $p < 0.03$ ). P4 pre-treatment was associated with a significant decrease in SARS-CoV-2 viral load ( $3.3 \cdot 7 \pm 0.2 \cdot 0.9$  log2fold,  $p < 0.03$ ), cell lysis ( $53 \cdot 60\% \pm 1 \cdot 4\%$ ,  $p < 0.03$ ) and a concomitant decrease in *ifnb* and *il6* expression ( $1.5 \cdot 2.7 \pm 0.5 \cdot 0.7$  log2fold,  $p < 0.03$ ). In contrast, P4 treatment was associated with a significant increase in the interferon stimulated gene, *mxr*, ( $1.7 \cdot 2.4 \pm 0.7$  log2fold,  $p < 0.03$ ) versus untreated cells; *ifit1* levels were not significantly different, but trended higher after P4 treatment.

**Conclusion:** Collectively, these data provide the first evidence that P4 may impart regulation of innate immune and inflammatory induction programs in SARS-CoV-2 infected lung cells. P4 signaling downregulated virus-induced IFN- $\beta$  and IL-6 expression. While induction of *mxr* may occur through the redundant actions of other Type I or III interferons, regulation of *ifit1* may result from P4 regulation of interferon regulatory factor activation and/or interferon signaling programs. Additional studies are ongoing to determine how P4 alters the antiviral response, which may explain in part the differential mortality by sex associated with COVID-19.

## W-257

**SARS CoV2 Nonstructural Proteins Modulate Autophagic Flux and Lipid Droplet Biogenesis in Placental Trophoblasts.** Deepak Kumar†, Indira U. Mysorekar\*. *Washington University SOM, St. Louis, MO, United States.*

**Introduction:** COVID-19, caused by SARS COV2 has been associated a high rate of pregnancy complications in infected women including premature birth and preeclampsia. The viral mechanisms driving these outcomes and the impact of viral infection in the placenta and the developing fetus remain to be elucidated. Like other RNA viruses including ZIKV, SARS CoV2 has been reported to modulate intracellular vesicular trafficking pathways for its replication and propagation. We have previously shown that basal autophagic activity is a crucial component of placental syncytiotrophoblast barrier function and plays a key role in vertical transmission of ZIKV and limits fetal damage. However, whether SARS COV2 alters intracellular trafficking and regulates autophagy in the placenta remains unknown.

**Methods:** Placental tissues from SARS COV2+ women were investigated for modulation of autophagic activity via monitoring the autophagy flux markers, LC3B and p62 by immunofluorescence. Frozen placental tissues from COVID19+ and negative patients were investigated for accumulation of lipid droplets by BODIPY staining. Trophoblast cell line, JEG-3, was

transfected with mammalian expression plasmids encoding SARS CoV2 non-structural proteins (NSPs), which are critical elements of the viral replication and transcription complex. The transfected cells were analyzed for autophagy flux markers by western blots and immunofluorescence. The expression levels of autophagy proteins were correlated with lipid droplet area and number.

**Results:** We found that SARS CoV2 partially utilizes the autophagy pathway and blocks the process at fusion step with lysosomes. SARS CoV2 infection is associated with upregulation of lipid droplets in placental cells. The NSP6 protein is a multipass transmembranous protein which is located in the endoplasmic reticulum, where it can associate with ATG5 and other autophagy pathway proteins. We show that overexpression of SARS COV2 non-structural proteins, NSP6 and NSP8 modulates autophagic response and upregulates lipid droplet biogenesis. NSP6 in particular restricts the expansion of autophagosomes, thereby favoring the reduced ability of autophagosome to deliver viral proteins to lysosomes. These altered pathways may trigger inflammatory responses during pregnancy which could drive adverse outcomes

**Conclusion:** Our findings suggest that SARS COV2 modulates autophagy machinery for its own replication and likely has an inhibitory effect on the autophagy process via blocking autophagosome/lysosome fusion and affecting lipid droplet biogenesis. Ongoing studies are investigating the mechanism of action of SARS COV2 including modulation of host processes via its nonstructural proteins. Studies of these NSPs will shed light on their specific role in COVID19 pathogenesis in the placenta and may also provide attractive drug targets for intervention.

## W-258

**The Early COVID-19 Era Negatively Impacted Symptoms, Stress, and Access to Care of Endometriosis Patients.** Paola M Ramos-Echevarria,<sup>1</sup> Denisse M Soto-Soto†,<sup>2</sup> Annelyn Torres-Reverón,<sup>3</sup> Caroline B Appleyard,<sup>1</sup> Tala Akkawi†,<sup>1</sup> Barbara D Barros-Cartagena,<sup>1</sup> Veronica López-Rodríguez,<sup>1</sup> Eida M Castro-Figueroa,<sup>1</sup> Idhaliz Flores\*.<sup>1</sup> *Ponce Health Sciences University - Ponce Research Institute, Ponce, PR, United States;* <sup>2</sup>*Centro Médico Episcopal San Lucas, Ponce, PR, Ponce, PR, United States;* <sup>3</sup>*DHR Health Institute for Research and Development, Edinburg, TX, United States.*

**Introduction:** Monitoring the impact of natural disasters such as pandemics on health and wellbeing is a public health priority. Stress is proven to affect pain intensity and quality of life of endometriosis patients. A cross-sectional study was conducted to determine whether the measures implemented to mitigate COVID-19 infections had a substantial impact on risk behaviors, endometriosis symptoms, stress, and access to healthcare.

**Methods:** Electronic questionnaires that measured COVID-19 impact and peri-traumatic stress were disseminated through social media over June-September 2020 and completed by 82 adult patients with endometriosis living in Puerto Rico, where strict lockdowns were implemented from March to May 2020. Descriptive data analysis and correlations were done in quantitative data using JMP Statistical Software, and systematic analysis of free text was done on qualitative responses using NVivo 10 Software.

**Results:** Many participants (72%) reported that their work was affected due to the pandemic, and 17% lost their job. Participants self-reported worsening of endometriosis symptoms (77%) and high levels of peri-traumatic stress (76%), as well as changes in risk behaviors such as less exercise (38%), eating less healthy (56%), increased sedentarism (65%), and sleeping less hours (65%) during the pandemic in comparison to the previous months. They also reported substantial barriers in access to medical appointments (44%), scheduled procedures (63%), and prescriptions (60%). Electronic health modalities (telemedicine, mobile apps) were considered acceptable alternatives for gynecologic care during natural disasters by 94% of the study participants.

**Conclusion:** The COVID-19 pandemic negatively impacted the health and wellbeing of endometriosis patients while imposing substantial restrictions on access to health care. These timely insights will guide the development and implementation of plans to address barriers to health care and minimize long-term detrimental effects of natural disasters on the health of those living with stress-related disorders such as endometriosis. This study was supported by 1R21HD098481-01

**W-259**

**The Impact of the COVID-19 Lockdowns on the Global Rates of Preterm Deliveries - A Systematic Review.** Rani Haj Yahya†, Stacey Peart, Jeanie Cheong, Brett Manley, Clare Whitehead. *The Royal Women's Hospital, Melbourne, Australia.*

**Introduction:** On the 11<sup>th</sup> of March 2020, COVID-19 was declared a pandemic by the World Health Organization (WHO). As a result, many countries entered harsh lockdowns in order to restrict the spread of the virus. Several studies were conducted to assess the implication of the lockdowns on the rates of preterm birth, stillbirths and other maternal and perinatal outcomes, with inconsistent results. We have conducted a systematic review to report the current evidence of the Indirect impacts of COVID-19 on the rates of preterm birth and perinatal mortality & morbidity.

**Methods:** We conducted a systematic review following the PRISMA guidelines. We searched electronic databases MEDLINE, Embase, PubMed for potentially relevant published and preprint studies. Two independent reviewers conducted title/abstract screening, full text screening, and data extraction using a pre-specified form. Conflicts were resolved by consensus or consultation with a third reviewer. The primary outcome was the rate of preterm births following the introduction of the COVID-19 restrictions, compared to the rate before these restrictions. Secondary outcomes included stillbirth and neonatal death rates, neonatal birthweight, NICU admissions and rates of caesarean deliveries. We have excluded studies assessing the direct effect of COVID-19 (positive cases) on pregnancy and perinatal outcomes.

**Results:** Our initial search has retrieved a total of 485 studies, of which 21 met inclusion criteria after screening. Studies from the Netherlands, Ireland, Japan, USA, Denmark, Italy and centres in USA and Israel demonstrated a reduction in preterm birth rates during “lockdown” periods compared to prior periods. Very (< 32 weeks) and extreme (<28) preterm birth rates were more affected than late preterm births. This did not appear to be at the expense of increased stillbirth or neonatal deaths. In contrast, studies from Uruguay, Nepal, the UK, Sweden, other centres in USA and Israel, and a 17 country international cohort study, found no difference in preterm birth rates. Stillbirth or neonatal death rates were increased in Italy, Nepal, UK, Israel and Kenya.

**Conclusion:** COVID-19 mitigation policies vary between regions which may have led to the inconsistencies in the reported effects on preterm birth and perinatal mortality across studies. It is vital to interrogate these differences to identify risk factors for preterm birth that may be potentially modifiable at the patient (reduced exposure to infection/inflammation), clinician (decision making) and societal levels (public health measures) to enable new strategies to prevent preterm birth in non-pandemic times.

**W-260**

**E-Health Modalities for Gynecologic Care during the COVID-19 Pandemic Are Well Accepted by Women of Reproductive Age.** Ariana Alvarado,<sup>1</sup> Paola Ramos,<sup>2</sup> Carlos Sierra,<sup>2</sup> Madeline Zapata,<sup>2</sup> Denisse Soto,<sup>3</sup> Idhaliz Flores\*.<sup>2</sup> <sup>1</sup>Pontifical Catholic University of Puerto Rico, Ponce, PR, United States; <sup>2</sup>Ponce Health Sciences University, Ponce, PR, United States; <sup>3</sup>Centro Médico Episcopal San Lucas, Ponce, PR, United States.

**Introduction:** Although health organizations worldwide have promoted and recommended the use of e-health modalities for monitoring symptoms and facilitating access to care, there is limited data on the adoption of these approaches for women of reproductive age. This study aims to assess if it would be acceptable for women of reproductive age to use e-health modalities, including mobile apps and telemedicine, for gynecologic consultations. This is of particular relevance currently as the COVID-19 pandemic has caused severe measures to mitigate this infection, including lockdowns and curfews, which limit access to care including gynecological consultation.

**Methods:** We conducted a cross-sectional study using an anonymous questionnaire to assess the perception of women of reproductive age regarding facilitators and barriers for using e-health modalities to monitor gynecologic health and book appointments with gynecologists. The questionnaire was sent electronically via social media (Facebook, Instagram, Whatsapp, Twitter).

**Results:** The questionnaire was completed by 402 participants from 10 countries. The COVID-19 pandemic impacted access to care: 34% reported difficulties obtaining medical attention (43% of them could not contact their physician; 59% reported limited office hours). Current use of a period tracker mobile app was reported by 60%; 92% to track the menstrual cycle, 49% to monitor ovulation, and 28% to monitor symptoms. The majority of participants (90%) would agree to have a telemedicine gynecological consultation. Facilitators of telemedicine use included: prevention of COVID-19 (94%), decreasing waiting time (83%), and flexible appointments (63%). The most useful functions of a period tracker app were: calendar (94%), monitoring fertile days (62%), and monitoring gynecological symptoms (48%).

**Conclusion:** This study showed that e-health modalities for gynecological care are acceptable for women of reproductive age. This is of particular relevance currently as the COVID-19 pandemic has caused severe measures to mitigate this infection, including lockdowns and curfews, which limit access to care. In the long-term, we expect that these data will help develop studies to demonstrate the effectiveness of e-health apps and telemedicine to mitigate the effects of pandemics on the health and wellbeing of women of reproductive age.

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**W-261**

**Impact of COVID-19 Pandemic on Mental Health of Patients Seeking Infertility Evaluation.** Alvin To†, Liubin Yang†, Janet Bruno-Gaston†, Schutt Amy\*. *Baylor College of Medicine, Houston, TX, United States.*

**Introduction:** The duration to achieve pregnancy during infertility treatment has been associated with significant mental health burden. During the COVID-19 pandemic, restrictive clinic policies have forced delays in access to care. As a result, patients undergoing infertility treatment may be at risk of significant psychological impact. We aim to investigate changes in depression screens among infertility patients during the COVID pandemic and characterize risk and protective factors.

**Methods:** Using an institutional database from an academic center in Houston, Texas, we performed a retrospective case-control study to evaluate the effect of the pandemic on depression screens in new patient visits. Women undergoing infertility treatment with completed Edinburgh Postnatal Depression Scale (EPDS) questionnaires during the pandemic (1/2020-9/2020) were compared with a pre-pandemic cohort (12/2018-3/2019). Patients seeking fertility preservation, endocrine management, and age less than 18 were excluded. Primary outcomes were positive screens (EPDS score ≥10). Secondary analysis was used to stratify positive screens by demographics (race, income, nulliparity, history or pregnancy loss, and history of live births), medical and psychiatric comorbidities. Chi-square, ANOVA, independent sample t Tests, and simple logistic regressions were calculated for EPDS scores and positive screens.

**Results:** Of the 807 patients in our database, 666 (83%) met the inclusion criteria with 267 (40%) of patients evaluated during the COVID pandemic. Overall, the rate of positive depression screens was unchanged during the pandemic (15.5% vs 13.9%) compared to pre-pandemic, and was unaffected by patient race or income. In the secondary analysis, patients with living children had a lower odds of screening positive for depression OR 0.55 (p=0.03, CI0.32-0.95, n=181) but exhibited an absolute increase in EPDS scores by +1.18 points (0.02-2.34, p=0.047) during the pandemic. Having no living children in combination with a history of SAB or RPL increased the odds of positive screens (OR1.67, 1.02-2.74, n=130 and OR2.38, 1.02-5.57, n=28 respectively). Additionally, patients with no medical comorbidities were more likely to screen positive OR 1.88 (1.14-3.09, n=429) for depression.

**Conclusion:** The prevalence of positive depression screens in our new patient population was unchanged during the COVID pandemic. Race or income did not affect the rate of positive screens. In our secondary analysis, having living children was identified as a potential protective factor for those with positive screens. Women with known risk factors of depression—particularly a history of spontaneous abortions or recurrent pregnancy loss—were at significant risk of screening positive for depression. Universal screening may help to identify psychological health needs, particularly when infertility treatment is delayed.

## W-262

**Disparities in SARS-CoV-2 Infection among Pregnant Women in a Large Integrated Healthcare System.** Michael J Fassett,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiaxiao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Darios Getahun.<sup>7</sup> <sup>1</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente West Los Angeles Medical Center; Keck School of Medicine, Los Angeles, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.

**Introduction:** Previous studies have demonstrated that the elderly, Latinas and African Americans are at increased risk for COVID-19 diagnosis. How these demographic characteristics may influence the risk of SARS-CoV-2 infection in pregnant women its consequences on pregnancy outcome is unclear. We examined how these characteristics may impact the risk of SARS-CoV-2 infection in pregnancy.

**Methods:** We performed a retrospective cohort study using data on 29,323 pregnant women extracted from the Kaiser Permanente Southern California electronic health records between April 6, 2020 through January 31, 2021. All women were universally tested for COVID-19 during pregnancy or upon admission to hospital by PCR-based tests performed prior to delivery. Multivariable logistic regression was used to estimate the odds ratios (OR).

**Results:** COVID-19 was diagnosed in 4% (1,185/29,323 of singleton pregnancies). Compared with women aged 25-29 years, women aged 18-24 were more likely to test positive (adj.OR: 1.29, 95% CI: 1.08-1.54) and women aged  $\geq 30$  years were less likely to test positive (adj.OR: 0.71, 95% CI: 0.61-0.82). Latinas were 2.13-fold (95% CI: 1.78-2.56) more likely to test positive than Whites. Compared to Whites, we observed numerically lower test positivity among Asian/Pacific Islanders and higher test positivity among Blacks, neither of which achieved statistical significance. As compared with nulliparous women, para 2 (adj. OR: 1.19, 95% CI: 1.04-1.38) or para  $\geq 3$  (adj. OR: 1.60, 95% CI: 1.36-1.87) were more likely to test positive. Prenatal SARS-CoV-2 infection showed a dose-response pattern among overweight (adj. OR: 1.34, 95% CI: 1.14-1.58), obese (adj. OR: 1.47, 95% CI: 1.23-1.76), and morbidly obese (adj. OR: 1.55, 95% CI: 1.28-1.88) women compared to normal weight women. Although underweight women were 33% less likely than normal weight women to test positive, results did not reach statistical significance.

**Conclusion:** The findings indicate the presence of SARS-CoV-2 infection disparities by maternal age, race/ethnicity, and parity. A dose-response relationship was seen for prenatal SARS-CoV-2 infection by parity and pre-pregnancy BMI categories.

## W-263

**Contraceptive Access for US Women in the Era of COVID19.** Lynae Maria Brayboy,<sup>1,2,3</sup> Rachel Michel†,<sup>4</sup> Amanda Shea,<sup>1</sup> Virginia Vitzthum\*,<sup>4,5</sup> <sup>1</sup>Clue by Biowink, Berlin, Germany, Germany; <sup>2</sup>Charité-Universitätsmedizin Berlin, corporate member of Freie Universi, Berlin, Germany, Germany; <sup>3</sup>Alpert Medical School of Brown University Providence, RI USA, Providence, RI, United States; <sup>4</sup>Indiana University, Bloomington, IN, United States; <sup>5</sup>Kinsey Institute, Bloomington, IN, United States.

**Introduction:** Women in the US have high rates of unintended pregnancy ranging from 45-69%. Prior to the pandemic 65% of US women aged 15-49 were using contraception. The effects of the pandemic include job and income loss, and a strain on mental health. Therefore, our team designed a survey to assess the impact of the COVID-19 pandemic on contraceptive access for Clue Period Tracking app users. Our hypothesis was that there is an increased need for alternative forms of contraception in the backdrop of job loss and in-person healthcare access.

**Methods:** Clue users were sent in-app messages prompting them to participate in the survey. Participants had to live in the USA, be 18 year of age or older, and speak English or Spanish. The survey was sent to 550,000 users in June 2020.

**Results:** The June 2020 survey was initiated by n=18,712 Clue users and completed by n=12,021 who affirmed that they had taken the survey seriously. The mean age was 27.36 years old and represented a range of ethnicities including 67.9% White, 12.8 % Latina or Hispanic, 6.4% biracial, 5.9% African-American or Black, 4.6% Asian-American, 0.6% Native American/Alaska native, 0.3% Native Hawaiian or Pacific Islander. 92% of the respondents had at least some education beyond high school, and 8.5% had no health insurance in 2019 vs 9.3% in 2020. 32% of the respondents used The Pill, other hormonal methods included the Hormonal IUD, Patch, Implant, Injection, Ring (28%), Condoms (25%), and Copper IUD (15.5%). 44% of respondents reported needing to get birth control between March and July 2020. Of those, 22% said there was no change in access to birth control as compared to 2019. While more than 4% could not get their preferred method, 7.1% needed contraception at some point, but did not have it. 3.3% reported using emergency contraception or had a pregnancy termination no matter the contraceptive method. 2.4% found it difficult or very difficult to get their method as compared to 2019.

**Conclusion:** Our initial results don't show that Clue users' access to contraception was impacted by the COVID-19 pandemic. However, this baseline survey was early in the pandemic. Potential confounders include education level and financial means. This survey also shows that 25% of women relied on condoms for contraception indicating a need for alternatives to hormonal contraceptives. The reports of emergency contraception or having a termination highlights that even in a global pandemic women still need access. We plan to continue sampling our survey responders to determine how contraceptive access may change.

## T-001

**Development and Validation of a Quantitative Assay for Neurosteroids and Steroid Hormones in Pregnancy.** Gabriella Mayne†,<sup>1</sup> Erik De Bloois†,<sup>2</sup> Dana Dabelea,<sup>2</sup> K. Joseph Hurt,<sup>3</sup> Uwe Christians\*. <sup>1</sup>University of Colorado, Denver, CO, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States; <sup>3</sup>University of Colorado School of Medicine, Aurora, CO, United States.

**Introduction:** Neurosteroids play critical roles during pregnancy for both maternal and fetal health. Stress-reactive neurosteroids are reportedly low in women with perinatal depression and may be associated with poor pregnancy outcomes in animal models. Neurosteroids are present at low serum concentrations, and a major research obstacle has been reliable methods for quantification especially for clinical/translational investigation. A simple selective assay for neurosteroids would allow diagnostic studies in pregnancy. Here we present a high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) assay to quantify allopregnanolone (3 $\alpha$ -hydroxy-5 $\alpha$ -pregnan-20-one), pregnanolone (3 $\alpha$ -hydroxy-5 $\beta$ -pregnan-20-one), epipregnanolone (3 $\beta$ -hydroxy-5 $\beta$ -pregnan-20-one), pregnenolone, progesterone, cortisol and cortisone in pregnancy.

**Methods:** We extracted serum analytes by methanol precipitation and separated via HPLC (Agilent Poroschell 120, 50 x 4.6 mm, 2.7  $\mu$ m particle size, EC-C18 analytical column) followed by identification and quantification using MS/MS. We used Clinical Laboratory Standards Institute and Food and Drug Administration (FDA) guidelines for bioanalytical assay validation to assess recovery, selectivity, lower limit of quantification, accuracy and precision (%CV), and to evaluate matrix effect and stability. Data analyses were performed using Analyst 1.6.2 and Sciex OS-MQ 1.6.1, with t-tests for log transformed patient data. We analyzed charcoal-stripped and regular human serum for assay development and quality controls (N=6). To test the assay in clinical samples we analyzed serum obtained from pregnant women (N=54).

**Results:** Extraction recovery was >95% for all analytes and there was no significant ion interference, demonstrating adequate selectivity. Range of quantification for allopregnanolone, pregnanolone and epipregnanolone was 0.78 ng/mL to 100 ng/mL, and for pregnenolone, progesterone and cortisone it was 1.56 ng/mL to 400 ng/mL. For cortisol it was 3.91 ng/mL

to 1,000 ng/mL. Accuracy was  $101.6\% \pm 5.5\%$  and  $102.6\% \pm 6.5\%$  for intraday and interday, respectively. Intraday %CV was  $9.5\% \pm 3.0\%$  and interday %CV  $11.5\% \pm 4.1\%$ . We evaluated matrix effects and accepted results which met FDA criteria. We demonstrated sample stability up to seven days at 4°C.

**Conclusion:** We developed a fast selective HPLC-MS/MS assay for validated simultaneous quantification of serum neurosteroids and steroid hormones in pregnancy. This assay may be useful in clinical pregnancy research and investigations.

## T-002

**Preeclampsia Is Associated with Increased Levels of Intrinsically Disordered Protein - Prostate Associated Gene (PAGE)-4.** Aslı Özmen†, Millena Levin, Alexa Taylor, Duygu Mutluay, Xiaofang Guo‡, Nihan Semerci†, Ozlem Guzeloglu Kayisli, Frederick Schatz, Charles Lockwood, Umit Kayisli. *University of South Florida, Morsani College of Medicine, Tampa, FL, United States.*

**Introduction:** Most proteins require a stable globular 3D structure to be functional. However, intrinsically disordered proteins (IDPs) are biologically active without stable 3D structures. IDPs regulate a wide range of cell functions involved in intracellular signaling, transcription, translation and the cell cycle. A member of Cancer/Testis Antigen family (CT-X antigens), PAGE4 is an IDP inflammatory stress-response protein expressed in the testis, prostate, and placenta. Our global RNAseq analysis revealed significantly increased expression in the CT-X family members PAGE4, XAGE2 and XAGE3 in placentas from patients with preeclampsia (PE) vs. gestational age (GA)- matched controls. Thus, we investigated PAGE4 expression in placentas from early pregnancy and compared PAGE4 levels in 3<sup>rd</sup> trimester normal and GA-matched PE placentas.

**Methods:** Paraffin sections from placentas of early pregnancy (n=2) or from PE and GA-matched idiopathic preterm birth controls (n=5/group) were immunostained for PAGE4. qPCR (n=7/group) and immunoblot (n=8/group) were performed in PE and GA-matched idiopathic preterm birth control placentas. Histologic score (HSCORE) was performed to evaluate total and nuclear PAGE4 levels whereas densitometric readings evaluated immunoblot bands. Results of qPCR were calculated using  $2^{-\Delta\Delta Ct}$  values. Data were compared by a *t*-test with  $P < 0.05$  considered statistically significant.

**Results:** In early pregnancy specimens, PAGE4 immunoreactivity is specifically expressed by villous cytotrophoblasts, but not by syncytiotrophoblasts and revealed a gradual decrease from proximal to distal trophoblastic columns in the anchoring villi. Villous cytotrophoblasts displayed both cytoplasmic and nuclear PAGE4 immunoreactivity with a significantly higher levels of nuclear PAGE4 HSCORE (Mean± SEM:  $219.84 \pm 14.79$  vs.  $130.23 \pm 17.46$ ;  $P=0.007$ ) in PE vs. control placentas. Immunoblot results confirmed significantly higher PAGE4 levels in PE vs. control placentas ( $143.37 \pm 22.70$  vs.  $86.99 \pm 7.85$ ;  $P=0.034$ ). These results were further confirmed by qPCR that PAGE4 mRNA expression is higher in PE vs. control placentas ( $2.75 \pm 0.56$  vs.  $1.04 \pm 0.16$ ;  $P=0.002$ ).

**Conclusion:** This is the first study showing PAGE4 upregulation in PE and suggesting that IDPs contribute to PE pathogenesis, likely in response to inflammatory stress. Moreover, a gradual decrease in PAGE4 expression from proximal (proliferative) to distal (invasive) trophoblasts of anchoring villi suggests a role for PAGE4 involving in proliferation. Further studies evaluating the cause-and-effect relationship of increased PAGE4 in trophoblasts will clarify its pathogenic vs. protective role(s) in PE pathogenesis.

## T-003

**Gardnerella vaginalis, but Not Lactobacillus crispatus, Disrupts Cervicovaginal Epithelial Cell Function and Immune Response through TLR2 Activation.** Lauren Anton, Amy G Brown, Kristin Gerson, Michal Elovitz\*. *University of Pennsylvania, Philadelphia, PA, United States.*

**Introduction:** Overabundance of *Gardnerella vaginalis* (GV) in the cervicovaginal (CV) space is associated with adverse clinical outcomes such as STIs, bacterial vaginosis and preterm birth, while a dominance of *Lactobacillus crispatus* (LC) is indicative of a healthy vaginal

environment. While we have shown that GV microbial supernatant disrupts the cervical epithelial barrier, the mechanisms regulating the effects of live bacteria on CV epithelial cells remain unclear. The study objective was to elucidate the host-microbiota interactions between GV and cervical/vaginal epithelial cells by investigating the effect of 1) LC and GV on the CV epithelial barrier and host immune response and 2) the role of the TLR2 receptor in perpetuating these effects.

**Methods:** Human ectocervical (Ect1/E6E7, Ecto), endocervical (End1/E6E7, Endo), vaginal epithelial cells (VK2) and HEK TLR2 reporter cells were co-cultured with LC (ATCC 33197) or GV (ATCC 14019) ( $\sim 1 \times 10^6$  -  $1 \times 10^4$  CFU/well) for 24 hours either alone or pre-treated with a monoclonal anti-TLR2 antibody (10 ug/ml). Bacterial abundance was quantified by CFU assays. Cell permeability assays (n=6) were performed following exposure to live bacteria with or without antibody. Media was used for LDH cytotoxicity assays (n=9) and measurement of cytokines/MMPs by Luminex (n=3). NFκB activation (n=6-9) and IL-8 (n=6-9) up-regulation in HEK TLR2 cells was measured using QuantiLuc and QuantiBlue assays.

**Results:** GV increased cell permeability in a dose-dependent manner in ecto ( $p < 0.0001$ ), endo ( $p < 0.0001$ ) and VK2 ( $p < 0.0001$ ) cells while LC had no effect. GV but not LC increased cytotoxicity ( $p < 0.0001$ ) in all three cell lines. Significant increases in 18 (out of 25 detectable) cytokines including IL-6, IL-8, IL-1a/b, IL-1RA, TNFα, MMP9 were observed after exposure to GV but were mostly unchanged with LC in ecto, endo and VK2 cells. LC and GV induced NFκB activation ( $p < 0.0001$ ) but only GV increased IL-8 ( $p < 0.0001$ ) in HEK TLR2 reporter cells. Pre-treatment with the anti-TLR2 antibody significantly mitigated: 1) GV-induced cytokine expression in ecto, endo and VK2 cells and 2) GV induced NFκB and IL-8 activation in HEK TLR2 cells.

**Conclusion:** Live GV reduces CV epithelial barrier integrity, increases epithelial cell death and initiates immune activation in host CV epithelial cells through TLR2-dependent mechanisms. These results provide evidence of a regulated and complex host-microbe response in the CV space that is both bacteria and epithelial cell type specific. Further investigation into CV host-microbial interactions will undoubtedly lead to new therapeutic approaches for adverse reproductive outcomes.

## T-004

**Is There a Threshold of Inflammation Needed to Trigger Preterm Labor?** M Cappelletti†, P Presicce†, F Ma†, P Senthamaikannan†, L Miller\*, M Pellegrini\*, A Jobe\*, S Divanovic\*, SS Way\*, C Chougnet\*, S Kallapur\*. <sup>1</sup>UCLA, Los Angeles, CA, United States; <sup>2</sup>Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, United States; <sup>3</sup>UCD, Davis, CA, United States; <sup>4</sup>University of California Los Angeles, Los Angeles, CA, United States.

**Introduction:** Intrauterine infection/inflammation (IUI) is a major contributor to preterm labor (PTL). However, IUI does not invariably cause PTL. We hypothesized that quantitative and qualitative differences in immune response exist in subjects with or without PTL. To define the triggers for PTL, we developed Rhesus macaque models of IUI with or without infection.

**Methods:** Groups of Rhesus macaques (*Macaca mulatta*) were delivered at ~135d gestational age (80-85% term gestation): 1. Intraamniotic (IA) saline (controls, n=15); 2. IA injection of lipopolysaccharide (LPS; *E. coli* O55:B5 Sigma, 1mg) (n=7); 3. IA live *E. coli* ( $10^6$ CFU) (n=13). A subgroup of IA *E. coli* animals were given antibiotics (Abx) (n=8) to understand the residual effects after elimination of live pathogen. Fetuses were delivered surgically 48h-72h after injections if not delivered vaginally and PTL was diagnosed with cervical changes. Amniotic fluid (AF) was collected for assessments of inflammation. RNASeq analysis was performed on Chorion-amnion decidua (CAD) tissues.

**Results:** PTL frequency was 0/15 in controls, 0/7 in LPS, 5/5 in *E. coli* without Abx and 5/8 in *E. coli* animals+ Abx. AF PGE2 level was significantly higher in *E. coli* compared to LPS animals (Fig. 1). RNASeq analysis revealed upregulation of specific pathways in the *E. coli* group compared to the LPS group (Fig. 2). Additionally, comparison within IA

*E. coli* animals with and without PTL showed significant increase in gene expression associated with inflammatory responses, specifically NF- $\kappa$ B signaling, IL-6 production and type I Interferon response.

**Conclusion:** Induction of PTL was correlated with both quantitative and qualitative changes in the intensity of the immune response to IUI. These IUI macaque models with or without PTL offer unique opportunities to unravel the IUI-specific pathways responsible for induction of PTL.

### T-005

**Immune Regulation in the Cervicovaginal Environment in Women with High Risk of Delivering Preterm.** Amirah Mohd Zaki<sup>†</sup>,<sup>1</sup> Alicia Hadingham,<sup>1</sup> Flavia Flaviani,<sup>1,2</sup> Deena Gibbons\*,<sup>1</sup> Yasmin Haque,<sup>1</sup> Jia Dai Mi,<sup>1</sup> Debbie Finucane,<sup>1</sup> Giorgia DallaValle,<sup>1</sup> Mansoor Saqi\*,<sup>2</sup> Rachel M. Tribe\*,<sup>1</sup> <sup>1</sup>King's College London, London, United Kingdom; <sup>2</sup>Guy's and St. Thomas' NHS Foundation Trust and King's College London, London, United Kingdom.

**Introduction:** There is a well reported ethnic disparity in spontaneous preterm birth (sPTB) rates both in the UK and elsewhere. Subsequently, there is growing interest in how the vaginal microbiome of women may differ by ethnicity and the underlying causes. The microbiome is a balance between the host immune response, the microorganisms present and the influence of other external factors. The aim of this study was to explore potential ethnic differences in the phenotype and transcriptome of immune cells in the cervix in pregnant women (stratified by self-reported ethnicity; White or Black African/Caribbean) at high risk of sPTB.

**Methods:** Cervical cytobrush samples were obtained from pregnant women attending a London prematurity clinic (INSIGHT study cohort). Single cell suspensions were stained with antibodies (CD45-APC, CD3-AF700, CD19-BV786, CD56-BV650, CD66b-FITC, CD14-BV421, CD16-PECy7 and HLADR-PE) for flow cytometry and analysed by FlowJo (n=30, White; n=20, Black). A subset of samples were FACS sorted for neutrophils (CD45<sup>+</sup>CD19<sup>-</sup>CD56<sup>+</sup>CD66b<sup>+</sup>) and prepared for RNA sequencing (RNA-seq). Extracted RNA was sequenced using the BGI platform BGISEQ-500 (White n=7; Black n=3 samples passing quality controls). RNA-seq samples were analysed using the bioinformatic tools hisat2, samtools, featureCounts and DESeq2.

**Results:** No significant differences in the percentage of T cells (p=0.90), B cells (p=0.99), NK cells (p=0.44), neutrophils (p=0.11) or monocytes (p=0.98) were observed in cervical samples between the two ethnic groups. In contrast, PCA plots of RNAseq data showed some ethnic differences; data from White women clustered closely together, whereas neutrophils from Black women were more scattered. 15,982 RNAs were expressed with 172 upregulated and 232 downregulated in neutrophils from white women versus black women. IL5RA, SOCS6 and HRH1 were in the top 10 genes that were upregulated. Ingenuity pathway analysis predicted an increased activation in immune related networks in neutrophils from White women.

**Conclusion:** Variations in the cervicovaginal environment are evident in women of different ethnicities. Given the disparity in sPTB rates in UK Black versus White women, the influence of immune cell function, in particular neutrophils, warrants further exploration.

### T-006

**Full Length Sequencing of Cervicovaginal Microbiota Associated with Spontaneous Preterm Birth.** Neha Satish Kulkarni<sup>†</sup>, Megan Cavanagh<sup>†</sup>, Emmanuel Amabebe<sup>†</sup>, Dilly OC Anumba\*. *University of Sheffield, Sheffield, United Kingdom.*

**Introduction:** Due to the inconsistencies in the relationship between vaginal microbial community composition and risk of spontaneous preterm birth (PTB). In order to determine which databases best identified PTB-associated microbiota, we characterised the vaginal microbiota of both term and preterm-delivered, predominantly Caucasian, women using a novel sequencing platform and five bacterial 16S rRNA sequence databases. Because not all the databases have complete taxonomy ranks, database curation and latest versions, comparison of the five databases was performed to ensure complete assessment of vaginal bacterial diversity in pregnant women.

**Methods:** The full length of bacterial 16S rRNA gene in cervicovaginal fluid samples of asymptomatic pregnant women (20-22 weeks' gestation) who delivered at term (n=16) and preterm (n=16) were sequenced using the Oxford nanopore MinION. The sequences were mapped and clustered into Operational Taxonomic Units (OTUs) using QIIME tool on Greengene, SILVA, RDP, EzBioCloud and NCBI databases individually. The relative abundance of bacterial species between the cohorts was compared by Wilcoxon rank sum test, and further expressed as a fold change (cut-off  $\pm 2$ ).

**Results:** Only *Lactobacillus vaginalis* and *Atopobium vaginae* were identified by all 5 databases. Only SILVA (p<0.001) and Greengene (p=0.03) showed significant differences in relative abundance of bacterial species between term and preterm-delivered women. These databases showed that *L. vaginalis*, *L. iners* and *L. jensenii* were more abundant in the preterm group, while *L. gasseri* and *L. acidophilus* were more abundant in the term group. Preterm birth was associated with more abundance of bacterial vaginosis-associated (e.g. *Shuttleworthia*, *P. amnii*, *Ureaplasma* etc.) and aerobic vaginitis-associated (e.g. *E. coli*, *Enterococcus*, *Streptococcus* and *Staphylococcus*) species. However, other BV-associated bacteria i.e. *A. vaginae* and *Dialister* sp. were more abundant in the term group.

**Conclusion:** These preliminary results indicate that SILVA and Greengene databases appear more useful in characterising the vaginal microbial community composition of this cohort as sequenced by nanopore MinION. Our observations regarding the relative abundance of *Lactobacillus* species and BV-associated anaerobes are consistent with our previous reports and those of others. The apparent abundance of other BV-associated bacteria in the term group and the microbial-metabolite interactions which influence the risk of preterm birth warrant further studies.

### T-007

**Administration of Statins Preceding the Maternal Exposure to Lipopolysaccharide Results in Anti-Inflammatory Effects in Fetal Brain.** Egle Bytautiene Prewit<sup>†</sup>, Mulampurath Achuthan Pillai Sureshkumar,<sup>1</sup> Tia Pearcy,<sup>1</sup> Mauricio F. La Rosa,<sup>2,3</sup> Maged Costantine.<sup>4</sup> <sup>1</sup>UT Health San Antonio, San Antonio, TX, United States; <sup>2</sup>UTMB, Galveston, TX, United States; <sup>3</sup>Universidad Peruana Cayetano Heredia, Lima, Peru; <sup>4</sup>The Ohio State University, Columbus, OH, United States.

**Introduction:** Inflammation is believed to be the primary driver of preterm birth and the associated neonatal complications, including neurological. Prevention of adverse neonatal outcomes remains a significant goal. Statins are known for their anti-inflammatory effects. We hypothesize that statins administered before lipopolysaccharide (LPS)-induced maternal inflammation will reduce pro-inflammatory (IL-6, IL-1 $\beta$ , TNF- $\alpha$ ) and increase anti-inflammatory cytokines (IL-4, IL-10) in fetal brain in a mouse model.

**Methods:** Day 15 pregnant CD-1 mice were randomly allocated for intraperitoneal injection with either simvastatin (SIM/LPS, n=9), pravastatin (PRAV/LPS, n=8) (both at 10  $\mu$ g/g of body weight in saline), or saline (SAL/LPS, n=9, positive control) 2 hours prior to administration of LPS (250  $\mu$ g in saline). Dams who received saline injections at both time points (SAL/SAL, n=9) served as negative controls. Six hours after the last injection, dams were euthanized, and fetal tissues collected. After determination of fetal gender, protein extraction was performed from the brains of 1 male and 1 female pup per dam. Concentrations for IL-6, IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and IL-10 in maternal plasma and fetal brain were measured using MILLIPIXEL multiplex immunoassays and analyzed by gender using One-Way or Kruskal-Wallis ANOVA followed by Dunn's multiple comparisons test (statistical significance: P<0.05).

**Results:** The concentrations of IL-6 (P=0.0005), IL-1 $\beta$  (P=0.01) and TNF- $\alpha$  (P=0.01) were significantly elevated in maternal plasma in SAL/LPS group in comparison to SAL/SAL dams. With SAL/SAL controls, administration of LPS significantly increased concentrations of IL-6 (males P=0.04, females P=0.03), TNF- $\alpha$  (males P=0.02, females P=0.04) and decreased quantities of IL-4 (females P=0.02) and IL-10 (males P=0.02, females P=0.03) in fetal brain. Treatment with pravastatin significantly decreased the brain concentrations of IL-6 (P=0.02) and TNF- $\alpha$  (P<0.0001) in male pups, IL-6 (P=0.03) in female pups, and

increased IL-4 ( $P=0.04$ ) in female pups as compared to SAL/LPS control. There were no statistically significant differences in the cytokine levels with maternal exposure to simvastatin. IL-1 $\beta$  in fetal brains was not affected by any treatment.

**Conclusion:** Administration of statins, especially pravastatin, before the exposure to maternal inflammation appears to suppress pro-inflammatory and increase anti-inflammatory cytokine quantities in the fetal brain. We propose a novel role for statins as prevention of fetal brain inflammation.

## T-008

**Increased Pro-Inflammatory Signaling of Stem Cells Correlating with the Phenotype of Uterine Fibroids.** Hoda ElHossiny Elkafas<sup>†,1,2</sup>, Mohamed Ahmed ALI<sup>†,1,3</sup>, Lauren Prusinski Fernung<sup>†,4</sup>, Sribalashubhini Muralimanoharan<sup>†,5</sup>, Hailian Shen<sup>†,5</sup>, Thomas G. Boyer<sup>\*,5</sup>, Nahed Ismail<sup>\*,1</sup>, Ayman Al-Hendy<sup>\*,1</sup>, Qiwei Yang<sup>\*,6</sup>. <sup>1</sup>University of Illinois at Chicago, Chicago, IL, United States; <sup>2</sup>Egyptian Drug Authority (EDA) formerly (NODCAR), Cairo, Egypt; <sup>3</sup>Faculty of Pharmacy, Ain Shams University, Cairo, Egypt; <sup>4</sup>Augusta University, Augusta, GA, United States; <sup>5</sup>University of Texas Health Science Center at San Antonio, San Antonio, TX, United States; <sup>6</sup>University of Texas Health Science Center at San Antonio, Chicago, IL, United States.

**Introduction:** Uterine fibroids (UFs) are a benign monoclonal tumor of the myometrium (MM) and recognized as the most prevalent gynecologic tumor among reproductive-age women. It is believed that abnormal myometrial stem cells (SCs) are the origin of UFs. However, the molecular mechanism underlying the conversion of normal myometrial SCs (MMSCs) to UF stem cells (UFSCs) is mostly unknown. We hypothesize that activation of pro-inflammatory pathways in SCs will create a microenvironmental niche contributing to the development of UFs.

**Methods:** Tissue microarray (TMA) of three matched pairs of UFs tissues (both *MED12* wild type and *MED12* mutant UFs), and adjacent MM tissues were used for immunohistochemical analysis of inflammatory pathway activation. Genome-wide RNA-seq analysis of isolated Stm1/CD44 stem cells from UFs (N=3) and matched MM (N=3) was performed to determine global gene expression profiles. Ingenuity Pathway Analysis (IPA) was used to determine the perturbed gene network linking to the inflammatory pathway. Additionally, the 65-plex Human Cytokine/Chemokine Discovery assay was performed to determine the secreted cytokine profiling between MMSCs and UFSCs. EdgeR (pair-wise comparisons) was used for statistical analysis of RNA-seq data.

**Results:** IHC-TMA staining showed significant upregulation of TSLP, IL1a, IL-1b, TNFa and INFg in both UF tissue (WT and *MED12* mutant UFs) as compared to adjacent MM tissues ( $P<0.05$ ). At the stem cell level, IPA of RNA-seq data demonstrated that the 12 inflammatory genes were upregulated ( $> 5$  folds change) in UFSCs relative to matched adjacent MMSCs. The production of 65 secreted cytokines showed a biologically significant increase in the expression of inflammatory mediators in UFSCs compared to MMSCs. Moreover, the output of (GM-CSF, G-CSF, IL-8RANTES, MCP-1, MCP-3, IP-10, IL-6). (\*\* $P<0.01$ ) are increased in UFSCs as compared to MMSCs.

**Conclusion:** These data strongly suggest that pro-inflammatory pathways are activated in UFs. Chronic inflammation plays a vital role in the conversion of MMSCs to UF initiated cells/UFSCs, resulting in UF-inducing niche, therefore leading to UF pathogenesis.

## T-009

**In Utero Exposure to *Ralstonia Insidiosa* Occurs during Normal Pregnancy and May Promote Tolerance.** Sonam Verma<sup>†,1</sup>, Rachel B Silverstein<sup>1</sup>, Elaine Parker<sup>1</sup>, Lindsay A Parnell<sup>1</sup>, Chetan S Joshi<sup>1</sup>, Matthew J Wargo<sup>2</sup>, Indira U. Mysorekar<sup>\*,1</sup>. <sup>1</sup>Washington University SOM, St. Louis, MO, United States; <sup>2</sup>University of Vermont Larner College of Medicine, Burlington, VT, United States.

**Introduction:** There is ongoing controversy regarding the presence or absence of microbes in the placenta and its possible effect on the developing fetus. Previous work in our lab has established the presence of bacteria within the placental basal plate, leading to rethinking of the sterile womb dogma. Bacteria were found localized to intracellular single-membrane vacuoles within trophoblast cells in term placentas.

16S rRNA sequencing confirmed the presence of gram-negative bacteria, *Ralstonia insidiosa* (*R. i*), within the basal plate. In this study, we sought to characterize the location and function of *R. i* at the maternal-fetal interface, to elucidate the effect of *R. i* on trophoblast cell viability and activity; and to assess whether *R. i* play has an adverse or beneficial role within the gravid intrauterine environment.

**Methods:** *R. i* presence and abundance within term placenta was investigated using species-specific bacterial probes and fluorescent in situ hybridization. JEG-3 cells were challenged with *R. i* in a dose dependent manner to study the impact on cell survival, invasion and pro-inflammatory responses in the trophoblasts. Pregnant mice were challenged with *R. i* to determine whether *R. i* home to the placenta and affect parturition. Potential cross talk between trophoblasts cells treated with *R. i* and decidual NK (dNK) cells was analyzed. Primary dNK cells were isolated from term basal plates and challenged with conditioned supernatant from primary extravillous trophoblast cells treated with *R. i* at differing multiplicities of infection. dNK cells HLA-G and KIR2DL4 expression was analyzed by flow cytometry and cytokine secretion (IL-8) measured using ELISA.

**Results:** We identify *R. i* as a bona fide resident at the maternal-fetal interface by species-specific FISH probes in term basal plate tissue. Furthermore, our studies provide *in vivo* evidence that viable *R. i* colonize and replicate within the murine placenta following intrauterine administration but did not lead to preterm birth. *R. i* challenge of JEG-3 cells did not induce apoptosis or induce pro-inflammatory responses. Excitingly, primary dNK cells challenged with conditioned media from *R. i* treated trophoblasts demonstrated elevated HLA-G expression in EVTs as well as IL-8 in the dNK cells, both of which are associated with immunotolerance.

**Conclusion:** Collectively, our findings demonstrate that *R. insidiosa* does not induce pro-inflammatory responses but may promote immune tolerance and support normal pregnancy outcomes. Our studies provide a foundation for understanding possible 'commensal' microbial-placental interactions and hint at the functional importance of *R. insidiosa* at the fetal-maternal interface in maintaining placental health and supporting fetal tolerance.

## T-010

***Gardnerella Vaginalis* Promotes Features of Epithelial-Mesenchymal Transition in the Cervicovaginal Space: Novel Pathways Underlying Premature Cervical Remodeling in Spontaneous Preterm Birth.** Kristin D Gerson<sup>†</sup>, Yusra Gimie, Lauren Anton, Michal A Elovitz<sup>\*,1</sup>. University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, United States.

**Introduction:** Bacterial vaginosis (BV), for which *Gardnerella vaginalis* (GV) is one of the main causative microbes, has long been associated with spontaneous preterm birth (sPTB). Clinical trials targeting eradication of BV have failed to decrease sPTB rates, likely in part due to their inability to target key molecular effects of GV. We previously reported that microbial supernatants from GV disrupt the cervical epithelial barrier. We hypothesize that GV promotes cervicovaginal (CV) epithelial barrier disruption through induction of epithelial-mesenchymal transition (EMT).

**Methods:** Vaginal (VK2), ectocervical (ecto), and endocervical (endo) cells were cultured in monolayer. Microbial supernatants from GV and *Lactobacillus crispatus* (LC), a healthy component of the CV ecosystem, were applied to cells for 48 hours. Supernatant media TSB-RB and NYC-III served as additional controls for GV and LC, respectively. Relative RNA abundance of EMT markers, including SNA11, ZEB1, and MMP-9, were quantified by qPCR and normalized to 18S. Statistical analyses using two-way ANOVA were performed to establish significance ( $p<0.05$ ).

**Results:** GV microbial supernatants increased expression of SNA11 ( $p=0.0004$ ), ZEB1 ( $p<0.0001$ ), and MMP-9 ( $p<0.0001$ ) in VK2 cells compared to non-treated, microbial, and media controls. Upregulation of MMP-9 by GV supernatants occurred in ecto ( $p<0.0001$ ) and endo ( $p<0.001$ ) cells compared to all controls. We observed a more potent effect on the induction of MMP-9 in VK2 cells vs ecto ( $p=0.005$ ) and endo ( $p<0.001$ ) cells. Induction of MMP-9 was not different between ecto and endo cells ( $p=0.116$ ).

**Conclusion:** Microbial supernatants from GV induce features of EMT, including upregulation of a protease that may comprise cervical integrity. Vaginal epithelial cells, the first line of defense against opportunistic microbes, mount the most robust response to GV microbial supernatants. These results demonstrate unique host-microbe interactions based on specific CV cell type. It is biologically plausible that MMP-9 produced by the vaginal epithelium in response to microbial supernatants induces breakdown of cervical epithelial cells and stroma without microbes directly contacting the cervix. Further research exploring the host-microbial interactions by cell type in the CV space may enhance our understanding of premature cervical remodeling and sPTB. (PENN MOD PRC)

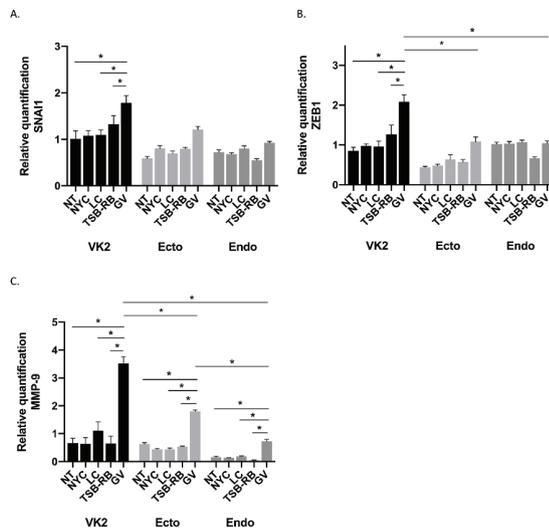


Figure 1. Relative mRNA quantification of EMT markers in vaginal and cervical cells. A. Relative quantification of SNAI1. B. Relative quantification of ZEB1. C. Relative quantification of MMP-9.

## T-011

**Myometrial-Derived CXCL12 Promotes and the CXCR4 Antagonist AMD3100 Prevents LPS Induced Preterm Labor by Regulating Macrophage Migration, Polarization and Function in Mice.** Ramanaiyah Mamillapalli, Lijuan Zhang, Shutaro Habata, Taylor S Hugh\*. Yale University School of Medicine, New Haven, CT, United States.

**Introduction:** Preterm birth is a major contributor to neonatal mortality and morbidity. Infection results in elevation of inflammatory cytokines followed by infiltration of immune cells into gestational tissue. These immune cells express CXCR4, a chemokine receptor for the ligand CXCL12. CXCL12 levels are elevated in preterm birth indicating it may have a role in preterm labor (PTL), however the pathophysiological correlations between CXCL12/CXCR4 signaling and premature labor are poorly understood. Pharmacological agents for PTL prevention frequently fail to prevent PTL. A better understanding of the molecular mechanisms involved in PTL, including the role of CXCL12/CXCR4, may allow the development of new therapeutic agents.

**Methods:** PTL was induced using LPS in a murine model. LPS was given by i.p to pregnant mice (N=8 per group) on gestational day 15.5 while PBS was used as a control. AMD3100 given 30 min before LPS injection and again after 3 hrs. Six hours after induction of PTL samples (blood, uterus, placenta and decidua etc.) were collected for further analysis. Experiments were also carried using macrophages collected from the abdomen of pregnant mice on day 15.5 using conditional medium collected from primary smooth muscle cell culture. Total RNA was extracted by Trizol reagent for gene expression analysis using qRT-PCR; protein levels were analyzed by ELISA, IHC and IF studies. Migration studies carried out using transwell plates and cell counts.

**Results:** LPS induced both CXCL12 RNA and protein levels specifically in myometrium compared to controls (3-fold and 3.5-fold respectively,  $P < 0.002$ ). Highest levels were found just before the start of labor. LPS also enhanced the infiltration of neutrophils, macrophages and T cells at 6 h after LPS injection, and induced macrophage M1 polarization. In vitro studies showed that condition medium from LPS treated primary smooth muscle cells (SMC) induced macrophage migration, M1 polarization and upregulated inflammatory cytokines such as IL-1, IL-6 and IL-TNF- $\alpha$ . AMD3100 treatment in pregnant mice led to a significant decrease in the rate of preterm labor (70%), prolonged pregnancy duration (21 vs 20 days) and suppressed macrophage infiltration into gestation tissue by 2.5 fold. Further, in-vitro treatment of SMC by AMD3100 suppressed the macrophage migration, polarization and decreased expression of IL-1, IL-6 and IL-TNF- $\alpha$ .

**Conclusion:** LPS treatment in pregnant mice induced preterm labor by increasing myometrial CXCL12 which recruits immune cells that in turn produce inflammatory cytokines. These effects stimulated by LPS were completely reversed by AMD3100 through blocking of CXCL12/CXCR4 signaling. Thus, the CXCL12/CXCR4 axis presents an excellent target for preventing infection and inflammation-related PTL.

## T-012

**Metabolic Profiling of Gardnerella vaginalis: A Vaginal Dysbiosis and Preterm Birth-Associated Bacteria.** Georgia R May†, Emmanuel Amabebe†, Dilly O Anumba\*, Steven Reynolds\*. University of Sheffield, Sheffield, United Kingdom.

**Introduction:** Preterm birth (PTB) is still a major obstetric and global health challenge with genital tract infections (e.g. bacterial vaginosis, BV) and inflammation contributing substantially to its pathogenesis. *Gardnerella vaginalis* and its metabolites are increased in vaginal samples of patients with BV. Hence, we determined the metabolic profile of *G. vaginalis* by examining its metabolism of glucose, L-lactic acid (LAC), and D-lactic acid (DAC).

**Methods:** *G. vaginalis* (n=6) was cultured under anaerobic conditions for 48 hours and then sub-cultured with combinations of  $^{13}\text{C}_6$ -glucose,  $^{13}\text{C}_3$ -LAC, and  $^{13}\text{C}_3$ -DAC. Following 48 hours in culture,  $^{13}\text{C}$ -NMR spectra were acquired with Broth only and *G. vaginalis* cultured without  $^{13}\text{C}$ -substrates as controls. Data was normalised to the lactate integral from the conversion of broth glucose after 48 hours of incubation.

**Results:** Greater concentrations of  $^{13}\text{C}_6$ -acetate were produced from  $^{13}\text{C}_6$ -Glucose when in single substrate culture compared to that formed in joint culture with either enantiomer of  $^{13}\text{C}_3$ -lactic acid. Greater concentrations of  $^{13}\text{C}$ -lactate were produced from  $^{13}\text{C}_6$ -glucose when in single substrate culture compared to that formed in joint culture with  $^{13}\text{C}_3$ -LAC only. No statistically significant difference was found between metabolites formed from  $^{13}\text{C}_3$ -LAC compared to  $^{13}\text{C}_3$ -DAC. Observations of raw spectra also indicated minimal production of formate, succinate and ethanol.

**Conclusion:** The findings suggested preferential metabolism of  $^{13}\text{C}_6$ -glucose over either enantiomer of  $^{13}\text{C}_3$ -lactic acid during the production of acetate. Acetate is a marker of vaginal dysbiosis (BV), infection and preterm birth. Further studies are required to determine whether *G. vaginalis* out-competes *Lactobacillus* species for glucose metabolism.

Figure 1.

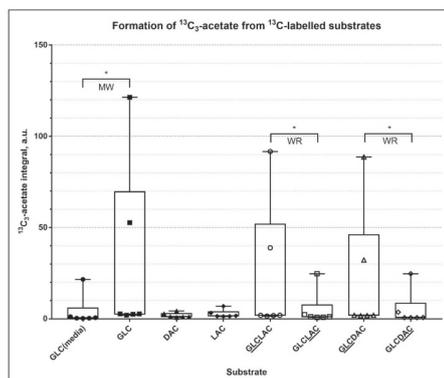
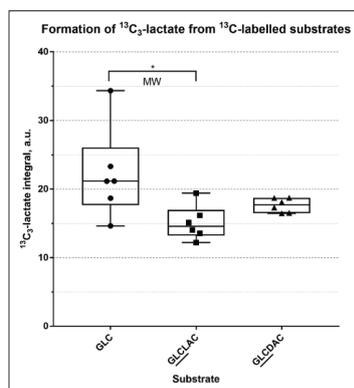


Figure 2.



### T-013

**Macrophage Density Is Increased in the Endocervix in Women at Term Compared to Preterm.** Sandra E Reznik,<sup>1</sup> Rachel Scales<sup>†</sup>,<sup>2</sup> Alison Yong<sup>†</sup>,<sup>1</sup> Gregory Dickinson<sup>†</sup>,<sup>3</sup> Pe'er Dar,<sup>3</sup> Steven M Yellon\*.<sup>2,1</sup> *St. John's University, Queens, NY, United States;* <sup>2</sup>*Loma Linda University, Loma Linda, CA, United States;* <sup>3</sup>*Albert Einstein College of Medicine, Bronx, NY, United States.*

**Introduction:** The cervix serves as the gate at the external maternal fetal interface for the developing pregnancy and remodels into the gateway for birth. In studies of rodent models and biopsies of the human cervix, reduced cell nuclei (CN) density and degradation of cross-linked collagen in the stroma are indices of inflammatory processes before birth at term. The novel approach in this study addresses the hypothesis that histomorphological structure and inflammation across subregions of cervix stroma are similar in women who deliver at term and preterm.

**Methods:** Hysterectomy specimens were obtained from multipara women with obstetrical complications at 32-34 weeks (Preterm not in labor, PT; n=4) or 37-39 weeks (Term, T; n=3, 2 post-induction and 1 not in labor). Blocks of tissue from the external cervical os to the lower uterus were processed and longitudinal sections were stained with methyl green for CN and picrosirius red (PSR) for collagen, and with antibodies to macrophages (M0s, CD68) and smooth muscle actin (SMA $\alpha$ ). CN and M0s were counted to assess density/area in the ectocervix, endocervix, vicinity of the internal os, and lower uterus. Optical density of PSR birefringence for collagen cross-linking and area of SMA stain/section were also assessed.

**Results:** CN and cross-linked collagen densities were similar in the stroma of the ectocervix, endocervix, and internal os subregions between PT versus T groups. However, in the T group, CN were less dense in the lower uterus versus ectocervix ( $P < .05$  ANOVA). No difference was found in density of M0s in the cervix stroma among all 4 subregions in the PT group ( $P > .05$  ANOVA). By contrast, M0s/area in the endocervix in the T group was increased compared to that in the ectocervix, internal os,

and lower uterus subregions, as well as versus the endocervix in the PT group ( $P < .05$  ANOVA). The percent area with SMA staining progressively increased from ectocervix to lower uterus in both groups. Moreover, the average SMA/area was the same in PT vs T groups ( $P > .05$  Student's t-test). **Conclusion:** These findings support the hypothesis that inflammation within subregions of the cervix are similar at term and preterm. However, at term, reduced CN density in the lower uterus vs ectocervix and abundance of M0s in the endocervix relative to other subregions and to endocervix at preterm support an alternative hypothesis. The data reveal that specific morphologic changes may distinguish remodeling in subregions of the lower reproductive tract of women at term from those who deliver preterm. This novel finding indicates that structural changes in the cervix associated with ripening before term may prove useful to evaluate risk of prematurity and identify a potential locus for anti-inflammatory therapeutic approaches to forestall preterm birth.

### T-014

**Differential Vaginal *Lactobacillus* Species Metabolism of Glucose, L- and D-lactate by <sup>13</sup>C-Nuclear Magnetic Resonance Spectroscopy.** Emmanuel Amabebe<sup>†</sup>, Dilly Anumba\*, Steven Reynolds\*. *University of Sheffield, Sheffield, United Kingdom.*

**Introduction:** Cervicovaginal dysbiosis can lead to infection/inflammation-associated spontaneous preterm birth. In conjunction with the host vaginal habitat, the vaginal bacteria produce unique metabolic by-products. In a healthy vagina, lactobacilli are the predominant species and have been linked to increased likelihood of term delivery. Although, lactobacilli are known to thrive by acidifying the vagina environment (pH < 4.5), the mechanism underpinning how lactobacilli, and other anaerobes, interact with each other through lactic acid metabolism remains unresolved. **Objective** To determine whether vaginal *Lactobacillus* species, *L. crispatus* and *L. jensenii*, differentially metabolise glucose, L- and/or D-lactic acid to propagate their survival/dominance, and prevent dysbiosis and infection-associated preterm birth.

**Methods:** *L. crispatus* and *L. jensenii* were incubated anaerobically for 24h at 37°C, with <sup>13</sup>C<sub>6</sub>-glucose, <sup>13</sup>C<sub>3</sub>-D-lactate or <sup>13</sup>C<sub>3</sub>-L-lactate (singularly or combined) for 24h. <sup>13</sup>C-spectra were acquired using a 9.4T NMR spectrometer. Metabolite integrals were normalised by the concentration of live bacteria present in the incubated samples i.e. the lactate integral produced from the conversion of broth glucose after 48 hours of incubation (pre and post addition of <sup>13</sup>C substrates).

**Results:** *L. crispatus* and *L. jensenii* (n=6 each) metabolised <sup>13</sup>C<sub>6</sub>-glucose to <sup>13</sup>C<sub>3</sub>-lactate and <sup>13</sup>C<sub>3</sub>-acetate. *L. jensenii* converted more <sup>13</sup>C<sub>3</sub>-D- or <sup>13</sup>C<sub>3</sub>-L-lactate to <sup>13</sup>C<sub>3</sub>-acetate than *L. crispatus*,  $p < 0.001$ . There was no significant difference in the amount of <sup>13</sup>C<sub>3</sub>-lactate produced by both species from <sup>13</sup>C<sub>6</sub>-glucose. Six of the *L. jensenii* <sup>13</sup>C spectra also showed succinate peaks at 32.4 and 180.6 ppm.

**Conclusion:** The conversion of glucose, L- and D-lactate to more acetate by *L. jensenii* compared to *L. crispatus*, suggests a possibly important pathomechanism of female genital tract dysbiosis, altered vaginal pH, infection and infection-associated spontaneous preterm birth.

### T-015

**Decreased Levels of Triggering Receptor Expressed on Myeloid Cells-Like (TREM-like) Transcript-1 (TLT-1) Are Present in Cord Blood from Premature Infants, and Deficiency in Mice Promotes the In-Utero Inflammatory Response to Maternal Systemic Lipopolysaccharide (LPS) Exposure.** Paola E Pena Garcia<sup>†</sup>,<sup>1</sup> Jessica Morales-Ortiz,<sup>1</sup> Barry A Finette,<sup>2</sup> Anthony V Washington,<sup>1</sup> Elizabeth A Bonney\*.<sup>2,1</sup> *University of Puerto Rico-Rio Piedras, San Juan, PR, United States;* <sup>2</sup>*University of Vermont, Burlington, VT, United States.*

**Introduction:** TLT-1 is a type 1 single Ig domain receptor that binds fibrinogen. Its long splice variant is specific to platelets and relocates to the cell surface from  $\alpha$ -granules upon stimulation. In adults, TLT-1 dampens the inflammatory response and facilitates platelet aggregation at sites of vascular injury. Preterm birth is associated with both bleeding and infection. **Hypothesis:** relative TLT-1 deficiency is associated with prematurity and fetal inflammation. To test this we examined the levels

of soluble TLT-1 (sTLT) in cord blood and compared the inflammatory response in C57BL/6 (WT) and TLT-1<sup>-/-</sup> (KO) pups of mothers given LPS during pregnancy.

**Methods:** Cord blood plasma samples from infants born 26-34 (n=7) or 38-42 (n=5) weeks of gestation to healthy mothers aged 17-38 were examined for sTLT. In mouse studies, WT or KO mothers were mated to same-strain males for 24 hours (day 0 gestation). On Day 15, females received ip injections of 10ug of LPS in PBS (control=PBS alone). Twelve to 16 hours later, we examined maternal and fetal tissues (blood, decidua basalis and amniotic fluid) for cytokine response by QT-PCR and multiplex assay. Hypothesis testing used ANOVA or Mann Whitney U as appropriate. We report median/ range or mean±SEM and p values.

**Results:** sTLT in preterm cord blood (median 0.82, range 0.43 to 1.56 pg/ml) was lower than in term babies (median 1.97, range 1.41-2.41, p=0.005). In mice, the unexposed litter size in KO vs WT mice was similar (7.6, vs 8.2±0.6 pups, n~6 p=0.5). Maternal inflammatory response (LPS vs. PBS) and rate of delivery of at least one pup by 16 hours after LPS was similar. However, in decidua of pups whose mothers received LPS 12 hours before, relative RNA expression of CXCL1 (KC) tended to be higher in KO vs WT tissues (five-fold, n~6; p=0.16) while that of GM-CSF tended to be 50% lower (n~5; p=0.07). In amniotic fluid, KO pups vs WT expressed higher levels of IL-6 (90.8 vs 10.7 ng/ml n~6; p=0.05) and  $\gamma$ -IFN (3.3 vs 0.8 ng/ml n~6; p=0.04) but not TNF (p=0.72). This effect remained when controlling for the maternal response (p=0.03 and 0.04).

**Conclusion:** The data suggest possible associations between lower TLT-1 and prematurity in infants and between TLT-1 deficiency and increased inflammation in fetal mice exposed to infectious stimuli. The data supports further investigation to probe mechanisms underlying the link(s) between bleeding, infection and subsequent preterm birth. *Supported by the March of Dimes Prematurity Research Initiative and NIH R01HL090933*

## T-016

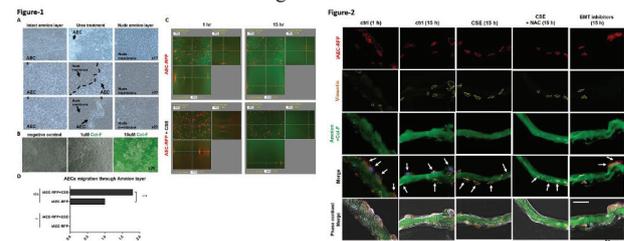
**3D Imaging of Epithelial-Mesenchymal Transition (EMT) Facilitated Amnion Epithelial Cells Migration Through Extracellular Matrix.** Enkhtuya Radnaa, Lauren Richardson, Ramkumar Menon\*. *University of Texas Medical Branch at Galveston, Galveston, TX, United States.*

**Introduction:** Human fetal membranes (amniochorion) maintain homeostasis via cyclic transition of cells (EMT & MET) throughout pregnancy. Amnion epithelial cells (AECs) become mesenchymal cells via EMT & migrate towards the matrix through membrane microfractures. However, EMT associated cell migration kinetics is not yet studied in fetal membranes. In this study, we tracked AEC migration through a nude (cell free) extracellular matrix under normal & oxidative stress conditions (inducer of EMT) using 3D imaging.

**Methods:** To create a nude membrane, the amniochorionic membranes were collected from term cesarean deliveries. The amnion layer separated from the chorion was treated with 5M Urea to remove cells (nude membranes). To test AEC migration, we used immortalized AEC grown under normal & oxidative stress conditions (OS & EMT inducer cigarette smoke extract treated AEC). Green fluorescence probe Col-F labeled nude (cell free) membrane was attached to a trans-well system & red fluorescent protein (RFP)-expressing AECs (iAEC-RFP; normal or OS induced) were seeded on the top of the membrane. Antioxidant N-acetyl cysteine (NAC, 15 mM), or EMT inhibitors (A83-01, 0.5 $\mu$ M & SB431542, 1 $\mu$ M) treatments were used as controls. Time-lapse imaging with Z-stack using a Keyence microscope (BZ-X800E) captured cellular migration through membrane matrix. The images were analyzed with 3D measurement using BZ-X800 Analyzer. iAEC-RFP cells migration, morphology & the EMT marker vimentin were analyzed with IHC.

**Results:** AECs maintained their epithelioid morphology when resting on nude membranes. Extracellular matrix of nude membrane permitted AEC migration as confirmed by image analysis of iAEC-RFP. CSE treated cells (OS & EMT) migrated ~2 times faster than normal AECs (Fig.1), showed mesenchymal morphology & expressed vimentin (Fig.2). CSE-induced migration was partially delayed with NAC treatment & EMT inhibitors-containing media treatment partially retained iAEC-RFP cells morphology as well as migration (Fig.2).

**Conclusion:** Using a live & cell free fetal membrane matrix in a transwell system, we determined epithelial cell transition, migration, & its kinetics under specific conditions. We report that OS can induce amnion epithelial cell EMT & increase their migratory potential leading to accumulation of proinflammatory mesenchymal cells in the matrix. Lack of transition of mesenchymal cells back to epithelial cells due to OS is a detrimental factor for membrane weakening.



## T-017

**Alarmin- and Endotoxin-Induced Intra-Amniotic Inflammation Induce Cervical Shortening without Altering the Cervico-Vaginal Microbiome.** Jose Galaz†, Roberto Romero\*, Andrew D Winters, Kevin R Theis\*, Nardhy Gomez-Lopez\*. *Wayne State University SOM, Detroit, MI, United States; Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, United States.*

**Introduction:** A mid-trimester sonographic short cervix is the strongest predictor for preterm birth, which is associated with intra-amniotic infection and/or inflammation, and disruption of the cervico-vaginal microbiome. While intra-amniotic infection is caused by microbes, sterile intra-amniotic inflammation (SIAI) is induced by alarmins. However, causal relationships between intra-amniotic inflammation, cervical shortening, and subsequent changes in the cervico-vaginal microbiome are lacking. Herein, we established a murine model to measure cervical length *in vivo* and studied the relationships between intra-amniotic inflammation, cervical shortening, and the cervico-vaginal microbiome.

**Methods:** Cervical length was measured in C57BL/6 dams using high-resolution ultrasound and compared with the excised cervix (n = 6). Dams received ultrasound-guided intra-amniotic injection of LPS on 16.5 days *post coitum* as a model of endotoxin-induced preterm birth (n = 6), IL-1 $\alpha$  as a model of SIAI (n = 6), or PBS (n = 6). RU486 (n = 6) was subcutaneously injected as a model of non-inflammatory preterm birth, or DMSO (n = 6). Cervical length was measured at time of injection and 6 h after. Cervical length difference between time points was reported as percentage of cervical shortening. A second cohort of dams receiving the same treatments (n = 6 per group) was used to collect cervical/vaginal tissues 6 h after injection for evaluation of the cervical and vaginal microbiome using 16S rRNA gene sequencing.

**Results:** First, we established a reliable *in vivo* method for measuring cervical length in mice. Dams that received intra-amniotic injection of LPS or IL-1 $\alpha$  displayed significantly greater cervical shortening compared to controls. In contrast, the administration of RU486 did not alter the cervical length. Microbiome analyses revealed taxonomic differences in the bacterial profiles of cervical and vaginal samples; however, generalized linear models showed no differences in the cervical or vaginal bacterial profile richness or heterogeneity for LPS vs. PBS, IL-1 $\alpha$  vs. PBS, or RU486 vs. DMSO. Similarly, PERMANOVA analyses revealed no differences in composition or structure of cervical or vaginal microbiomes between these three contrasts. Lastly, based on LEfSe and ANCOM-BC analyses, there were no differentially abundant bacterial taxa in the cervix or vagina for any comparison.

**Conclusion:** Cervical length can be measured by ultrasound in mice. Alarmin- and endotoxin-induced intra-amniotic inflammation led to cervical shortening, which did not occur in a non-inflammatory model. Notably, cervical shortening was not associated with an altered cervico-vaginal microbiome. Further research is needed to study microbiome perturbations closer to delivery.

## T-018

**Ureaplasma parvum Induces Cervical Epithelial and Stromal Cell Inflammation and May Propagate via Exosomes.** Ourlad Alzeus Gaddi Tantengco†, <sup>1,2</sup> Richard B Pyles, <sup>3</sup> Kathleen Vincent, <sup>2</sup> Paul Mark B Medina, <sup>1</sup> Ramkumar Menon\*. <sup>1</sup>University of the Philippines Manila, Manila, Philippines; <sup>2</sup>The University of Texas Medical Branch at Galveston, Galveston, TX, United States; <sup>3</sup>The University of Texas Medical Branch, Galveston, Galveston, TX, United States.

**Introduction:** *Ureaplasma parvum* (UP), an intracellular organism, is one of the most frequently isolated organisms from the cervicovaginal space of patients with preterm birth. The exact mechanisms by which *Ureaplasma* spp. infect the cells in the female genital tract and establish ascending infection are yet to be fully elucidated. We hypothesize that UP can promote inflammation in the cervical epithelial and stromal cells and be packaged inside cervical cell-derived exosomes for propagation and establishment of ascending infection.

**Methods:** Human ectocervical and endocervical epithelial cells, and cervical stromal cells (Stroma) were exposed to  $10^9$ - $10^{10}$  CCU UP culture per milliliter of media for 24h and 48 h. Fluorescence microscopy was used to determine the entry of DiO-labeled UP and its co-localization with the endosome-specific tetraspanin CD9 in all cervical cells. The cytotoxicity of UP to cervical cells were assessed by LDH assay while inflammation was determined using ELISA for GM-CSF, IL6, and IL8. Exosomes were isolated from UP-infected Ecto cells using differential ultracentrifugation and size exclusion chromatography and characterized by determining *U. parvum* antigen and mba by western blot, a virulence factor in UP. LPS was used as a positive control. Statistical analyses using one-way ANOVA and posthoc test were performed to establish significance ( $p < 0.05$ ).

**Results:** Regardless of exposure time, UP infection did not cause cytotoxicity in cervical epithelial or stromal cells. LPS did not induce inflammation in cervical epithelial cells, whereas UP infection caused a significant increase in GM-CSF ( $p < 0.0001$ ), IL6 ( $p < 0.0001$ ), and IL8 ( $p < 0.01$ ) compared to the control. Gentamicin treatment dampened this inflammatory response in the epithelial cells. Conversely, LPS induced a significant increase in GM-CSF, IL6, and IL8 ( $p < 0.0001$ ) in cervical stromal cells compared to UP infection. Fluorescence microscopy showed the intracellular colonization UP in both epithelial and stromal cells. UP was co-localized with the endosome-specific tetraspanin, CD9. Western blot analyses showed that exosomes isolated from UP-infected Ecto cells were positive for CD9, CD63, *U. parvum*, and mba.

**Conclusion:** UP infection promotes cervical epithelial cell inflammation. This study also determined that UP can be packaged inside the exosomes released from cervical epithelial cells. This can be a mechanism used by UP to evade the immune system and establish ascending infection to the uterine and amniotic cavity during pregnancy.

## T-019

**Quantification of Cervical Microstructure Change in Normal Pregnancies Using Diffusion Basis Spectrum Imaging.** Hansong Gao, Wenjie Wu, Zhexion Sun, Sicheng Wang, Zichao Wen, Qing Wang, Pamela K Woodard, Yong Wang. Washington University School of Medicine, St. Louis, MO, United States.

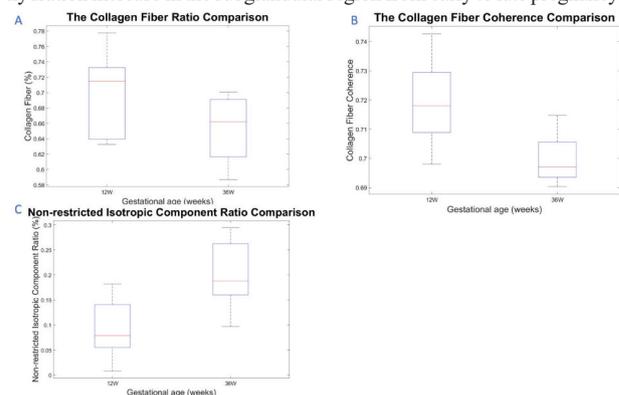
**Introduction:** Cervical remodeling is key to the timing of birth. The cervical collagen fiber and hydration change dramatically from early pregnancy to late pregnancy. We use diffusion basis spectrum imaging (DBSI) to quantitatively measure the microstructural components change in cervical remodeling by characterizing the water diffusion property. Temporal differences in cervical remodeling were compared between normal early and late pregnancies.

**Methods:** In each normal early (12-14 weeks' gestation) and late pregnancy group (36-38 weeks' gestation), ten healthy women underwent imaging on a 3.0 Tesla MRI scanner using a spin-echo sequence with 23-direction b value encoding. To compare the temporal difference, DBSI was performed on both the early and late pregnancy patients in both the subglandular and outer stromal regions.

**Results:** In the subglandular region, the collagen fiber ratio (early pregnancy 0.715 [0.640,0.732] vs. late pregnancy 0.662[0.617 0.691],  $p$ -value  $< 0.05$ ) and the collagen fiber coherence (early pregnancy

0.718[0.701 0.729] vs. late pregnancy 0.697[0.693 0.701],  $p$ -value  $< 0.05$ ) were significantly lower in late pregnancy than in early pregnancy, suggesting that fibers in this region in late pregnancy become disorganized. In addition, the non-restricted isotropic components ratio (early pregnancy 0.079 [0.055 0.141] vs. late pregnancy 0.188[0.160 0.262],  $p$ -value  $< 0.0005$ ) significantly increases, which suggests that subglandular hydration increases significantly from early to late gestation. In the outer stroma region, all three DBSI features (the collagen fiber ratio: early pregnancy 0.649[0.629 0.675] vs. late pregnancy 0.641[0.615 0.675]  $p$ -value = 0.889; the collagen fiber coherence: early pregnancy 0.705[0.700 0.721] vs. late pregnancy 0.703[0.697 0.712],  $p$ -value = 0.258; Non-restricted isotropic components ratio: early pregnancy 0.158[0.104 0.173] vs. late pregnancy 0.173[0.135 0.207],  $p$ -value = 0.178), showed no significant difference between early and late gestation time points.

**Conclusion:** Our DBSI findings suggest that the microstructural compartments are significantly different in early and late pregnancies in the subglandular region, but insignificantly different in the outer stroma zone. DBSI can non-invasively quantify the change in microstructural components, suggesting that cervical collagen fiber disorganization and hydration increase in the subglandular region from early to late pregnancy.



## T-020

**Sulforaphane Abrogates ROS Mediated Nrf2 Decrease in Mechanically Stretched Primary Amnion Cells.** Justin G Padron†, Chelsea Saito-Reis, Claire E Kendal-Wright\*. Chaminade University of Honolulu, Honolulu, HI, United States.

**Introduction:** Nuclear-factor-E2-related factor 2 (Nrf2) is a key transcription factor for the regulation of cellular responses to cellular stress and its expression is significantly lower after spontaneous term labor in human fetal membranes. As the fetal membranes are known to stretch *in vivo*, and we have previously shown that total Nrf2 decreases with *in vitro* stretch, we sought to abrogate stretch mediated Nrf2 downregulation associated with cellular stress using the phytochemical sulforaphane.

**Methods:** Human fetal membranes were collected at term repeat Cesarean section (with IRB approval) and primary amnion epithelial cells (AEC) isolated. Expression of Nrf2 in isolated AECs was confirmed using immunocytochemistry. AECs were grown on distensible silicon plates coated with collagen IV and subjected to 4 hrs of 20% stretch using a Flexcell-FX500TM tension system. Cells were treated with 0, 1, and 2 uM of Nrf2 activator sulforaphane during stretch. Cytoplasmic ROS production was quantified by DCF formation using OxiselectTM Intracellular ROS Assay Kit (Cell Bio Labs). Cytotoxicity as a result of mechanical stretch was quantified using the Pierce LDH Cytotoxicity Assay Kit (Abcam; Cambridge, MA, USA).

**Results:** Robust expression of Nrf2 in AECs was confirmed using immunocytochemistry (n=3). 4 hrs of 20% of stretch increased ROS by 41.7% (n=5,  $p=0.047$ ). LDH activity was increased with stretch by 34.7% (n=5,  $p < 0.05$ ). Nuclear Nrf2 was decreased by 39.2% (n=4,  $p < 0.05$ ) with mechanical stretch, however 1uM sulforaphane treatment increased nuclear Nrf2 by 33.1% (n=4,  $p < 0.05$ ) in the stretch treatment condition.

**Conclusion:** As stretch forces have been postulated to contribute to fetal membrane weakening and Nuclear Nrf2 decreases with *in vitro* mechanical

stretch; phytochemicals like sulforaphane may provide potential dietary therapeutics, to rescue the effects of the loss of this transcription factor *in vivo*. Nrf2 is known to have anti-inflammatory effects in several cell types including primary amnion cells, thus the manipulation of the activity of this transcription factor may be key to controlling inflammation during parturition.

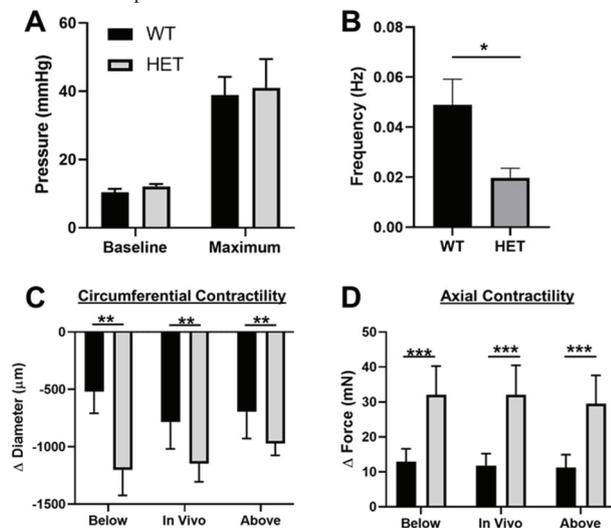
### T-021

**Determining the Role of Elastic Fibers on Cervical Contractility *In Vivo* and *In Vitro*.** Cassandra K. Conway<sup>†</sup>, Kristin S. Miller\*. Tulane University, New Orleans, LA, United States.

**Introduction:** Cervical smooth muscle cells (cSMCs) work in coordination with the extracellular matrix (ECM) to perform normal reproductive function. However, the relationship between cSMC function and ECM structure and function remains unclear. Specifically, elastic fibers may interact with SMCs to maintain contractility [1]. Additionally, elastic fiber degeneration is associated with cervical pathologies [2]. Therefore, this study seeks to utilize Fibulin-5 haploinsufficient mice to determine the role of elastic fibers in cSMC function.

**Methods:** A total of n=16 Fibulin-5<sup>+/+</sup> wildtype (WT) and Fibulin-5<sup>+/-</sup> haploinsufficient (HET) female mice at estrus aged 4-6 months were utilized for this study (Tulane IACUC Approved). To determine an appropriate pressure loading environment and *in vivo* contractile behavior, WT and HET (n=5/group) mice were anesthetized with isoflurane, a catheter placed transcervically, and measurements of pressure and contraction frequency were recorded. A separate cohort of mice (n=3/group) were euthanized, the cervix isolated and cannulated within an inflation-extension device while submerged within aerated Krebs Ringer Buffer at 37°C. At the physiologic length and the *in vivo* (mean±SD) pressure, biaxial maximum contraction was induced with 20mM KCl [3]. T-tests compared *in vivo* pressure and frequency measurements between genotypes. A 2-way ANOVA (Genotype, Pressure) compared changes in outer diameter and force with maximum contraction. Posthoc t-tests with Bonferroni correction were utilized where appropriate.

**Results:** *In vivo* frequency of contractions increased ( $p<0.05$ ) in the WT compared to the HET, however, baseline and contractile pressure did not significantly change with genotype. Maximum axial ( $p<0.001$ ) and circumferential contractility ( $p<0.01$ ) increased in the HET compared to the WT for all pressures.



**Figure 1:** (A,B) *In vivo* (n= 5 per genotype) and (C,D) *in vitro* (n=3 per genotype) cervical contractility in the WT (black) and HT (grey). (A) *In vivo* baseline (10.5±1.64mmHg, WT; 12.1±1.56mmHg, HET) and maximum pressure (38.9±11.2mmHg, WT; 41.0±18.8mmHg, HET) did not significantly change with haploinsufficient genotype. (B) However, frequency of contractions decreased significantly ( $p<0.05$ ) in the HET cervixes (0.02±0.01Hz) compared to WT (0.05±0.03Hz). (C, D) *In vitro* maximum cervical contractility significantly increased circumferentially (C) and axially (D) in the HET cervix (-1145±275μm and 32.1±14.5mN, respectively) compared to the WT cervix (-784±404μm and 11.8±5.89mN, respectively) at the physiologic length and *in vivo* pressure. Significance denoted by \* $p<0.05$ , \*\* $p<0.01$ , and \*\*\* $p<0.001$ .

**Conclusion:** Fibulin-5 haploinsufficiency increased *in vivo* frequency of contractions and *in vitro* maximum circumferential and axial contractile potential. Contrastingly, axial contraction decreased within vaginal samples when exposed to elastase [4]. This may suggest a compensatory mechanism for contractile potential in the HET mouse through cSMC and elastic fiber remodeling. Therefore, further understanding the interplay between elastic fiber remodeling and cSMC function may inform normal and pathological cervical function.

[1] Karnik *et al.*, Development, 2003. [2] Leppert *et al.*, Am J of Obst and Gyn, 1987. [3] Murtada *et al.*, J of Biomech Eng, 2016. [4] Clark *et al.*, Interface Focus, 2019.

### T-022

**Polarized Light Imaging of the Pregnant Cervix.** Jessica Ramella-Roman\*, Ilyas Saytashev<sup>†</sup>, Sudipta Saha<sup>†</sup>. Florida International University, Miami, FL, United States.

**Introduction:** The current global level of preterm infants born every year is 15 million. Of these, about 1 million will die before the age of five due to complications that result from premature birth prior to 37 weeks of gestation. Many survivors will face life-long challenges including neurological disorders, long-term cognitive impairment, defects in hearing, vision and digestion, as well as respiratory disease. There is an absence of clinical tools for early and accurate detection of spontaneous preterm birth risk which limits our understanding of the pregnant cervix. Modalities such as Second Harmonic Generation (SHG) can visualize the cervix ultra structure but are costly and not easily applicable in human studies. Here we propose the use of polarized light imaging to monitoring the pregnant cervix. The main advantage of this approach is in its ease of translation to clinical use.

**Methods:** We have developed an instrument that combines two polarization imaging techniques, Muller Matrix microscopy and Muller Matrix confocal polarimetry, and integrated these modalities into a Nonlinear Microscope (NLM). We utilize a femtosecond laser with a home-built laser scanning microscope. Reflected light at fundamental wavelength (700-900 nm) is separated from epi-detected TPEF/SHG by a short-pass dichroic mirror and directed to an output port by a beam splitter. Mueller Matrix Polarimetry imaging is enabled by an addition of a polarization state generator at the microscope input, and a polarization state analyzer before the camera. Mice cervixes at different gestational time were imaged with this system. MMI and SHG is ultimately compared.

**Results:** Our results show that the mouse cervix collagen anisotropy and directionality can be captured through Mueller Matrix imaging. These results are validated by co-registered Second Harmonic data collected simultaneously. The SHG data provides direct visualization of the collagen fibers while the pregnant cervix ultra-structure is correctly reconstructed by utilizing Mueller Matrix decomposition. Mueller matrix decomposition extracts constituent polarization properties from a Mueller matrix of any unknown complex system. The decomposition of the sample's Mueller matrix, yields three canonical matrices accounting for material depolarization  $M\Delta$ ; retardance, optical activity  $MR$ , and diattenuation  $MD$ . In this study, retardation and depolarization were used to localize area of compact collagen from area of loosely connected fibers.

**Conclusion:** Our imaging systems can relay information about the cervix mechanical properties and structure by measuring birefringence and the general anisotropy of the tissue ultrastructure. Preliminary data on pregnant mice demonstrate that Mueller Matrix polarimetry is an effective technique for imaging the cervix. This modality has the advantage of being easily translatable to the clinical setting and in-vivo pilot studies in humans are underway.

**T-023**

**Methods to Quantify the Genetic Architecture of Cervical Length.** Hope M Wolff<sup>†</sup>,<sup>1</sup> Roberto Romero\*,<sup>2</sup> Jerome F Strauss, III\*,<sup>1</sup> Sonia S Hassan\*,<sup>3</sup> Shawn J Latendresse\*,<sup>4</sup> Bradley T Webb\*,<sup>1</sup> Aaron R Wolen\*,<sup>5</sup> Adi L Tarca\*,<sup>3</sup> Timothy P York\*.<sup>1</sup> <sup>1</sup>Virginia Commonwealth University School of Medicine, Richmond, VA, United States; <sup>2</sup>Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Rockville, MD, United States; <sup>3</sup>Wayne State University School of Medicine, Detroit, MI, United States; <sup>4</sup>Baylor University, Waco, TX, United States; <sup>5</sup>The University of Tennessee Health Science Center, Memphis, TN, United States.

**Introduction:** A short cervix (cervical length < 25 mm) in the midtrimester (18 to 24 weeks) of pregnancy is a powerful predictor of preterm birth. Although the biological mechanisms of cervical change over the course of pregnancy have been the subject of extensive investigation, very little is known about the genetic architecture of cervical length, or the extent to which genetic factors contribute to premature cervical shortening. We have developed longitudinal models of cervical length change across pregnancy that, when combined with genomic data, can be used to estimate the heritability of cervical length and its genetic covariance with gestational age at birth.

**Methods:** Latent growth curves were established to model cervical change for a large cohort of women with longitudinal assessments of cervical length across pregnancy. Cervical length was measured in millimeters using transvaginal ultrasound. Baseline cervical length was measured between 18 and 24 weeks of gestation, and 1 to 15 additional measurements were collected for each subject during follow-up. Demographic characteristics, including relevant medical history and birth outcome data, were collected for each subject. Gestational age at birth was measured from the first day of the woman's last menstrual period and confirmed by obstetrical ultrasound.

**Results:** The latent growth curve describes cervical change as a function of time using two classes of parameters: intercept terms, which account for inter-individual variability in the baseline value of cervical length, and polynomial terms, which capture individual departures in the rate of change from the population mean. We describe how the parameters of the model can be combined with genomic and birth outcome data to make predictions about the genetic architecture of cervical change across pregnancy and its genetic relationship to spontaneous preterm birth.

**Conclusion:** An estimate for the genetic contribution to cervical length and its role in mediating the timing of birth is essential to understanding the pathophysiology of spontaneous preterm birth. The methods we have developed will allow us to estimate the heritability of cervical length and its bivariate genetic correlation with gestational age at birth. Extensions of this model can be used to develop a polygenic risk score which could be applied in clinical practice to rapidly assess a patient's genetic risk for developing a short cervix and delivering preterm.

**T-024**

**Oxytocin Receptor Is Degraded via the Ubiquitin-Proteasome System Following Prolonged Agonist Exposure.** Kevin Prifti, Manasi Malik<sup>†</sup>, Sarah K England, Antonina I Frolova\*. Washington University School of Medicine in St. Louis, St. Louis, MO, United States.

**Introduction:** Oxytocin (OT) administration during labor is clinically challenging due to the decreased response seen with continuous exposure. This attenuation may result from oxytocin receptor (OXTR) desensitization, a process common to most G-protein coupled receptors (GPCRs), where an agonist-occupied receptor undergoes phosphorylation and temporary internalization. Prior studies have shown that following endocytosis, the OXTR recycles back to the cell surface via the endosomal recycling pathway. However, other GPCRs, in response to prolonged agonist exposure, are targeted for degradation. The objective of this study was to determine whether prolonged agonist stimulation leads to OXTR degradation.

**Methods:** HEK-293 cells stably transfected with OXTR\_GFP were pretreated with cycloheximide (CHX) to stop *de novo* protein synthesis and then treated with OT at different concentrations ( $10^{-9}$ - $10^{-6}$ M) and durations (2-8hrs). Cells were pretreated with MG132 and PP-I for 30 minutes prior

to OT exposure to inhibit the ubiquitin-proteasome degradation pathway. Protein content was quantitated by immunoblot. Confocal microscopy was used to determine colocalization between OXTR and lysosomes labeled with a LysoTracker probe. To detect OT-stimulated OXTR ubiquitination, OXTR\_GFP was immunoprecipitated and probed with antibody to ubiquitin using standard immunoblotting techniques. Site-directed mutagenesis was used to generate lysine-deficient OXTR mutants to additionally determine the role of ubiquitination in receptor trafficking. **Results:** In HEK-293 cells stably expressing OXTR\_GFP, OXTR protein levels decreased significantly following treatment with 100nM OT for 6hr or longer in the presence of CHX, consistent with protein degradation ( $n=6$ ,  $p<0.001$ ). OXTR\_GFP did not colocalize with LysoTracker following exposure to OT for up to 4 hrs, suggesting that the receptor is not targeted to the lysosomal compartment for degradation. Proteasome inhibitors MG132 and PP-I reversed the oxytocin-induced OXTR degradation. Additionally, OXTR\_GFP ubiquitination was detected following 4 hour treatment with OT. Unlike the WT, protein expression of the lysine-deficient OXTR mutants OXTR-4K\_GFP and OXTR-0k\_GFP was not decreased following prolonged treatment with OT, further implicating receptor ubiquitination in receptor trafficking and proteasomal degradation.

**Conclusion:** Our data suggest that the OXTR is degraded following prolonged agonist exposure *in vitro*. Additionally, we show that the receptor is not targeted to the lysosome, but rather ubiquitinated and targeted to the proteasome for degradation following prolonged exposure to OT. These results present a novel target for improving OT response in labor management to improve uterine contractility.

**T-025**

**A Patient-Specific Multi-Scale, Multi-Physics Simulation of Whole Uterine Contraction.** Yiqi Lin<sup>†</sup>,<sup>1</sup> Jazmin Aguado-Sierra,<sup>2</sup> Zhexion Sun,<sup>1</sup> Constantine Butakoff,<sup>3</sup> Sicheng Wang,<sup>1</sup> Mariano Vazquez,<sup>2</sup> Yong Wang.<sup>1</sup> <sup>1</sup>Washington University, St. Louis, MO, United States; <sup>2</sup>Barcelona Supercomputing Center, Barcelona, Spain; <sup>3</sup>Elem Biotech, S.L., Barcelona, Spain.

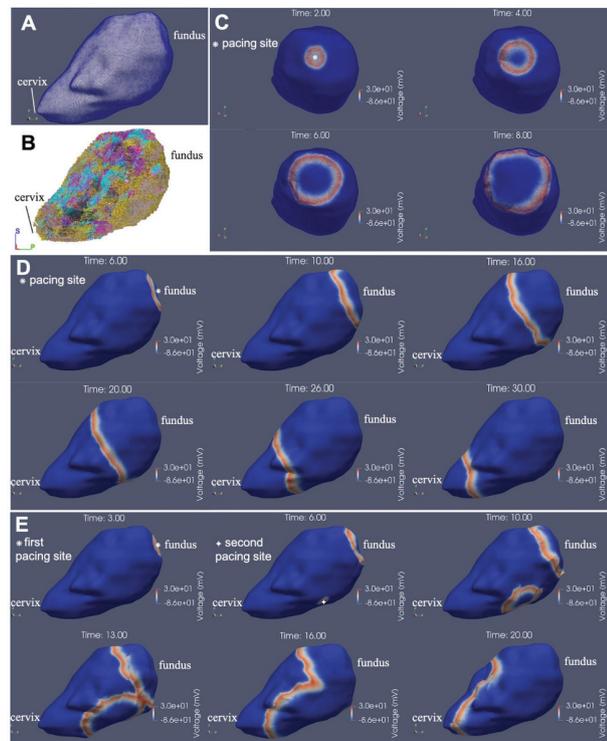
**Introduction:** We employed the patient-specific anatomical and diffusion MRI data together with the uterine surface pacing to conduct an electro-mechanical coupled whole uterine contraction simulation. Our simulation system was built using the Alya platform, a numerical solver for coupled systems of partial differential equations discretized on unstructured meshes.

**Methods:** We generated the body-uterus geometry and detailed uterus segmentation using patient-specific MRI data. A tetrahedral mesh was created on myometrium from the segmented image using ANSA software with at least 4 transmural elements to apply boundary conditions onto the exterior muscles. We refined and interpolated DTI-derived myometrium fiber orientation data to the meshed uterus geometry. Then, we built a electrophysiology model as a non-linear reaction-diffusion system that generates transmembrane potential based on the modified Fitzhugh Nagumo model. We coupled the electrophysiology simulation with the electro-mechanical contraction using excitation-contraction coupling model by Hunter-McCulloch. The whole process was simulated on Alya based on an automatic mesh partition.

**Results:** Based on patient's MRI data, meshed uterus geometry and interpolated fiber orientation data were derived and shown in **Fig. 1A** and **1B**. After a single stimulus paced in the fundus region, **Fig. 1C** shows the mechanical deformation in the fundus view. **Fig. 1D** shows the transmembrane potential propagation and the mechanical deformation from fundus to cervix. The electrical wave travels 38 seconds in this simulation. Under the similar simulation environment, we paced a second stimulus on the uterus side 0.5 second after the first pacing. **Fig. 1E** shows the propagation and interaction of two transmembrane potential waves, which merge together starting from the thirteenth second. The simulation takes around 3 hours to finish on a 48-cores server.

**Conclusion:** Our system can reproduce the electrical and mechanical activation patterns of the whole uterine contraction using patient-specific data. It helps us better understand the underlying biology of human uterine contraction and potentially provide more clinical insight about the

labor progression. We aim to use this system to conduct patient-specific diagnosis of dysfunctional labor, and optimize treatment plan by predicting patient's responses to candidate intervention or drugs.



**Fig 1. Patient-specific whole uterus simulation.** (A) Meshed whole uterus geometry. (B) Uterine myometrium fiber tracking using diffusion tensor imaging. (C) Four frames of simulations were shown to indicate the mechanical deformation of the uterus, following one single uterine surface pacing at fundus. (D) (E) Six frames of simulations were shown to indicate the dynamics of electrical and mechanical activation sequences, following one or two uterine surface pacing sites at fundus. Mechanical wave was also demonstrated following electrical wave propagation.

**T-026**

**Contraction Synchronization Predicts the Onset of “True” Labor.** Ponnila S Marinescu†, Roger C Young, David Adair, Braxton Hern, Eva K Pressman, Neil S Seligman\*. <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>PreTeL, Inc., Chattanooga, TN, United States; <sup>3</sup>University of Tennessee College of Medicine, Chattanooga, TN, United States.

**Introduction:** Global synchronization of uterine activity is necessary for labor, but little is known about how often synchronized contractions are expressed in “true” vs “false” labor. Contraction synchronization can be assessed using multichannel uterine electromyography (uEMG). Here, we use uEMG to compare the frequency of synchronized contractions among women who presented for evaluation of “true” versus “false” labor.

**Methods:** Multi-center observational cohort study of women from 2 academic medical centers. Women with uncomplicated singleton pregnancies between 30w0d - 42w0d and chief complaint of contractions were included. Those with cervical dilation ≤ 2 cm and no further cervical change were in the “false” labor (FL) group, which was divided into < 36w0d and ≥ 36w0d sub-groups. The “true” labor (TL) group was a comparison cohort of 6 term women with cervical exams > 2 cm and subsequent dilation. Primary outcome was number of synchronized contractions within a 10-minute period. Contractions were reported by toco. A 6-channel uEMG was performed using directional sensors for > 60 minutes and filtered between 0.15 and 1.2 Hz to isolate uterine signals. A computer algorithm identified contractions by uEMG and then assessed each channel for uEMG activity through the first 5-15 seconds of each toco-positive contraction. A contraction was “synchronized” if EMG activity occurred in > 50% of the channels.

**Results:** 19 women were included, 6 in the TL group and 13 in the FL group. There were 5 in FL subgroup < 36 weeks and 8 in FL subgroup ≥ 36 weeks. Ratios of the number of synchronized contractions to total contractions for each group are presented in Table 1. The FL subgroups

did not differ from one another ( $P = .28$ ) but a higher frequency of synchronized contractions was observed in the TL group compared to the collective FL group ( $P = .0005$ ). Additionally, the FL subgroups were individually different from the TL group ( $P = .003$  for < 36 weeks;  $P = .0003$  for ≥ 36 weeks).

**Conclusion:** Contraction synchronization is more frequent in “true” labor than in “false” labor, even when gestational age is considered. We hypothesize that transition to more synchronized contractions may be necessary for the onset of “true” labor. Future work will be required to determine the value of synchronization analysis as a prospective predictor for “true” labor.

**Table 1. Ratio of synchronized contractions among “True Labor” and “False Labor” groups and subgroups.**

	n	Synchronized Contractions Total Contractions	P Values
Group “True Labor”	6	0.72 ± 0.08	.0005 .003 .0003 .28
Group “False Labor”	13	0.30 ± 0.24	
Subgroup “False Labor” < 36 weeks	5	0.30 ± 0.23	
Subgroup “False Labor” ≥ 36 weeks	8	0.43 ± 0.12	

**T-027**

**Somatostatin Receptor Type 2 Expression in Pregnant and Labouring Human, Non-Human Primate, and Mouse Myometrium.** Oksana Shynlova,<sup>1</sup> Adam Boros-Rausch†,<sup>2</sup> Anna Dorogin,<sup>2</sup> Tsung-Yen Wu,<sup>3</sup> Kristina Adams Waldorf,<sup>3</sup> Stephen Lye\*. <sup>1</sup>Sinai Health System, Toronto, ON, Canada; <sup>2</sup>SHS, Toronto, ON, Canada; <sup>3</sup>U of W, Seattle, WA, United States.

**Introduction:** Uterine contractility is regulated by the interaction of myogenic, neurogenic, and hormonal mechanisms. Labour (both term and preterm) is an inflammatory process. Factors affecting contractility of the inflamed uterus are not fully understood. The hormonal peptide somatostatin (SST) is significantly increased in human plasma during late pregnancy. SST mediates its effects by family of G-protein coupled receptors (SSTR1-5). Activation of SSTR2 mediates contractility of smooth muscle of the human gut and the non-pregnant pig uterus. We studied the presence of SSTR2 during gestation and term labour (TL) using human, non-human primate (NHP), and mouse uterine tissues. We also assessed SSTR2 expression in preterm human myometrium, a NHP model of (Group B Streptococcus)-induced preterm labour (PTL) and murine models of infectious (LPS-induced) and sterile (RU486-induced) PTL.

**Methods:** Myometrial biopsies were collected from term and preterm labouring women undergoing emergency caesarean section (TL/PTL), preterm and term not in labour women undergoing elective caesarean section (PTNL/TNL) following informed consent (N=2-4/group). Primary myocytes were isolated by enzymatic digestion from TNL tissues. Mouse TL model: myometrial tissues were collected at gestation day (GD) 15, 18, 19/TNL, TL (N=4; term GD19.5). PTL models: intraperitoneal injection of LPS (50µg/dam) or subcutaneous injection of RU486 on GD15, sterile saline or corn oil used as vehicle (N=4/group). Myometrial tissues were collected on GD16 during PTL. NHP PTL model: on GD117-125 chronically catheterized pregnant *Macaca nemestrina* received choriodecidual inoculations of either vehicle (saline, N=7) or GBS (5x10<sup>8</sup> colony forming units/ml; N=5), cesarean section was performed at PTL or 4 days after inoculation. Tissues were fixed in formalin for immunohistochemistry, or flash frozen; total RNA was extracted for RT-qPCR.

**Results:** Immunostaining of human myometrium revealed strong SSTR2 protein expression by smooth muscle cells and resident macrophages; the presence of SSTR2 protein was increased in both, term and preterm labour, as compared to tissues from not in labour patients. Similarly, murine gestational tissues showed an increase in myometrial SSTR2 expression at term (GD19 and TL) as compared to GD15, and during PTL (LPS- and RU486-induced) compared to control animals. *SSTR2* transcripts were detected in pregnant non-labouring NHP myometrium and in primary human myometrial cells, however, *SSTR2* mRNA expression was not affected by GBS infection (NHP), or treatment with Lipopolysaccharide (primary human myocytes).

**Conclusion:** SSTR2 transcripts and protein was detected in human, NHP and mouse myometrium. Expression of SSTR2 protein was associated with the inflammatory process of labour.

## T-028

**BK<sub>Ca</sub> Channels Are Involved in Both Spontaneous and LPS-Stimulated Uterine Contractions in Pregnant Mice.** Junjie Bao,<sup>1,2</sup> Xiaofeng Ma,<sup>1</sup> Monali Wakle-Prabakaran,<sup>1</sup> Ronald McCarthy,<sup>1</sup> Sarah K England\*,<sup>1</sup> <sup>1</sup>Washington University School of Medicine, St. Louis, MO, United States; <sup>2</sup>Guangzhou Women & Children's Medical Center, Guangzhou Medical University, Guangzhou, China.

**Introduction:** Uterine activation arises in response to the electrical activity of the smooth muscle cells of the myometrium. BK<sub>Ca</sub> channels, as one of the most abundant potassium channels in the myometrium, are implicated to play an essential role in uterine contraction. However, it remains controversial and elusive if and how BK<sub>Ca</sub> channels are involved in basal versus inflammatory states of the uterus. Here we investigated the role of the BK<sub>Ca</sub> channel in spontaneous and LPS-stimulated uterine contraction *in vitro*, to better understand the relationships between BK<sub>Ca</sub> channels and uterine contractile activity.

**Methods:** Female C57BL/6J mice were paired with a male for 2 h, those that developed a copulatory plug were designated as gestation day (GD) 0 of pregnancy. Mice uterine samples were collected on GD18.5. A subset of samples were embedded in paraffin and detected for the expression of BK<sub>Ca</sub> with immunofluorescence microscopy. The others were dissected to obtain 4 x 8 mm strips of longitudinal muscle, and mounted onto force transducers in organ baths. Primary myocytes were also isolated and channel activity measured and analyzed for open-state probability ( $P_o$ ) using pCLAMP software. All the data were normalized for comparison. Statistical comparisons were performed using Student's t test (two groups), or one-way ANOVA (three or more groups) with appropriate post hoc tests.

**Results:** The BK<sub>Ca</sub> channel expressed in the myometrium of pregnant C57BL/6J mice at GD 18.5. Tension recording results showed that LPS increased the area under the curve (AUC) and the amplitude of contraction ( $p < 0.05$ ). While blocking BK<sub>Ca</sub> channels with paxilline decreased both spontaneous and LPS-stimulated uterine contractions ( $p < 0.05$ ). Patch-clamp results showed that LPS markedly increased  $P_o$  of mice MSMCs at a holding potential of 60 mV ( $0.00011 \pm 0.00006$  vs  $0.26570 \pm 0.05796$ , Baseline vs LPS,  $p < 0.05$ ), while paxilline decreased  $P_o$  gradually and reached maximal effect in 15 min [ $0.26570 \pm 0.05796$  vs  $0.15210 \pm 0.08756$  vs  $0.02134 \pm 0.01259$  vs  $0.00304 \pm 0.00167$ , LPS vs LPS + Pax (0-1 min) vs LPS + Pax (4-5 min) vs LPS + Pax (14-15 min),  $p < 0.05$ ]. Furthermore, LPS could not increase  $P_o$  with pre-treatment of paxilline as usual (LPS alone) ( $p > 0.05$ ).

**Conclusion:** BK<sub>Ca</sub> channels are deeply involved in both basal and LPS-stimulated uterine contraction in pregnant mice. Further elucidation of the mechanisms may provide new insights into potential strategies to regulate uterine contraction.

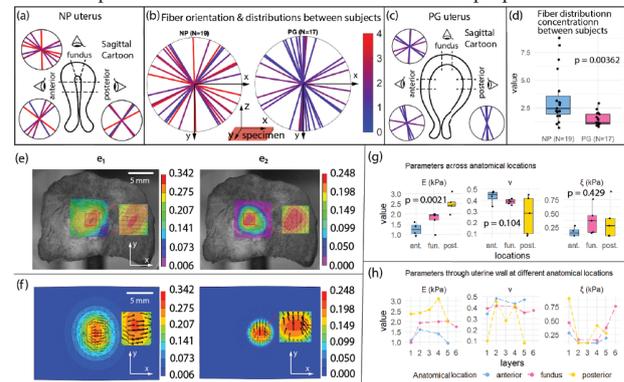
## T-029

**The Anisotropic Mechanical Properties of Human Uterus.** Shuyang Fang,<sup>1</sup> James McLean,<sup>1</sup> Joy Vink,<sup>2</sup> Christine Hendon,<sup>1</sup> Kristin Myers,<sup>1</sup> <sup>1</sup>Columbia University, New York, NY, United States; <sup>2</sup>Columbia University Medical Center, New York, NY, United States.

**Introduction:** The mechanical function of the uterus is critical for a successful pregnancy. It is hypothesized the magnitude of uterine tissue stretch triggers the onset of contractions. Using imaging, mechanical test, and computational modeling, we investigate how the tissue's fiber distribution and material properties impact its mechanical responses to loadings.

**Methods:** We collected 27 specimens from a non-pregnant (NP) and a pregnant (PG) patient through the uterine wall thickness at 3 anatomical locations: anterior, fundus, and posterior (Fig. 1ac). We used optical coherence tomography and von Mises fitting to characterize fiber (collagen and SMC) distributions. We used spherical indentation and uniaxial tension to test its mechanical behavior under various loading conditions.

We modeled the tissue as a fibrous network embedded in a compressive ground substance and fitted this model to the experimental data using an iterative optimization to uncover the tissue's material properties.



**Figure 2:** Fiber distribution and comparison of the first (e1) and second (e2) principal strain fields between the experiment and FEA. (a)-(c) Each line represents a fiber family, and the concentration kappa about the preferential direction is indicated by the color bar. Blue denotes a concentration factor equal to zero for a uniform distribution, while red denotes a larger factor for a more anisotropic distribution. (a)(c) NP & PG tissue fiber distributions viewed by anatomical locations. (b) All fiber distributions of NP and PG tissue are combined for each, respectively. The dominant fiber directionality of NP tissue appears to be at around  $\pi/4$  &  $3\pi/4$  while PG is at  $\pi/2$ . (d) PG tissue shows significantly smaller concentration factors, indicating a more uniform distribution. (e) The experimental data are plotted with color bar indicating the magnitude. The red-square areas are viewed close to show the vector directions indicated by the lines. (f) The corresponding FEA data are plotted with the arrows indicating the vector directions. (g) The NP tissue has the largest E at its posterior wall, followed up the fundus and the anterior. (h) The NP tissue's E is larger at the middle layers

**Results:** Fiber distributions are significantly different between the NP and PG tissue, both are anisotropic (Fig. 1a-d). The best-fit material parameters under indentation are averaged and listed in Table 1 and plotted in Fig. 1e. Heterogeneous mechanical properties are seen in human uterine tissue at different anatomical locations and across different uterine wall layers (Fig. 1fg).

IFEA & von Mises fitted material parameters		
Parameter	Mean Value	Standard Deviation
Young's modulus (kPa)	1.756	0.685
Poisson's Ratio	0.329	0.124
Fiber Stiffness (kPa)	0.393	0.347
Fiber Concentration	2.46	1.99

**Conclusion:** PG fibers are more dispersed than NP fibers (Fig. 1d). NP fibers exhibit a circularly arranged, meanwhile, basket-interweaving architecture (Fig. 1a). PG fibers exhibit a longitudinally or meridionally arranged architecture (Fig. 1c) as the uterus undergoes a 6-fold increase in length during pregnancy. NP tissue, on average, is less compressible than PG tissue, but the difference is not significant. The force response of the human uterus under all loading conditions is time-dependent and non-linear, and uterine fiber architecture dictates 2-D principal strain fields. Human uterine tissue also shows heterogeneous mechanical properties at different locations.

## T-030

**Genome-Wide Changes Accompanying Myometrial Contraction and Labor: Integrated Analysis of ChIP-seq and RNA-seq Data Reveals Critical Steroid-Target Genes.** Ariel J Dotts†, Ping Yin\*, William A Grobman\*, Serdar E Bulun\*. Northwestern University, Chicago, IL, United States.

**Introduction:** Preterm birth is the leading cause of infant morbidity worldwide. Approximately 380,000 babies are born prematurely in the USA every year. Estrogen (E2) and progesterone (P4) play important roles during pregnancy and labor, but a clear understanding of the underlying mechanisms is lacking. E2 and P4 function by activating their cognate nuclear receptors ESR1 and PGR, respectively, to affect their binding to regulatory regions of target genes and control their transcription. Here, we sought to identify steroid hormone target genes and pathways critical for myometrial quiescence and contraction. Elucidating the molecular

mechanisms whereby the myometrium transforms from a quiescent to a contractile state would fill an important evidence gap and be beneficial in advancing treatment for the prevention of preterm birth.

**Methods:** We performed RNA-seq using human myometrial tissue biopsies obtained at cesarean delivery from pregnant women at term who were not in labor (TNIL; n=7) and women at term who were in labor (TIL; n=4). ChIP-seq for active histone modification marks (H3K27ac and H3K4me3) and ESR1 and PGR were conducted on myometrial tissues of TNIL and TIL (n=3 subjects for each factor).

**Results:** Principal component assay of RNA-seq data distinguished the TNIL and TIL transcriptomes as two distinct clusters. Over 1600 genes were differentially expressed between TIL and TNIL tissues (FDR <0.05), with 799 (57%) upregulated and 610 (43%) downregulated in TIL tissues. Pathway analysis of these differentially expressed genes uncovered that genes and pathways key for labor were significantly enriched for those related to the acute inflammatory response and positive regulation of cytokine-mediated signaling pathway. ChIP-seq for ESR1 and PGR revealed common or unique genomic regions occupied by each transcription factor in TNIL and TIL myometrial samples. Genes associated with these regions were enriched in pathways similar to those identified in RNA-seq analysis, suggesting that ESR1 and PGR, via interaction with their target genomic loci, directly mediate the expression of genes associated with myometrial function. Integrative analysis of RNA-seq and ChIP-seq data of hormone receptors and histone marks uncovered ESR1/PGR downstream target genes potentially regulating myometrial contraction and labor. We are currently further characterizing the functions of these genes.

**Conclusion:** ChIP-seq and RNA-seq data identified steroid hormone receptor target genes that are actively regulated during labor. These genes and pathways may serve as potential therapeutic targets to prevent preterm birth.

### T-031

**Uterine Electromyography: Novel Uterine Bioelectrical Signaling Patterns Lead to Advances in Understanding of Uterine Contractile Activity.** Ponnala S Marinescu<sup>†</sup>,<sup>1</sup> Lauren A Miller,<sup>2</sup> Roger C Young,<sup>3</sup> Braxton Hern,<sup>4</sup> Eva K Pressman,<sup>1</sup> Neil S Seligman\*.<sup>1</sup> <sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>St. Luke's Clinic, Boise, ID, United States; <sup>3</sup>PreTel, Inc., Chattanooga, TN, United States; <sup>4</sup>University of Tennessee College of Medicine, Chattanooga, TN, United States.

**Introduction:** Uterine electromyography (uEMG) has been used in the assessment of myometrial contractile activity. Historical methods were based on propagation velocity or power spectrum amplitude and frequency analyses. Newly discovered uEMG signals, however, have enriched our understanding of the bioelectric relationship with myometrial activation. Here, we present these novel uEMG signals and their possible clinical correlates.

**Methods:** Prospective observational study of women with singleton pregnancies between 16w0d-22w6d (mid-trimester analysis) and between 30w0d-42w0d (preterm-term analysis); women in the latter group were evaluated on Labor and Delivery for contractions. Four to seven-channel uEMG recordings were obtained using directional EMG sensors for  $\geq 30$  minutes. Uterine signals were isolated using a bandpass filter between 0.15 and 1.2 Hz.

**Results:** We identified four novel uterine EMG signals in addition to the previously identified "contraction associated signals"; criteria for each are shown in Table 1. These new signaling patterns are independent of tocodynametry (toco)-observed contractions and are distinct from one another, although some (e.g., spike-like signals) fall on a continuum with larger patterns (e.g., fasciculation-like signals). Each pattern is associated with a unique clinical implication. Spike- and burst-like signals were observed in mid-trimester patients; however, these women did not have recorded contractions, and therefore, the presence of other signals in the mid-trimester cannot be excluded.

**Conclusion:** Discovery of novel uterine bioelectrical signaling patterns has enriched our understanding of uterine contractility and myometrial

activation. Future work should focus on the overarching goal of correlating the relationships among these, and other undiscovered bio-signals, with specific clinical applications.

Table 1. Uterine bioelectric signals identified via uterine electromyography

	Signal Name	Definition	Physiologic Correlate
Signals associated with Toco-observed contractions	Contraction-Associated Signals	Mixed amplitude and mixed frequency with amplitudes usually $\geq \pm 150 \mu\text{V}$ ; lasts through most of contraction duration	Tissue-level activity that coordinates into organ-level effects
	Signal Name	Definition	Physiologic Correlate
Signals independent of Toco-observed contractions	Spike-Like Signals (SLS)	Isolated voltage transients lasting 3-15 seconds; $> 50 \mu\text{V}$ amplitudes	Unknown
	Fasciculation-Like Signals	Higher frequency transients than SLS, with a more complex structure	Brief, localized uterine muscle activity
	Burst-Like Signals	Repetitive, largely monophasic transients, lasting 10-20 seconds	Seen in the mid trimester and during the mid 3 <sup>rd</sup> trimester
	Uterine Oscillatory Signals	Pseudo-sinusoidal oscillations with 50 to 200 $\mu\text{V}$ amplitudes	Related to degree of cellular stimulation (e.g., exposure to $\text{IP}_2$ -mediated drugs)

### T-032

**HIF-1 $\alpha$ , but Not HIF-2 $\alpha$ , May Modulate Myometrial Contraction via Upregulating Oxytocin Receptor during Labor.** Bolun Wen<sup>†</sup>,<sup>1</sup> Xueya Qian,<sup>1</sup> Lele Wang,<sup>1</sup> Xiaodi Wang<sup>†</sup>,<sup>1</sup> Junjie Bao,<sup>1</sup> Binsheng Wu<sup>†</sup>,<sup>1</sup> Wenfeng Deng<sup>†</sup>,<sup>1</sup> Fan Yang<sup>†</sup>,<sup>2</sup> Lina Chen<sup>†</sup>,<sup>2</sup> Huishu Liu\*.<sup>1</sup> <sup>1</sup>Guangzhou Women & Children Medical Center, Guangzhou Medical University, Guangzhou, China; <sup>2</sup>Guangzhou Women & Children Medical Center, South China University of Technology, Guangzhou, China.

**Introduction:** A fine uterine contraction can decide how successful a delivery goes. With every contraction narrowing the vessels that transport oxygen, the womb is challenged with hypoxia, leading to uterine hypoxic stress in which has been shown that hypoxia-induced factor (HIF) plays a crucial role. Our work aims at defining how HIF modulates the myometrial contractility so as to explore the potential targets for improving parturition.

**Methods:** 6 pieces of myometrium tissues were collected from women undergoing caesarean section (3 of labor and 3 of non-labor). Western blot was performed to analyze the protein level of HIF-1 $\alpha$ , HIF-2 $\alpha$  and oxytocin receptor in myometrium. In addition, primary uterine smooth muscle cells were cultured and divided into 3 groups: C (control), H (hypoxia for 2h with 3%O<sub>2</sub>) and I (HIF-1 $\alpha$  inhibitor 2-MeOE2 treatment before and during hypoxia). Cells were harvested after hypoxia. Western blot was run for the expression of HIF-1 $\alpha$ , HIF-2 $\alpha$  and oxytocin receptor.

**Results:** For laboring myometrial tissues, HIF-1 $\alpha$ , and oxytocin receptor showed significantly higher expression than those in non-labor. And the expression of HIF-2 $\alpha$  was opposite to the result of HIF-1 $\alpha$ . Additionally in cellular experiment, group H showed the highest expression of HIF-1 $\alpha$  whereas it could barely be detected in either group C or group I. Meanwhile, the expression of oxytocin receptor was significantly higher in group H. However, the expression of HIF-2 $\alpha$  in laboring myometrium was dramatically lower whereas it showed no differences among groups of cells.

**Conclusion:** Hypoxic stress is one of the cause of triggering labor as HIF-1 $\alpha$  may be the key factor in this process. Furthermore, HIF-1 $\alpha$  is likely to strengthen the myometrial contractility via upregulating oxytocin receptor.

### T-033

**HIF1 $\alpha$  Activation Modulates Autophagy during Labor by Inhibiting RAB7B, Enlarging Mitochondrial Fuel Oxidation.** Xiaodi Wang<sup>†</sup>, Bolun Wen<sup>†</sup>, Junjie Bao<sup>†</sup>, Huishu Liu\*, LeLe Wang<sup>†</sup>. Guangzhou Women & Children Medical Center, Guangzhou, China.

**Introduction:** Contractions are the decisive factor in childbirth, and energy is needed to maintain effective contractions, but the exact mechanism is unclear. In the exploration of the mechanism of contractions during labor, uterine smooth muscle HIF-1 $\alpha$  is increased under postpartum hypoxia stress. There are indications that induction of HIFs could directly affect the EV biogenesis pathways involving RABs. Ras proteins are

small GTPases of the Ras GTPase superfamily, which regulate vesicle transport pathways. Rab protein is involved in autophagy by controlling the fusion and transport of autophagosomes and is an important organizer of the membrane between autophagosomes and endosomal compartment. However, its effect on lipid metabolism in muscle cells is still unclear. Here, we investigate the promotive role of HIF- $\alpha$  in uterine smooth muscle autophagy and its basis.

**Methods:** HIF- $\alpha$  activation promotes mitochondrial function by promoting autophagy in human uterine muscle or in vitro primary uterine smooth muscle cell models in labor and nonlabor. The uterine muscle transcriptome data enriched the gene set related to "autophagy". Autophagy flux was significantly increased in uterine myocytes with HIF $\alpha$  gene in labor. Mechanically, HIF- $\alpha$  inhibited RAB7B, which is responsible for the formation of autophagosomes and autosomes, by inducing miR-17-3P\_R+1.

**Results:** Chromatin immunoprecipitation assay enabled us to identify HIF- $\alpha$  as a transcription factor for miR-17-3P\_R+1. In addition, the 3'UTR luciferase assay confirmed the direct inhibition of miR-17-3P\_R+1 on RAB7B. Concordantly, HIF- $\alpha$  activation or miR-17-3P\_R+1 transfection increased mitochondrial oxygen consumption and mitochondrial transmembrane potential and played a role in contractions energy support.

**Conclusion:** In conclusion, we demonstrate for the first time that peripartum HIF-1 $\alpha$  inhibits RAB7B via transcriptional induction of miR-17-3P\_R+1. Depletion of RAB7B increases autophagic flux as indicated by the increased size of autophagic structures as well as the magnitude of macroautophagic sequestration and degradation. In addition, we found that regulation of lipid homeostasis during contractions and the molecular linkage between miRNAs and key molecules control autophagy and its coupled fuel oxidation in mitochondria. Therefore, this pathway provides a therapeutic target for contractions regulation and a theoretical basis for targeted energy supplementation during labor.

#### T-034

**Neuropathic Pain Marker Expression in Endometriosis Patients with Chronic Pelvic Pain.** Ian Waldman<sup>†</sup>, Emily R Disler, Ankrish Milne, Kha U Dam, Nicholas W Ng, Xinjie Chen, Marian Damian Cruz, Maya Seshan, Bradley J Quade, **Raymond M Anchan\***. *Brigham & Women's Hospital, Harvard Medical School, Boston, MA, United States.*

**Introduction:** Endometriosis deleteriously impacts patients' quality of life with common symptoms being pain, abnormal uterine bleeding, and infertility. Given the wide range of surgical disease load that is poorly correlated to clinical pelvic pain symptoms, we hypothesize that *the specific neurogenesis of pain neurons in endometriosis tissue correlates with phenotypic variability of pain*. In this study, we aim to [1] investigate patient-specific neuronal growth in endometriotic tissue and [2] correlate these findings with self-reported preoperative quality of life (QOL) questionnaires.

**Methods:** Patients with a clinical diagnosis of endometriosis were consented for this study and asked to complete the World-Endometriosis Research Foundation (WERF) Quality of Life (QOL) questionnaire. Patients underwent operative laparoscopy with excision of endometriosis and endometriomas as well as an endometrial biopsy for eutopic tissue. These samples were processed for immunohistochemistry (IHC), RT-PCR, and qPCR. Tissue from patients 30 years or older with the highest reported pain scores (greater than 7 out of a 10 point scale) were evaluated for varying expression levels of pain markers and nerve markers.

**Results:** [1] A qualitative IHC analysis of neuronal markers reveals greater expression in eutopic endometrial tissue relative to endometriomas, whereas greater expression of pain-specific neuronal markers was observed in endometriomas compared to eutopic endometrial tissue. [2] The differential IHC expression of pain-specific neuronal markers between eutopic endometrium and endometriomas was further validated using Qt-PCR, which demonstrated an increased expression of the genes for the pain neuronal markers, Tyrosine hydroxylase (fold change=7), Tropomyosin receptor kinase A (fold change=4), and Neural cell adhesion molecule (fold change=4).

**Conclusion:** These preliminary data suggest patient- and tissue-specific expression patterns for pain neurons, which may in part explain variability

of pelvic pain symptoms. These findings provide exciting opportunities to establish patient-specific pelvic pain therapies. This translational approach offers insight into the development and progression of the disease, leading to advances in the field of Precision Medicine.

#### T-035

**Patient-Derived Xenograft Murine Model for Precision Medicine in Endometriosis.** Valerie Flores<sup>†</sup>, Cagdas Sahin, Hugh S Taylor\*. *Yale School of Medicine, New Haven, CT, United States.*

**Introduction:** Endometriosis is a debilitating, gynecologic disease affecting 10% of reproductive-aged women. Response to medical therapy is variable as lesions do not consistently respond to first-line, progestin-based therapy. We showed in a retrospective study that progesterone receptor (PR) status in lesions can predict response to progestins. Low PR status was associated with a 6% chance of response, suggesting some women may benefit from alternative hormonal therapy as first-line. Here, we utilize patient-derived xenograft (PDX) murine models to test human endometriotic lesion response to GnRH antagonist, compared to endogenous progesterone (P4) exposure or medroxyprogesterone acetate (MPA). We aimed to test the ability of PR status to predict response to different hormonal therapies, as this could allow for individualized treatment of endometriosis.

**Methods:** 8wk old NOD/SCID mice underwent transplantation of endometrioma lesions (5mm) collected from women undergoing surgery for endometriosis. Mice were not ovariectomized. IHC was performed to determine PR expression in lesions. The Histo-Score was used to quantify PR status as High or Low as previously described. Two wks following transplantation, mice underwent daily subq injections with vehicle (DMSO), MPA (50ug), or Cetrotide (100ug); n=6-9 lesions per group. After 1 month of treatment mice were sacrificed, lesions collected, and measured. Welch's t-test used for statistical analysis.

**Results:** Lesions with high PR showed near-complete response to P4 or MPA compared to lesions with low PR (p=0.01). Avg post-treatment size in high PR lesions was 4.7mm<sup>3</sup> compared to 35mm<sup>3</sup> in low PR lesions. High PR lesions responded completely to Cetrotide (i.e., 0 mm) (p=0.009). Lesions with low PR responded poorly to P4 or MPA. The low PR lesions also had a lower response to Cetrotide when compared to high PR lesions, showing only a 50% decrease in size with Cetrotide compared to complete regression of high PR lesions. Overall, lesions with low PR showed more aggressive growth and resistance to multiple treatments compared to those with high PR.

**Conclusion:** Use of PDX models to test clinical response is a novel approach to endometriosis. We have previously shown that women with low PR lesions respond poorly to progestins. Here we validate our prior work in a mouse xenograft model by prospectively showing that lesions with low PR expression do not respond to progestin-based therapy. We show that lesions with low PR have a better response to antagonist therapy, with a more than 2-fold decrease compared to treatment with P4 or MPA; we expect this response remains clinically meaningful for patients. However these results demonstrate a distinct behavior of lesions with low PR that extends beyond progesterone resistance. The use of murine avatars can allow clinicians to predict an individual's response to therapy, avoid trialing futile treatments, and allow a precision medicine approach to endometriosis.

#### T-036

**Neuropeptide S Receptor 1 Is a Novel Non-Hormonal Treatment Target in Endometriosis.** Thomas T Tapmeier,<sup>1</sup> Nilufer Rahmioglu,<sup>1</sup> Jianghai Lin,<sup>2</sup> Maik Obendorf,<sup>3</sup> Bianca de Leo,<sup>3</sup> Grant Montgomery,<sup>4</sup> Udo Oppermann,<sup>1</sup> Stephen Kennedy,<sup>1</sup> Thomas Zollner,<sup>3</sup> Christian M Becker,<sup>1</sup> Joseph Kemnitz,<sup>5</sup> Jeffrey Rogers,<sup>6</sup> Krina T Zondervan\*. <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Jinan University, Guangzhou, China; <sup>3</sup>Bayer AG, Berlin, Germany; <sup>4</sup>University of Queensland, Brisbane, Australia; <sup>5</sup>University of Wisconsin, Madison, WI, United States; <sup>6</sup>Baylor College of Medicine, Houston, TX, United States.

**Introduction:** Endometriosis affects up to 10% of women of reproductive age world wide. Treatment options are limited and symptomatic at best. We set out to identify new treatment targets in endometriosis, and to test

candidate targets *in vitro* and *in vivo* using suitable inhibitors. We have conducted genetic analyses in human family- and population-based studies complemented by a study of large-pedigree of rhesus macaque with endometriosis to identify novel rare and common associations in *NPSR1*. We investigated functional effects of *NPSR1* *in vitro* and *in vivo* including laboratory inhibition experiments and endometriosis mouse models.

**Methods:** Germ-line sequencing of 32-families with 3+ endometriosis cases was complemented by association analyses in 3194 cases and 7060 controls, and linkage/sequencing of 849 rhesus macaques with endometriosis. Laboratory analyses were conducted on relevant patient tissues. Target inhibition was performed in cell-based assays and in endometriosis mouse models including pain readouts.

**Results:** Family-based sequencing revealed significant over-representation of predicted deleterious rare coding variants in *NPSR1* in endometriosis cases vs. controls ( $P=7.8 \times 10^{-4}$ ), involving three missense variants: novel C7\_34698148, rs34705969 and rs116825950. Association analysis also revealed a common insertion/deletion variant, rs142885915, associated with stage III/IV endometriosis ( $P=5.15 \times 10^{-3}$ , Odds Ratio=1.23, 95% Confidence Interval=1.09-1.39). *NPSR1* was expressed in glandular epithelium from eutopic and ectopic endometrium, and on immune cells in peritoneal fluid, in particular monocytes from endometriosis cases. The *NPSR1* inhibitor, SHA 68R reduced *NPSR1* mediated  $Ca^{2+}$  and cAMP signalling, pro-inflammatory TNF- $\alpha$  release and monocyte chemotaxis *in vitro* ( $P<0.01$ ), and in endometriosis models *in vivo*, with significant reductions of inflammatory cell infiltrate and abdominal pain observed ( $P<0.05$ ).

**Conclusion:** Genetic variants in *NPSR1* are associated with endometriosis, and *NPSR1* inhibition in relevant inflammatory pain models is consistent with a functional role of *NPSR1* in endometriosis. The receptor presents a new non-hormonal target for the treatment of endometriosis.

### T-037

**Immune Dysfunction in the Menstrual Effluent of Women with Endometriosis: Implications for Disease Pathogenesis.** Jessica E. Miller†, Harshavardhan Lingegowda†, Danielle Sissett†, Christine N Metz, Peter K Gregerson, Madhuri Koti, Chandrakant Tayade\*. <sup>1</sup>Queen's University, Kingston, ON, Canada; <sup>2</sup>Northwell Health, Manhasset, NY, United States.

**Introduction:** Endometriosis (EMS) is a common gynaecological condition that affects approximately 176 million women worldwide. While the pathogenesis of EMS remains unknown, the theory of retrograde menstruation is widely accepted. This theory suggests that menstrual effluent is refluxed into the peritoneal cavity where it adheres to peritoneal structures and develops into an endometriotic lesion. Therefore, the analysis of menstrual effluent (ME) can provide insights into the early time points of the pathogenesis of EMS. Here, we analyzed ME from EMS patients and healthy, fertile donors (HD) to further understand the role of immune dysfunction in the pathogenesis of EMS.

**Methods:** ME was collected using a menstrual cup from HD (n=12) and from surgically confirmed EMS patients (n=12). Serum was extracted from ME. ME serum underwent multiplex cytokine analysis (Eve Technologies, Alberta, Canada). CD45<sup>+</sup> cells were separated using cell sorting and underwent the EasySep dead cell remover (STEMCELL). CD45<sup>+</sup> cells underwent flow cytometric analysis to identify T helper 17 cells (CD4<sup>+</sup>CD3<sup>+</sup>CCR6<sup>+</sup>IL-17A<sup>+</sup>), regulatory T cells (CD4<sup>+</sup>CD3<sup>+</sup>CCR6<sup>-</sup>CD25<sup>+</sup>Foxp3<sup>+</sup>), macrophages (CD11b<sup>+</sup>CD14<sup>+</sup>CD68<sup>+</sup>), and neutrophils (CD11b<sup>+</sup>CD14<sup>-</sup>CD16<sup>+</sup>CD66b<sup>+</sup>). Non-parametric student's t-test with Mann-Whitney correction was used to test for significance. With the remaining CD45<sup>+</sup> cells, total RNA was extracted, and normalized to 20ng/ul. The RNA was analyzed using Nanostring nCounter® and nCounter® Digital Analyzer by means of nCounter® Human PanCancer Immune Panel. Resultant RCC files were uploaded to the ROSALIND software and advanced analysis took place, as per manufacturer instructions.

**Results:** ME serum from EMS had significantly elevated IL-1a compared to HD. EMS patients had significantly fewer T<sub>H</sub>17 cells in the ME compared to HD and while not significant, T<sub>REG</sub> cells were increased in EMS patients compared to HD. Macrophages and neutrophils did not show significant differences between EMS and HD. Transcriptomic profiling

revealed 20 differentially expressed (DE) genes in the ME CD45<sup>+</sup> cells of EMS compared to HD. 19 DE genes were significantly decreased in EMS patients and many of these decreased DE genes were associated with the T<sub>H</sub>17 axis including *Rora*, *IL23A*, and *IL6*.

**Conclusion:** For the first time, we show that ME from EMS patients display drastic immune dysfunction compared to HD: specifically a decrease in the T<sub>H</sub>17 axis. Therefore, this study highlights the T<sub>H</sub>17 axis as an important area for future research in the pathogenesis and therapeutics of EMS.

### T-038

**The Effect of Peritoneal Fluid-Derived Exosomes from Endometriosis Patients on Mesothelial Cells.** Kayla Y Li†, Kavita S Subramaniam, Hannah M Nazri, Thomas T Tapmeier, Krina T Zondervan, Christian M Becker\*. <sup>1</sup>University of Oxford, Oxford, United Kingdom; <sup>2</sup>Wellcome Centre for Human Genetics, Oxford, United Kingdom.

**Introduction:** Endometriosis is a gynecological inflammatory disease associated with severe pain and infertility. The most accepted theory for its pathophysiology is Sampson's theory of retrograde menstruation and subsequent lesion growth. Reports suggest that peritoneal mesothelial cells (PMCs) of the mesothelial lining have to be altered to enable lesion establishment, similar to PMCs in cancer metastasis. Peritoneal fluid (PF) from endometriosis patients has been shown to alter PMCs, but the active constituent remains unknown. We hypothesize the effect to be mediated by PF-derived exosomes and compared the effect of PF and PF-derived exosomes from endometriosis patients on PMCs in a pilot study.

**Methods:** PF samples were obtained from Stage I/II endometriosis (n=3) and non-endometriosis (n=3) patients of varying menstrual phases undergoing surgery at the John Radcliffe Hospital, Oxford, United Kingdom and processed using size-exclusion chromatography. Exosomes from all samples were characterised by Nanoparticle-Tracking Analysis. Primary LP-9 and immortalised MeT-5A mesothelial cell lines were exposed to PF and exosomes from each patient. The effects were confirmed by bright-field microscopy and quantified using a FITC-dextran cell permeability assay. Results were compared using paired t-tests.

**Results:** Exosomes were successfully isolated from PF from endometriosis and non-endometriosis patients. There were no significant differences (p > 0.05) in concentration and size of PF-derived exosomes from both groups. Following exposure to PF and isolated exosomes from endometriosis and non-endometriosis samples, it was observed by bright-field microscopy that isolated exosomes from both groups induced changes in LP-9 cell morphology, resembling retraction and gap formation, while PF with exosomes exhibited a stimulating effect on LP-9 growth. As quantified by the FITC-dextran cell permeability assay, PMCs exposed to samples from endometriosis patients experienced gap formation and an increase in permeability as judged by higher fluorescence levels, but these levels did not significantly differ (p > 0.05) from those induced by non-endometriosis samples.

**Conclusion:** PF and PF-derived exosomes from endometriosis patients potentially have distinct functional effects on PMCs. Further investigation is needed to elucidate the roles of exosomes and other PF constituents in endometriosis.

### T-039

**Collagen I Triggers Directional Migration, Invasion and Matrix Remodeling of Stroma Cells in a 3D Spheroid Model of Endometriosis.** Stejskalova Anna, Fincke Victoria†, Sebastian D. Schäfer, Ludwig Kiesel, Martin Götte\*. Muenster University Hospital, Muenster, Germany.

**Introduction:** Endometriosis is a painful gynecological condition characterized by ectopic growth of endometrial cells. Little is known about its pathogenesis, which is partially due to a lack of suitable experimental models. Here, we use endometrial stromal (St-T1b), primary endometriotic stromal, epithelial endometriotic (12Z) and co-culture (1:1 St-T1b:12Z) spheroids to mimic the architecture of endometrium, and either collagen I or Matrigel to model ectopic locations.

**Methods:** In vitro study on the immortalized epithelial endometriotic cell line 12Z, the immortalized stroma cell line ST-T1b and primary ectopic endometriotic stroma cells. Cells were cultured using the hanging drop

method prior to 3D culture on collagen I or matrigel, and subjected to treatment with pharmacological inhibitors and microRNAs. Cells were observed by conventional and immunofluorescence microscopy.

**Results:** Stromal spheroids, but not single cells, assumed coordinated directional migration followed by matrix remodeling of collagen I on day 5 or 7, resembling ectopic lesions. While generally higher area fold increase of spheroids occurred on collagen I compared to Matrigel, directional migration was not observed in co-culture or in 12Z cells. The fold increase in area on collagen I was significantly reduced by MMP inhibition in stromal but not 12Z cells. Inhibiting ROCK signalling responsible for actomyosin contraction increased the fold increase of area and metabolic activity compared to untreated controls on Matrigel. The number of protrusions emanating from 12Z spheroids on Matrigel was decreased by microRNA miR-200b and increased by miR-145.

**Conclusion:** This study demonstrates that spheroid assay is a promising pre-clinical tool that can be used to evaluate small molecule drugs and microRNA-based therapeutics for endometriosis.

#### T-040

**Artificial Intelligence for Diagnosis and Quantification of Adenomyosis: Can Robots Assist?** Joseph Huang\*,<sup>1</sup> Yan-Ru Su,<sup>2</sup> Chun-Yen Huang,<sup>1</sup> Yu-Chun Yu,<sup>1</sup> Yi-Wu Chiang,<sup>3</sup> Nari Kay.<sup>1</sup> *E-Da Hospital, Kaohsiung, Taiwan; <sup>2</sup>National Sun Yat-Sen University, Kaohsiung, Taiwan; <sup>3</sup>E-National Sun Yat-Sen University, Kaohsiung, Taiwan.*

**Introduction:** Adenomyosis is defined as the existence of endometrial tissue in myometrium and manifested by menorrhagia and dysmenorrhea with defective endometrial receptivity. Although various classifications are used to describe the extensiveness of adenomyosis, all are relatively subjective and inconsistent among different examiners. Thus, this study aimed to generate an algorithm for recognizing and quantifying the lesions that will provide more objective communication among examiners. Since TGF- $\beta$ 1 plays a crucial role in the pathogenesis of adenomyosis, the effects of anti-TGF- $\beta$ 1 on adenomyosis formation in a mouse model was used to validate the algorithm.

**Methods:** Adenomyosis in mice was induced by injecting tamoxifen (1  $\mu$ g/gm body weight) in the first four postnatal days (PNDs). At PND42, anti-TGF- $\beta$ 1 (10  $\mu$ g) or ddH<sub>2</sub>O was injected into left or right uterine horn, respectively. Uteri were collected at PND64 for H & E staining. Deep computer learning using convolutional neural network (CNN), leaky rectified linear unit (ReLU) and dense atrous spatial pyramid pooling (DASPP) was used to establish the algorithm for identifying the lesions. The accuracy of the algorithm was tested by mean intersection over union (mIOU). The quantification of the lesions was established using four parameters, including distance ratio, area index, skeleton ratio and shape index ratio.

**Results:** The computer successfully detected adenomyotic lesions and showed that anti-TGF- $\beta$ 1 significantly reduced their development. A software was generated for the quantification of adenomyotic lesions.

**Conclusion:** Deep computer learning is proven feasible for the quantification of adenomyotic lesions. The information gathered in this study will be used to further develop an algorithm for human tissues.

#### T-041

**Immortalization of Murine Uterine Stromal and Epithelial Cell Lines for Endometriosis Research.** Danielle Peterse†, Samuel Garrard†, Victor Fattori†, Aram Ghalali†, Michael Rogers\*. *Boston Childrens Hospital / Harvard Medical School, Boston, MA, United States.*

**Introduction:** Endometriosis is an estrogen-dependent gynecological disorder, characterized by the presence of endometrial-like tissue outside the uterus. For a better understanding of the pathogenesis and pathophysiology of this disease, cell- and animal models are essential. Currently, the number of models is limited and there are no murine cell lines available, although these cell lines would be of great value for endometriosis research, both *in vitro* and *in vivo* research. Therefore, we immortalized murine uterine stromal and epithelial cells in this study.

**Methods:** Uteri from 9-week old C57Bl/6J mice were harvested and dissociated using the GentleMACS (Milteny Biotech). Murine uterine epithelial cells were selected using magnetically labelled EpCAM beads

and stromal cells were collected in the flow-through. The isolated cells were cultured on Matrigel coated plates. After passaging, the cells were immortalized by transduction with retrovirus containing SV40/large T. Next, growth conditions were optimized, and the created cell lines validated by Western Blot, Immunofluorescence and RNA-sequencing.

**Results:** The cells have currently been in culture for 19 passages, and they have been keeping their respective stromal or epithelial appearance. Additionally, the cells have shown to be able to withstand the freezing and thawing process. Western blot and Immunofluorescence showed that the epithelial cells expressed E-cadherin, N-cadherin, Vimentin and Estrogen-receptor alpha. The stromal cells also showed expression of N-cadherin and Vimentin. RNA-sequencing indicated high expression of cytokeratins and cadherins in the epithelial cells. Additionally, these cells showed a high expression of genes in the PI3K-Akt signaling pathway. Pathway enrichment analysis indicated that the actin cytoskeleton-, extracellular matrix-, and regulation of cell adhesion- pathways were enriched in the epithelial cells.

**Conclusion:** In this study, stromal and epithelial cell lines were successfully created from murine uterine tissue. The results of the Western blot, immunofluorescence and RNA-sequencing validated the epithelial characteristics of the created cell lines. In the future, these cell lines can be used for *in vitro* experiments, but also for inducing endometriosis in mice. These created cell lines have the advantage that it will be very easy to study the influence of certain genes on the development and progression of endometriosis in an animal model by using CRISPR techniques to knock out genes of interest in the cells before introducing endometriosis in the animals.

#### T-042

**Endometriosis pathoetiology: The Role of microRNAs in the Dysregulation of Endometrial Function.** Bhuchitra Singh†,<sup>1</sup> Jiahui Zhang†,<sup>2</sup> Isabelle Baptista†,<sup>3</sup> Ping Xia,<sup>1</sup> James Segars\*.<sup>1</sup> *Johns Hopkins University School Of Medicine, Baltimore, MD, United States; <sup>2</sup>Renaissance School of Medicine, Stony Brook, NY, United States; <sup>3</sup>Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** The clinical diagnosis of endometriosis is often delayed 8 to 10 years after the onset of symptoms due to the lack of a reliable non-invasive diagnostic test. In recent years, a number of research studies have reported using microRNAs (miRNAs) as potential non-invasive biomarkers for diagnosing endometriosis. As miRNAs regulate gene expression, we hypothesized that a systematic review of differentially expressed miRNAs in endometriosis might identify candidate markers for further study.

**Methods:** A systematic search of PubMed and EMBASE was conducted from January 2007 to July 2, 2019. Search keywords included "endometriosis", "microRNA", "miRNA", and "biomarkers". Using PRISMA guidelines, abstracts and later full-text articles were selected for review if they evaluated the roles of microRNAs in endometriosis. The differential expression of miRNAs in endometriosis and their potential as biomarkers of endometriosis from relevant published studies were synthesized and discussed.

**Results:** Out of 210 studies identified, 28 met our inclusion criteria. The differential expression of miR-9, -34, -29c, -135, and let-7 were found to contribute to the pathogenesis of endometriosis through progesterone resistance and dysregulation of the cell cycle. Aberrant levels of miR-145, -141, -200b, -126-5p were found to be related to the proliferation, migration, and invasion of endometriotic cells. Dysregulation of miR-17-5p, -20a, -22, -449b-3p are hypothesized to affect the expression of angiogenic factors in endometriotic tissue. Furthermore, miRNAs let-7b and miR-342-3p were thought to contribute to the lipid dysfunction and thus lower BMI phenotype commonly observed in endometriosis patients. Several miRNAs in serum, including the let-7 family, miR-145, and miR-199a, emerged as potential diagnostic biomarkers for endometriosis.

**Conclusion:** This study provides a foundation of candidate microRNAs that offer diagnostic utility. Additional functional and validation studies are needed to establish the external and internal validity of microRNAs as biomarkers and to better understand their role in pathoetiology of endometriosis.

**T-043**

**The G Protein-Coupled Receptor 55 (GPR55) as Putative Target for the Treatment of Endometriosis.** Frank Sacher\*,<sup>1</sup> Gernot Langer\*,<sup>1</sup> Bernd Bojahr\*,<sup>2</sup> Martin Fritsch\*,<sup>1</sup> René Wenzl\*,<sup>3</sup> Thomas M Zollner\*,<sup>1</sup> Maik Obendorf\*,<sup>1</sup> Jens Nagel\*.<sup>1</sup> *Bayer AG, Berlin, Germany; <sup>2</sup>MIC, Berlin, Germany; <sup>3</sup>Medical University of Vienna, Vienna, Austria.*

**Introduction:** The G-protein coupled receptor GPR55 binds and becomes activated by the phospholipid lysophosphatidylinositol (LPI), is expressed on pro-inflammatory macrophages and NK cells, endometrial cells and sensory neurons

**Methods:** First, we study GPR55 expression in eutopic and ectopic endometrium as well as LPI levels in the peritoneal lavage of endometriosis patients and controls. Furthermore, we study the effect of the GPR55 agonist LPI in various *in vitro* and *in vivo* inflammatory models. Finally, we characterized the link between GPR55 and inflammatory pain in experimental models including an endometrial explant and an *in vivo* model of mechanical allodynia

**Results:** Our results indicate that GPR55 is expressed in endometriosis lesions and that the endogenous agonist LPI is slightly increased within endometriosis patients. Furthermore, GPR55 agonist LPI induces the secretion of pro-inflammatory cytokines and ERK1/2 phosphorylation in human endometrial explants. Injection of LPI into the skin resulted in the formation of skin edema as sign of induced inflammation; this effect could be blocked by co-application of the anti-inflammatory drug dexamethasone. Finally, injection of LPI into the paw of mice induced mechanical allodynia as effective as the injection of Zymosan

**Conclusion:** GPR55 is a pro-inflammatory receptor involved in inflammatory response to LPI. The results of the inflammatory *in vitro* and *in vivo* models suggesting therapeutic benefit of GPR55 antagonists in inflammatory conditions such as endometriosis

**T-044**

**Simvastatin Suppresses Wnt/ $\beta$ -catenin Pathway in Human Leiomyoma Cells.** Malak El Sabeh†, Subbroto Kumar Saha†, Sadia Afrin†, Mostafa Borahay\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Uterine leiomyomas (UL) are the most common tumors in the female reproductive tract. The Wnt/ $\beta$ -catenin pathway is a conserved pathway implicated in proliferation, differentiation and stem cell renewal. Wnt ligand binds to frizzled receptor (FZD) and its co-receptor, LRP5, resulting in the release of  $\beta$ -catenin from its destruction complex.  $\beta$ -catenin translocates to the nucleus where it alters gene expression. Several proteins in this pathway are upregulated in leiomyomas, and it was shown to be implicated in the paracrine signaling between UL stem cells and surrounding mature cells. Simvastatin is used to treat hypercholesterolemia but has promising effects as a treatment for UL as it was shown to induce apoptosis, reduce proliferation, and alter extracellular matrix deposition. The aim of this work is to check the effect of simvastatin on the Wnt/ $\beta$ -catenin pathway.

**Methods:** Primary and immortalized leiomyoma (HuLM) cells were treated with simvastatin (1  $\mu$ M) for 48 hours. The effect of simvastatin on the expression of several mRNA and proteins in the Wnt/ $\beta$ -catenin pathway was examined using RT-qPCR and western blot, respectively. Since  $\beta$ -catenin translocates to the nucleus, we performed subcellular fractionation to isolate nuclear protein. Leiomyoma cells were stained with anti-Wnt4, anti-total- $\beta$ -catenin, and anti-non-phosphorylated- $\beta$ -catenin for immunocytochemistry. Immunohistochemistry was done on human tissue collected from the clinical trial to test the effect of simvastatin on uterine leiomyoma (NCT03400826). The student's t-test was used to determine statistically significant differences ( $P < 0.05$ ).

**Results:** Simvastatin significantly reduced the mRNA expression of Wnt5a in primary leiomyoma tissue by 47% and the protein expression of Wnt4 by 57% in HuLM cells. Simvastatin also reduced the protein expression of the co-receptor LRP5 by 78%. Total and nuclear  $\beta$ -catenin were reduced after simvastatin treatment by 49% and 43%, respectively. These results were also confirmed through immunocytochemistry with a decrease in the expression of Wnt4, total  $\beta$ -catenin, and non-phosphorylated- $\beta$ -catenin, the active form of  $\beta$ -catenin, in both HuLM and primary cells. Moreover,

simvastatin reduced the estrogen and progesterone's induced increase in protein expression of Wnt4, total  $\beta$ -catenin, and non-phosphorylated- $\beta$ -catenin seen with immunocytochemistry. c-Myc, a downstream target of the Wnt/ $\beta$ -catenin pathway, was also affected by simvastatin treatment with a 51% decrease in its mRNA expression in HuLM cells. The effect of simvastatin on the Wnt/ $\beta$ -catenin pathway was confirmed using human tissue a decrease in non-phosphorylated- $\beta$ -catenin after simvastatin treatment for 12 weeks.

**Conclusion:** We conclude that simvastatin may have a therapeutic impact on UL through suppression of the Wnt/ $\beta$ -catenin pathway.

**T-045**

**Integrative Cistrome and Transcriptome Analysis Identifies Tryptophan-Kynurenine-AHR Pathway as a Novel Regulator of Leiomyoma Growth.** Azna Zuberi†. *Northwestern University, Feinberg School of Medicine, Chicago, IL, United States.*

**Introduction:** Uterine leiomyoma (LM) is the most common tumor in reproductive age women. Two distinct subtypes, one with MED12 mutation (mut-MED12) and the other with HMGA2 rearrangement (re-HMGA2), comprise 85% of all LM. Estrogen and progesterone, via their receptors ESR1 and PGR, play key roles in LM growth. Here, we aimed to identify hormone-dependent genes and pathways dysregulated by distinct driver mutations in the hope of finding druggable targets for LM treatment.

**Methods:** ChIP-seq for ESR1, PGR, and MED12 was performed using fresh-frozen tissues of G44D (the most frequent MED12 mutation) mut-MED12 LM (n=3); re-HMGA2 LM (n=2); LM without these two mutations (designated as WT LM, n=3), and their matched normal myometrium (MM) (n=8). RNA-seq was performed using the same samples. Hydrophilic metabolites were quantified using LC-MS. CCK-8 kit was used to measure cell proliferation.

**Results:** RNA-seq identified 2568 genes significantly differentially expressed only in G44D LM vs MM. Adjacent to these genes, we uncovered 785 sites that were co-bound by PGR, ESR1, and MED12 with their strongest occupancy in G44D LM. The genes associated with these sites were enriched with pathways key for LM pathogenesis. Specifically, Tryptophan (TRP) 2,3-Dioxygenase (TDO2) was only significantly upregulated in mut-MED12 LM. TDO2 protein catalyzes the first and rate-limiting step of kynurenine (KYN) production, which activates aryl hydrocarbon receptor (AHR) and plays important roles in tumorigenesis in general. Consistent with TDO2 transcript level, LC-MS assay revealed lower TRP level in G44D LM vs MM. Interestingly, KYN level was increased not only in G44D (90% of cases) but also in WT LM (71% of cases) compared with MM. KYN (25, 50, 75, 100, 150, and 200  $\mu$ M) treatment of primary LM cells for 24h dose-dependently induced CYP1B1 (known AHR target gene) expression with the highest induction at 200  $\mu$ M (n=3), indicating that it can activate AHR in LM cells. KYN (200  $\mu$ M) treatment for 48h significantly increased LM cell proliferation (n=5). TDO2 specific inhibitor 680C91 (5, 10, and 15  $\mu$ M) treatment for 48h dose-dependently decreased LM cell proliferation, reaching statistical significance at 10  $\mu$ M; whereas KYN only significantly inhibited MM cell proliferation at the highest dose (n=5). Similar treatment with AHR specific inhibitor CH223191 also significantly inhibited LM cell proliferation at 10 and 15  $\mu$ M; in contrast, MM cell proliferation was not altered by any of the doses used (n=5). These findings indicate that TDO2 and AHR inhibitors affect the function of LM cells more robustly than that of MM cells.

**Conclusion:** Our data suggest that TRP-KYN-AHR pathway was dysregulated and may play key roles in LM growth. The observed differential effect of AHR and TDO2 inhibitors on LM vs MM cells sheds light on the anti-tumorigenic roles of the compounds, which may serve as novel therapeutics for the disease.

**T-046**

**Tissue Factor Pathway Inhibitor 2 Expression in Uterine Fibroids.** Papri Sarkar†, Xiaofang Guo, Ozlem Guzeloglu-Kayisli, Asli Ozmen, Alexa Taylor, Erika New, Anthony Imudia, Charles Lockwood, Umit Kayisli\*. *University of South Florida, Tampa, FL, United States.*

**Introduction:** Tissue Factor Pathway Inhibitor-2 (TFPI2), a member of the Kunitz-type serine proteinase inhibitor family, acts as an anti-coagulant by inhibiting factor VIIa/tissue factor, factor Xa, plasmin, trypsin, and plasma kallikrein. TFPI2 is also reported to block extracellular matrix degradation (ECM) by inhibiting matrix metalloproteinase (MMP)-1, 2, 9 and 13 activity in human atheroma. TFPI2 is secreted predominantly by syncytiotrophoblasts and weakly by endothelial and smooth muscle cells. Abnormal uterine bleeding occurs in women with uterine fibroids, which also have increased ECM production and deposition. We recently found that uterine fibroid vs. their paired myometrial tissues show elevated levels of FK506-binding protein 51 (FKBP51), a co-chaperone that inhibits progesterone receptor transcriptional activity. Our global RNA-seq analysis revealed that FKBP5-silencing inhibits TFPI2 levels in fibroid cultures. Thus, we hypothesized that increased TFPI2 expression in fibroids promotes both abnormal bleeding via its anti-coagulant effects and excess ECM deposition via its anti-MMP effects.

**Methods:** Primary cultures of smooth muscle cells (n=4) isolated from uterine fibroid tissues were transfected with either scrambled or FKBP5-siRNA. Cells were then treated with 10<sup>-8</sup> M estradiol (E<sub>2</sub>) or 10<sup>-7</sup> M medroxyprogesterone acetate (MPA) and then TFPI2 levels measured by qPCR. Total RNAs from paired uterine fibroid vs. myometrial tissues from proliferative (n=8) and secretory (n=6) phases were isolated and TFPI2 levels using TaqMan Gene Expression Assay. Data were compared by a paired t-test or One-Way ANOVA with P<0.05 considered statistically significant.

**Results:** Significantly lower TFPI2 levels were detected in FKBP5-silenced vs. control siRNA treatment (Mean± SEM: 0.66±0.1 vs. 1.0±0.01; P<0.05), consistent with our RNA-seq results. Moreover, both E<sub>2</sub> and MPA treatment significantly reduced TFPI2 levels (P<0.05) in fibroid cultures. TFPI2 levels did not differ significantly in uterine fibroid vs. their paired myometrial tissue (1.32±0.6 vs. 3.16±1.3; P=0.26). Comparison of TFPI2 levels in proliferative vs. secretory phase were also not different in uterine fibroids (P=0.7) vs. myometrial tissues (P=0.1).

**Conclusion:** These results demonstrate FKBP5-mediated upregulation of TFPI2 levels, as well as E<sub>2</sub> and MPA-mediated inhibition of TFPI2 mRNA, suggesting that sex steroid withdrawal during menstruation together with enhanced FKBP5-mediated inhibition of progesterone receptor activity in fibroids increases TFPI2, which may contribute to fibroid associated abnormal uterine bleeding.

**T-047**

**Glucocorticoids Repress Vitamin D Receptor Expression in Human Uterine Fibroid Cells: Implications for the Benefit of Vitamin D.** Erin Silva†, Tanya Glenn†, Pablo Suarez†, Natalie A DeWitt†, Andrew Nguyen†, Andreanna Burman†, James Segars, Shannon Whirlledge\*. <sup>1</sup>Yale University, New Haven, CT, United States; <sup>2</sup>Johns Hopkins School of Medicine, Baltimore, MD, United States.

**Introduction:** Uterine fibroids are a significant burden for reproductive aged women. Yet, effective medical treatments suitable for long-term use remain limited. There is an urgent need to better understand the signaling pathways involved in uterine fibroid pathogenesis and how these pathways could be exploited for targeted therapeutics. Glucocorticoids are emerging transcriptional regulators in uterine fibroid cells and have been shown to promote fibroid cell proliferation. We hypothesize that glucocorticoid signaling in uterine fibroids regulates the expression of genes critical to fibroid pathogenesis.

**Methods:** A bioinformatics analysis of COREMINE Medical, MetaCore, Ingenuity Pathway Analysis, and the Comparative Toxicogenomics Database was performed to identify novel glucocorticoid-associated genes that are linked to uterine fibroids. Immortalized human uterine fibroid cells (UtLM) and primary fibroid cells were treated with the glucocorticoids cortisol and dexamethasone (10 or 100nM) for RNA and protein measurements. Parallel experiments were performed in

immortalized uterine smooth muscle cells (UtSMC). The mechanism of glucocorticoid regulation was explored via siRNA knockdown, and actinomycin D treatment was employed to evaluate the effect on RNA stability. For all experiments, biological replicates n>3.

**Results:** We identified four genes that were associated with the search term “glucocorticoid receptor” and key uterine fibroid features. Glucocorticoid treatment significantly repressed the expression of all four genes in UtLM cells (p<0.05-0.0001). Notable among the repressed genes was the vitamin D receptor (VDR), which inhibits fibroid proliferation upon activation with vitamin D. Repression of VDR mRNA was specific to fibroid cells, as glucocorticoid exposure enhanced VDR expression in UtSMCs, and was validated in primary human uterine fibroid cells. By knocking down the glucocorticoid receptor (GR) gene, we determined that repression of VDR requires GR. Co-treatment of glucocorticoids and actinomycin D demonstrated that glucocorticoids do not significantly alter the stability of VDR mRNA. In addition, glucocorticoid-mediated repression occurred within hours and was significant for nascent VDR transcripts, supporting a potential direct transcriptional regulation by GR. Interestingly, co-treatment of glucocorticoids with the active form of vitamin D, calcitriol, blunted expression of VDR-responsive genes.

**Conclusion:** These studies are the first to demonstrate that glucocorticoids may interfere with vitamin D signaling in uterine fibroid cells by repressing the expression of VDR. Thus, glucocorticoids may promote fibroid growth by inhibiting the actions of vitamin D.

**T-048**

**The Effect of Isolation Method on the Phenotype of Uterine Fibroid Cells in Culture.** Tanya L Glenn†, Erin Silva†, Pablo Suarez, Clare Flannery, Shannon Whirlledge\*. *Yale University, New Haven, CT, United States.*

**Introduction:** Despite the prevalence and severity of uterine fibroids, their pathogenesis is poorly understood. This lack of insight regarding the mechanisms leading to uterine fibroid formation and growth has impeded the development of long-lasting, effective, non-surgical alternatives for the treatment of uterine fibroids. *In vitro* studies with primary patient cells are a fundamental aspect to identifying the uterine fibroid signaling pathways driving pathogenesis. Yet, we have found over 30 different published methods for isolating primary uterine fibroid cells from patient tissue. As such, no standardized protocol has been established, and potential variations among protocols may influence the reproducibility of outcomes. The objective of this study was to determine whether variables in cell isolation methods result in differences in fibroid cell proliferation or the expression of uterine fibroid markers.

**Methods:** We performed fibroid cell isolation from patient-derived tissue, comparing primary variables among digestion protocols: timing of digestion (day of tissue procurement versus the next day), type of collagenase used for digestion (Type II versus Type IV), and base media for digestion (DMEM/F-12 versus Hanks' Balanced Salt Solution-HBSS). We assessed the different methods by plating an equivalent volume of digested cells and measuring cell viability with an MTT assay, cell death by LDH release, and the transcript levels of the sex steroid receptors, estrogen receptor alpha (ESR1) and progesterone receptor (PGR), as well as uterine fibroid marker genes dermatopontin (DPT), versican (VCAN), transforming growth factor beta 3 (TGFβ3), and cytochrome P450 26A1 (CYP26A1) by qRT-PCR.

**Results:** Compared to HBSS, cells from tissue digested in DMEM/F-12 media released less of the cell death marker LDH 24hr after plating. Correspondingly, cells digested in DMEM/F-12 media demonstrated the greatest rate of proliferation. While waiting to digest tissue until the next day did not result in increased release of LDH, proliferation rates were lower when compared to tissue processed the same day, and tissue digested next day in HBSS with collagenase IV demonstrated the slowest rate of cell proliferation. We found no statistical difference in the expression of ESR1, PGR, VCAN, TGFβ3, or CYP26A1 mRNA between cells digested the same day versus those digested the next day, although transcript levels of DPT were significantly lower (p<0.033) in the next day digested cells.

**Conclusion:** These data are the first to demonstrate that the method by which uterine fibroid cells are isolated from patient tissue can alter the

cellular phenotype. This discovery suggests that efforts to standardize uterine fibroid cell isolation may greatly improve the reproducibility and rigor of uterine fibroid research.

#### T-049

**Correlation of Methylation Status and Gene Expression Shows Epigenetics Involvement in Key Biological Processes of Uterine Leiomyoma Development.** María Cristina Carbajo-García†,<sup>1,2</sup> Ana Corachán†,<sup>1,2</sup> Elena Juárez-Barber†,<sup>2</sup> Javier Monleón\*,<sup>3</sup> Vicente Payá\*,<sup>3</sup> Alexandra Trelis†,<sup>3</sup> Alicia Quiñonero\*,<sup>2</sup> Antonio Pellicer\*,<sup>1,4</sup> Hortensia Ferrero\*.<sup>2</sup> <sup>1</sup>University of Valencia, Valencia, Spain; <sup>2</sup>IVI Foundation, Health Research Institute la Fe, Valencia, Spain; <sup>3</sup>La Fe University and Polytechnic Hospital, Valencia, Spain; <sup>4</sup>IVIRMA Rome, Rome, Italy.

**Introduction:** Uterine leiomyoma (UL) is a multifactorial disease with an unclear pathogenesis and high prevalence. The gold standard treatment is surgery, which is associated with high costs and impact in global health diminishing reproduction options. For these reasons, to determine new mechanisms involved in UL pathogenesis could allow us to define new molecular targets to treat them. In this regard, epigenetics through DNA methylation could be a new therapeutic approach because of it could be reversed. Thereby, we focused on RNA sequencing (RNAseq) and genome-wide DNA methylation (GW-DNAmet) integration to describe new molecular pathways and define driver genes as epigenetic targets for UL treatment.

**Methods:** Prospective study of DNA methylation by GW-DNAmet and gene expression by RNAseq of UL versus adjacent myometrium (MM) tissue. Samples were collected from women aged 27-49 years (n=31). RNAseq and GW-DNAmet results were statistically analyzed (FDR < 0.05) and validated by quantitative real-time PCR and pyrosequencing, respectively.

**Results:** Principal Component Analysis (PCA) revealed a differentiated behavior of global methylation in UL versus MM tissue, demonstrating an effect of DNA methylation on tumorigenesis. Accordingly, a characteristic global transcriptome of UL versus MM was observed by PCA in RNAseq. Among the most statistically significant differentially expressed genes, we found *MEX3B*, *MAGED1*, *CAPN6* and *PPARG1A* to be upregulated and hypomethylated (Fold change [FC]= 5.36, adjusted pvalue [p]= 0.0037; FC=1.49, p= 0.0045; FC= 47.94, p= 0.0054; and FC=2.21, p= 0.0063, respectively). These genes were all associated with cancer and involved in cell migration, invasion, proliferation and tumor metabolism, respectively. Accordingly, functional analysis showed enriched functions deregulated in UL versus MM (FDR < 0.05), involved in biological processes such as migration, invasion, immune response, cell activation and exocytosis.

**Conclusion:** This study showed that DNA methylation status and gene expression exhibit a significant global effect on UL, altering key biological processes implied on tumor development. Thereby, epigenetics through DNA methylation is an important regulator of UL pathogenesis and its reversion could be a promising new therapeutic approach to treat UL patients. This study was funded by PI18/00323, ACIF/2019/139, APOSTD/2020/123, FI19/00110 and CP20/00120.

#### T-050

**Obesity-Related Leptin and Ghrelin Alterations on Leiomyoma Growth.** Lauren Reschke†, Sadia Afrin†, Malak El Sabeh†. Johns Hopkins University, School of Medicine, Baltimore, MD, United States.

**Introduction:** Uterine leiomyomas or fibroids, are the most common benign smooth muscle tumors of the female reproductive system, with an estimated lifetime incidence of 70-80%.<sup>1</sup> Obesity is a chronic disease that represents a global health burden and major public health concern. Recent evidence suggests a positive association between body mass index (BMI) and uterine leiomyomas, with a two-threefold increased risk of uterine leiomyomas in obese women.<sup>2,3</sup> Leptin and ghrelin are key hormones involved in energy homeostasis, with leptin elevated in obese individuals, while ghrelin is decreased. We hypothesized that adipose tissue dysfunction-associated alterations in leptin and ghrelin influence leiomyoma proliferation in vitro.

**Methods:** Human immortalized uterine leiomyoma (HuLM) cells were treated with leptin (100ng/mL) and ghrelin (10nM) for 8 days. Proliferative

effects were examined using western blotting for cellular proliferation marker, PCNA, and collagen I, a marker of extracellular matrix (ECM) deposition. Student's t-test was used to determine statistically significant differences ( $p < 0.05$ ).

**Results:** Compared to controls, the levels of PCNA significantly increased in cells treated with leptin by 62% ( $p=0.04$ ), and trended to have more ECM deposition noted by an increase in collagen I ( $p=0.20$ ). Cells treated with ghrelin had the opposite effect, with a significant 83% reduction in PCNA ( $p=0.001$ ), and trended to have less ECM deposition with lower levels of collagen I compared to controls ( $p=0.84$ ).

**Conclusion:** Treatment of HuLM cells with leptin suggests increased leiomyoma proliferation and ECM deposition, while treatment with ghrelin demonstrates decreased proliferation and ECM deposition. Further experimentation is warranted to determine the underlying mechanisms of these changes. Obesity and obesity related alterations in leptin and ghrelin may be a potential target for conservative therapeutic interventions for women with uterine leiomyomas. **References:** 1. Baird, D.D., et al., *High cumulative incidence of uterine leiomyoma in black and white women: ultrasound evidence.* Am J Obstet Gynecol, 2003. 188(1): p. 100-7. 2. AlAshqar, A., et al., *Cardiometabolic Risk Factors and Benign Gynecologic Disorders.* Obstet Gynecol Surv, 2019. 74(11): p. 661-673. 3. Borahay, M.A., et al., *Estrogen Receptors and Signaling in Fibroids: Role in Pathobiology and Therapeutic Implications.* Reprod Sci, 2017. 24(9): p. 1235-1244.

#### T-051

**Evaluating the Inhibitory Effect of Elagolix & Relugolix on Leiomyoma Growth in 2D Cell Culture.** Danielle Wright, Joy Britten-Webb, Minnie Malik, William Catherino. Uniformed Services University of Health Sciences, Bethesda, MD, United States.

**Introduction:** GnRH antagonists directly compete with endogenous GnRH at receptor sites and cause inhibition of sex hormone production. Collectively, GnRH antagonists pose as treatment options for various gynecologic conditions including uterine leiomyoma through suppression of estradiol and progesterone, hormones that contribute to the morphogenesis of leiomyoma through increased expression of proteins that make up its extracellular matrix (ECM). Elagolix and relugolix are both orally administered GnRH antagonists that provide a new and innovative way to treat uterine leiomyoma. The half-life of elagolix is 4-6 hours with an IC50 of 1.5 nM, while the half-life of relugolix is 49.4 hours with an IC50 of 0.33 nM. Given the differing pharmacological properties of these two drugs, the objective of this study is to determine the direct effect elagolix and relugolix have on the extracellular matrix of human leiomyoma.

**Methods:** Leiomyoma cells were exposed to elagolix and relugolix for 24 and 48 hours. Expression of leiomyoma extracellular matrix proteins (collagen, fibronectin, and versican) were analyzed using western blot analysis.

**Results:** Suppression of ECM proteins was not sustained at 48 hours in leiomyoma cells treated with Elagolix, suggesting that leiomyoma cells directly metabolize elagolix to an inactive metabolite. Leiomyoma cells treated with Relugolix for 24 hours showed maximal, decreased expression of collagen (0.59 +/- 0.16-fold,  $p=0.000032$ ) and fibronectin (1.09 +/- 0.88-fold,  $p=0.0000074$ ) at 100 nM. For versican maximal, decreased expression was achieved at 10 nM (0.99 +/- 0.19-fold,  $p=0.000015$ ); at 100 nM protein expression increased perhaps due to a cytotoxic effect noted at this concentration. At 48 hours, a concentration dependent suppression was seen in collagen (0.59 ± 0.16-fold,  $p=0.0005$ ) and fibronectin (1.09 ± 0.88-fold,  $p=0.000026$ ) up to 100 nM. For versican, a concentration dependent suppression was achieved up to 10 nM (0.55 ± 0.00); at 100 nM protein expression, again, increased. Finally, when comparing performance of elagolix and relugolix, relugolix maintained suppression of our proteins' expression at 48 hours.

**Conclusion:** Our findings suggest that treatment with relugolix directly regulated collagen, fibronectin and versican production in our 2D human leiomyoma cell culture model. The reduction in protein expression caused

by relugolix treatment provides a direct mechanism whereby leiomyoma size may be decreased by disrupting the aberrant fibrosis that characterizes the leiomyoma phenotype.

### T-052

**Single Cell Transcriptomes from Uterine Fibroids and Fibroid-Free Myometrium Elucidate Myometrial Tumorigenesis.** Wanxin Wang<sup>†,1</sup>, Aymara Mas,<sup>2</sup> Patricia Escorcia,<sup>2</sup> Javier Monleon,<sup>3</sup> Stephen Quake\*,<sup>1,4</sup> Carlos Simon\*,<sup>5,2,6</sup> <sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>Igenomix Foundation, Valencia, Spain; <sup>3</sup>Hospital Universitario La Fe, Valencia, Spain; <sup>4</sup>Chan Zuckerberg Biohub, San Francisco, CA, United States; <sup>5</sup>Harvard University, Boston, MA, United States; <sup>6</sup>Valencia University, Valencia, Spain.

**Introduction:** Uterine fibroids represent the most common benign tumors in women in reproductive age. It has been reported that they arise from a single dysregulated myometrial smooth muscle cell. However, the underlying tumorigenic mechanism remains unclear and surgery has been the gold standard for treatment. Our primary motivation stems from the need to better understand the cellular hierarchy of uterine fibroids and myometrium, leveraging the high resolution of single cell RNAseq. We aim to identify cell types and states that are unique to the fibroids, based on their molecular signatures. This may point towards more targeted and less invasive treatment strategies for the disease, and better elucidate the mechanism of myometrial tumorigenesis.

**Methods:** After tissue dissociation, single cell RNA-seq analysis was performed to profile a total of 5432 single cells from uterine fibroids (F), fibroid-free matched myometrium (M) and healthy myometrium (hM) from 7 patients. Full length cDNA libraries of individual cells were prepared using an adapted SmartSeq2 protocol. Nextera XT DNA Sample Preparation kit was used for library preparation. Each cell was sequenced on a Novaseq to ~1e<sup>6</sup> reads/cell. Quality control and bioinformatic analyses were performed using custom R scripts.

**Results:** Dimensional reduction revealed that F, M and hM consist of 14 cell types and states. Canonical markers and highly differentially expressed genes identified major lineages of smooth muscle cells (SMC), fibroblasts (FB), vascular smooth muscle cells, lymphatic endothelia (LEC), vascular endothelia, macrophages/dendritic cells and mast cells. We discovered that the tumor (F) and the non-tumor (M/hM) tissues differ most drastically in FB, SMC, and LEC. LEC is more abundant in the tumor. For both SMC and FB, we identified states that are enriched in tumor and non-tumor, respectively, and report signatures that differentiate the two states.

**Conclusion:** Single cell transcriptomic analyses revealed cellular hierarchies that are common or different among F, M, and hM. Cell types and states that are unique to F and their expression signatures might provide molecular targets for less invasive treatment of these benign tumors. (Wang W & Mas A contributed equally.)

### T-053

**Nintedanib Alters Hippo Signaling in Uterine Fibroid Cells Leading to Decreased Levels of Connective Tissue Growth Factor and Cyclin D1, and Reduced Fibroid Cell Proliferation.** Md Soriful Islam, Ha Vi S Nguyen, Jacqueline Y Maher, Joshua T Brennan, James H Segars\*. Johns Hopkins University, School of Medicine, Baltimore, MD, United States.

**Introduction:** Recent studies showed that uterine fibroids exhibit dysregulation of the Hippo signaling pathway, a key signaling pathway regulating cell proliferation and apoptosis. Hippo cytoplasmic modules include MST1/2 kinases and SAV1 that form a complex to phosphorylate and activate LATS1/2, which then phosphorylates YAP/TAZ resulting in 14-3-3-mediated cytoplasmic retention and degradation of YAP/TAZ. Conversely, dephosphorylated YAP/TAZ translocates to the nucleus to induce expression of genes promoting cell proliferation, such as CTGF and cyclin D1 (CCND1). Nintedanib is a multi-target tyrosine kinase inhibitor that is FDA-approved for treatment of idiopathic pulmonary fibrosis. Here, we tested the hypothesis that nintedanib treatment of uterine fibroid cells would alter Hippo signaling to influence cell proliferation.

**Methods:** To assess cell viability, human myometrial (P51M) and fibroid (P51F) cells were treated with nintedanib at different concentrations (0.1, 0.5, 1, 5 and 10  $\mu$ M) for 24 or 48hrs. Next, following 24hrs of serum

starvation, we treated both myometrial and fibroid cells with nintedanib at 5  $\mu$ M for 24hrs or 48hrs in media supplemented with 10% serum. Then we measured mRNA and/or protein expression of PCNA, LC3B, SAV1, MST1, LATS1, YAP, p-YAP, CTGF and CCND1. ImageJ was used to quantify protein expression and differences in mRNA levels were assessed using the  $2^{-\Delta\Delta CT}$  method with an alpha of  $p < 0.05$ .

**Results:** We found a differential effect of nintedanib treatment at 5  $\mu$ M for 24 or 48 hrs with a  $\geq 20\%$  reduction of fibroid cell growth, and no effect on myometrial cells. In addition, protein levels of the proliferation marker, PCNA, were decreased after 48hrs of nintedanib treatment in fibroid cells. Nintedanib treatment also significantly elevated protein expression of the autophagy marker LC3B in fibroid cells. The Hippo pathway kinases MST1 and LATS1 were not affected by nintedanib treatment while SAV1 mRNA was significantly induced in fibroid cells. The protein levels of YAP were significantly reduced after 24hrs of nintedanib treatment in fibroid cells. As expected, p-YAP protein expression levels were slightly increased by nintedanib treatment. Of note, the YAP-responsive genes, CTGF and CCND1, were decreased by nintedanib treatment in fibroid cells.

**Conclusion:** We found that nintedanib treatment had a differential effect on fibroid cell growth compared to myometrial cells. Hippo signaling was altered, resulting in reduced levels of genes involved in cell proliferation. Nintedanib promoted fibroid cell death, at least in part, through autophagy. These results provide evidence to support additional evaluation of nintedanib using *in vivo* models of uterine fibroid growth.

### T-054

**Impact of Vilaprisan on Well-Being in Mice in the Induced Menstrual Bleeding Model.** Oliver M Fischer, Frank Sacher, Jens Nagel, Thomas M Zollner. Bayer AG, Berlin, Germany.

**Introduction:** Abnormal uterine bleeding (AUB) or heavy menstrual bleeding (HMB) are common gynecological problems that have significant impact on quality of life of affected women and on the activities of their daily living. The most prominent cause of AUB & HMB are uterine fibroids (myomas or leiomyomas), steroid-dependent benign tumors. Besides the heavy bleeding during menstruation, dysmenorrhea, generally known as painful periods, is a common symptom of the disease. Vilaprisan is selective progesterone receptor modulator currently in development for the treatment of uterine fibroids and endometriosis. Available clinical data demonstrate good efficacy of vilaprisan in controlling uterine bleeding in uterine fibroid patients. Back-translation of clinical data into preclinical settings is critical advance preclinical research in the field. Here, we used an established murine model to study menstrual bleeding in rodents. In this murine model hormone substitution and induced decidualisation of the uterus are used to simulate menses in rodents. This experimental set up is combined with a behavioral analysis of the animals to assess overall well-being depending on vilaprisan treatment.

**Methods:** Artificial menstrual cycle phases of ovariectomized mice were induced by E2 and P4 supplementation/ withdraw schedule including the induction of decidualisation with intra-uterine oil application. Concomitantly, animals were treated with vehicle or vilaprisan at doses of 0.3, 1 and 3 mg/kg. To study the well-being, animals were subjected after P4 withdraw to an open field test, which is generally used to assess locomotion and exploratory behaviors.

**Results:** Treatment of mice with vilaprisan significantly reduced blood loss of the simulated menses in a dose-dependent manner compared to vehicle-treated animals and in line with the observed efficacy in clinical trials. Interestingly, while simulated menses result in a strong reduction of locomotor activity (Nagel et al, SRI 2019), treatment with vilaprisan significantly improved activity behavior of the animals which is associated with an improved overall well-being of the animals. In clinical trials an improvement in quality-of-life related endpoints has been reported.

**Conclusion:** Taken together we conclude that the murine heavy menstrual bleeding model translates well into the clinical setting as demonstrated by the observed efficacy of vilaprisan on both endpoints, blood loss as well as locomotor activity which mirror the results observed in clinical trials.

## T-055

**Complementary and Alternative Medicine Use among Women with Symptomatic Uterine Fibroids.** *Elia Marina Rubio†, Joan Hilton, Vanessa Jacoby\*. University of California San Francisco, San Francisco, CA, United States.*

**Introduction:** Uterine fibroids represent a significant health burden among reproductive-aged women. Fibroids can cause heavy menstrual bleeding, urinary incontinence, pelvic pain, emotional distress, and fertility problems. Minimally invasive surgeries and pharmaceutical therapies exist to provide temporary symptom relief. The use of complementary and alternative medicine (CAM) for a wide range of medical conditions is well documented, but CAM use specifically for fibroid-associated symptomatology has not been adequately described. This study aims to examine CAM use among women with image-confirmed, symptomatic uterine fibroids in the United States.

**Methods:** We conducted a cross-sectional analysis of baseline data from a multi-center, prospective cohort study of premenopausal women with symptomatic fibroids enrolled in the Uterine Leiomyoma Treatment with Radio Frequency Ablation (ULTRA) study from 2017-2019. Participants were queried about CAM use in the prior six months. Women were asked to indicate any use of 10 CAM modalities (acupuncture, massage, exercise, diet, herbs, tea, yoga, chiropractor, physical therapy, other) specifically for fibroid symptoms, or for other reasons. A multivariate logistic regression model was performed to identify the independent factors associated with CAM-use among those who used CAM to treat fibroid symptoms and those who did not use CAM for fibroids.

**Results:** Among 204 women, 55% were Black/African American with a mean age of 42 (SD 6.6) years. CAM use was common with 42% (95% CI 35-49%) reporting use of CAM modalities specifically to treat fibroid symptoms. Popular CAM treatments used were diet (65%), herbs (52%), tea (48%), and exercise (35%); massage (29%), yoga (13%), acupuncture (17%), and chiropractor (8%) were also readily used. On average, each individual utilized 3 different types of CAM regardless of its use for fibroids symptoms only, other reasons only, or both. In our multivariable model, there were no differences in age or race/ethnicity among CAM users versus non-users. CAM users were 3 times more likely to report pelvic pressure or pain as a primary fibroid symptom than those who did not use CAM (OR 3.1 95% CI: 1.2-8.3). We also found that women with a body mass index above average were less likely to use CAM for fibroids (OR: .94 95% CI .88-.98). Other notable but non-significant associations were CAM-users being almost twice more likely to desire future pregnancy (OR: 1.95 95% CI .91-4.18) and report experiencing urinary frequency or nighttime urination as a primary fibroid symptom (OR 1.49 .73-3.0).

**Conclusion:** In a racially, ethnically, and geographically diverse sample of women seeking surgical intervention for uterine fibroids, CAM use was highly prevalent. Our findings highlight the use of CAM to mitigate the severity of symptoms. Clinical trials are needed to understand the effectiveness of CAM for fibroids symptoms.

## T-056

**Increased FK506-Binding Protein 51 (FKBP51) Promotes Uterine Fibroid Cell Differentiation Leading to Extracellular Matrix Abundance.** *Erika P New†, Xiaofang Guo,<sup>1</sup> Nihan Semerci,<sup>1</sup> Ozlem Guzeloglu-Kayisli,<sup>1</sup> Anthony N Imudia,<sup>1,2</sup> Charles J Lockwood,<sup>1</sup> Umith A Kayisli\*.<sup>1</sup> University of South Florida, Tampa, FL, United States; <sup>2</sup>Shady Grove Fertility Center Tampa Bay, Tampa, FL, United States.*

**Introduction:** Uterine fibroids are benign growths that arise in the myometrium, causing significant morbidity. Fibroids are associated with enhanced extracellular matrix (ECM) production and composed of clonally derived smooth muscle cells, as well as heterogeneous cell types including fibroblasts, blood vessels, nerves, inflammatory cells, mesenchymal cells, and stem cells. Our prior research demonstrated that fibroids compared to matched myometrial tissues express significantly higher levels of FK506-binding protein 51 (FKBP51), an immunophilin protein that inhibits transcriptional activity of the progesterone receptor. The clinical significance of increased expression of FKBP51 in fibroid vs.

myometrial tissues however is unknown. Thus, our aim is to determine the impact of increased FKBP51 levels in uterine fibroid pathogenesis by measuring global transcriptional changes in uterine fibroid cells.

**Methods:** Human primary smooth muscle cells isolated from fibroid tissues obtained from patients with uterine leiomyoma were cultured to 70% confluence. Cultures (n=4) were transfected with either scrambled siRNA as control or FKBP5-specific siRNAs to silence FKBP5 expression. After 72 h, RNAs were isolated and processed for global RNA-Seq analysis to obtain differentially regulated genes. Ingenuity Pathway Analysis (IPA) software was used as a bioinformatic tool to describe dynamic molecular changes between groups. Results were confirmed by qPCR using TaqMan Gene Expression assays. Data were compared by a paired t-test and p<0.05 was considered statistically significant.

**Results:** A 25-fold decrease in FKBP5 levels was detected in fibroid cultures after FKBP5 siRNA vs. scrambled siRNA transfection for 72 h. Bioinformatic analysis of global RNA-seq results revealed that FKBP5-silencing was associated with downregulation of several ECM genes, including collagen type1 A1 (*COL1A1*), *COL4A5*, *COL7A1*, *COL10A1*, fibronectin (*FN1*), and laminin c1 (*LAMC1*). Analysis of qPCR revealed a 1.68-fold reduction in *COL4A5*, 1.76-fold reduction in *COL7A1*, 1.54-fold reduction in *FN1*, and 1.49-fold reduction in *LAMC1* levels in FKBP5-silenced vs. control transfected uterine fibroid cells (P<0.05).

**Conclusion:** These results indicate that FKBP51 expression is involved in collagen, fibronectin, and laminin over-production in uterine fibroid cells. Compared to myometrial tissue, increased FKBP51 in fibroids promotes cell differentiation toward a secretory smooth muscle cell type leading to increased ECM deposition. Silencing FKBP51 reduces expression of these proteins, suggesting a novel therapy targeting cellular differentiation.

## T-057

**Effect of Selective Progesterone Receptor Modulator (SPRM), Ulipristal Acetate (UPA) on Uterine and Fibroid Volume Measured by Unbiased Stereology and MRI.** *Hilary Critchley\*,<sup>1</sup> Kaiming Yin,<sup>1</sup> Lucy Whitaker,<sup>1</sup> Suzanne McLenachan,<sup>2</sup> Jane Walker,<sup>2</sup> Graham McKillop,<sup>2</sup> Neil Roberts.<sup>1</sup> <sup>1</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>2</sup>Royal Infirmary, Edinburgh, United Kingdom.*

**Introduction:** Heavy Menstrual Bleeding (HMB) adversely affects the quality of life of one in four women of reproductive age and represents a substantial burden and cost to society. The aim of the present study was to determine whether the Selective Progesterone Receptor Modulator (SPRM) Ulipristal Acetate (UPA) is effective in reducing the volume of the uterus in women with HMB, and if this is influenced by whether fibroids are present.

**Methods:** Nineteen women with HMB (with and without uterine fibroids) were recruited (informed written consent) for the embedded mechanism of action study in the UCON clinical trial (EUDRACT 2014-003408-65, REC -14/LO/1602). Women were treated with three 12 week cycles of 5 mg daily UPA with 4 weeks off medication between each cycle. Structural T2-weighted Magnetic Resonance Imaging (MRI) was performed at baseline (pre-treatment) and after six, and twelve, months and which corresponds to after 2.00 (SD = 0.16), and 2.96 (SD=0.09), cycles, respectively. The volume of the body of the uterus excluding cervix, and of uterine fibroids when present, was estimated from the MR images using the Cavalieri method of modern design stereology in combination with point counting.

**Results:** The measurements, which were shown to have excellent intra-rater repeatability (Pearson's r 0.997 p<0.001 for uterus and 0.982 for fibroids p<0.001) and inter-rater reproducibility (Pearson's r 0.994 p<0.001 for uterus and 0.984 for fibroids p<0.001), were performed blind to whether the MR images had been acquired at baseline or after 6 or 12 months. For the total group of 19 patients, 8 of whom had fibroids, a significant reduction in the volume of the body of the uterus was observed after three, but not two, cycles of UPA (p = 0.02).

**Conclusion:** SPRM UPA treatment for 12 months produced a significant reduction in the volume of the uterus. Further analysis is in progress to assess whether the response may be different depending upon whether fibroids are present in the uterus.

Disclaimer The views expressed are the view of the author(s) and not necessarily those of the MRC, NIHR, or the Department of Health and Social Care.

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### T-058

**Differential Hypoxic Response in Uterine Leiomyoma & Myometrial Cells.** Mariko Miyashita-Ishiwata<sup>†</sup>, Malak El Sabeh<sup>†</sup>, Sadia Afrin<sup>†</sup>, Mostafa Borahay. *The Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Uterine leiomyomas are one of common tumor of the female reproductive tract. Angiogenesis is the physiological process that forms new blood vessels from pre-existing vessels, and is a crucial step for tumor growth and development. Multiple angiogenic growth factors are differentially expressed in leiomyoma compared with myometrium, but the mechanism about leiomyoma angiogenesis remains still unclear. Some angiogenic factors are induced under hypoxia chiefly and oxygen concentration in uterine leiomyoma was reported significantly lower than in the surrounding myometrium. The mechanism of leiomyoma pathogenesis and physiology under hypoxic condition is unknown. The aim of this study was to assess the differential expressions of angiogenic, fibrosis and proliferative factors under hypoxic condition between uterine leiomyoma cells and myometrial cells.

**Methods:** Primary leiomyoma cells and myometrial cells were isolated from leiomyoma and the surrounding myometrium. Cells were cultured in a hypoxic chamber (2% O<sub>2</sub>) or stimulated with cobalt chloride (CoCl<sub>2</sub>) to mimic hypoxic condition. The protein expressions of angiogenic factors (vascular endothelial growth factor: VEGF, endothelin-1: ET-1 and adrenomedullin: ADM) and hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) were measured by western blotting (WB). The expressions of transforming growth factor (TGF)- $\beta$ 3 and its downstream (SMAD signaling pathway) were evaluated by WB. Proliferation was valued by using proliferating cell nuclear antigen (PCNA) and extracellular matrix (ECM) production was valued by using fibronectin.

**Results:** HIF-1 $\alpha$  was induced in both of leiomyoma and myometrial cells under hypoxic condition. Protein expressions of VEGF, ET-1, ADM and TGF- $\beta$ 3 were increased under hypoxic condition in leiomyoma cells by 2.33-fold, 4.22-fold, 3.48-fold, and 1.27-fold compared to normoxic condition, while those expressions in myometrial cells were decreased under hypoxic condition. Phosphorylation of SMAD 3 was upregulated under hypoxic condition in leiomyoma cells by 2.3-fold, though SMAD3 pathway was not phosphorylated in myometrial cells under hypoxic condition. PCNA and fibronectin expressions were increased under hypoxic condition in leiomyoma cells by 1.9-fold and 1.23-fold, not in myometrial cells.

**Conclusion:** We conclude that angiogenic factors are increased in leiomyoma cells under hypoxic condition and decreased in myometrial cells. Proliferation and fibrosis are induced in leiomyoma cells under hypoxic condition. The results indicate that angiogenesis, ECM production and proliferation are induced in uterine leiomyoma more under hypoxic condition than normal condition and suggest that leiomyoma is more capable to grow under low oxygen concentration than normal oxygen concentration. Further studies are warranted to show the association between tumor growth and hypoxia in uterine leiomyoma.

### T-059

**Insights Into Primary Ovarian Insufficiency Through Clinical and Biochemical Data at the NIH.** Jamie Merkison, Ninet Sinaii, Veronica Gomez-Lobo, Jacqueline Maher\*. *National Institutes of Health, Bethesda, MD, United States.*

**Introduction:** Primary ovarian insufficiency (POI) is a heterogenous condition causing progressive hypergonadotropic hypogonadism in women prior to age 40. POI has significant health and reproductive consequences for patients. The aim of this analysis was to provide a descriptive summary of clinical/biochemical information collected from patients to detail their presentation and fertility outcomes.

**Methods:** From 1991 - 2017, a NIH protocol collected data from POI patients under the direction of PI Lawrence Nelson, MD. Relevant data were extracted from 899 patients within the NICHD Data and Specimen Hub (DASH). A diagnosis of POI was confirmed via documentation of oligomenorrhea and at least 1 FSH value > 40 OR 2 FSH values > 25. 530 patients met diagnostic criteria. Data are described as frequency (%) and median (IQR).

**Results:** A diverse group were represented: 79% white (n=417), 12% black (n=65), 4% Asian (n=23), 4% Hispanic (n=22), 0.4% Native Hawaiian/Pacific Islander (n=3). Age at time of study entry ranged from 17.3-42.9, with a median of 33 years. Median age of menarche was 13(12-14) years, and median age at onset of menstrual abnormality was 25 (17-32) years. POI was diagnosed at median age 29 (24-34) years, with 19 (7-54) months elapsed from diagnosis to study entry. There were 61 single live births by 44 women prior to POI diagnosis. After POI diagnosis, 9 women reported 11 live births. Three patients conceived spontaneously and 6 underwent ovulation induction/IVF or used donor eggs. Seven spontaneous abortions (SAB) occurred in 5 patients. Therefore, 2.6% conceived following POI diagnosis. Of the 101 (19%) patients who underwent ovulation induction/IVF, 2% (n=2) had live births and 3% (n=3) had SAB. Of the 14 patients using donor eggs, 28% (n=4) had live births and 7% (n=1) had SAB. The most common menopausal symptoms were: 76% (n=404) hot flashes, 58% (n=307) vaginal dryness, 48% (n=253) insomnia, 15% (n=79) mood changes, 14% (n=72) night sweats, 7% (n=39) cognitive effects. Karyotype was performed in 515 patients: 91% (n=469) had a normal karyotype, 5.0% (n=26) had Turner mosaicism, 1.4% (n=7) had a single chromosome deletion/inversion, and 2.5% (n=13) had other abnormalities. Fragile X testing was performed in 188 patients, of which 93.6% (n=176) had normal (5-44) CGG repeats in the FMR1 gene, 2.1% (n=4) had intermediate (45-54) repeats, and 4.3% (n=8) had a premutation (55-200 repeats), a known cause of POI. No patients had a full mutation.

**Conclusion:** This descriptive analysis of 530 women with POI showed the variability of clinical presentation and outcomes. Pregnancy rates were 2.6% following POI diagnosis. Compared to postmenopausal women in the general population, the incidence of hot flashes in POI appears to be similar (79 vs 76%) and vaginal dryness is higher (20 vs 58%). A Fragile X premutation as a cause was consistent with the literature at 4.3%.

### T-060

**Advancing Equity and Diversity in Gynecologic Biobanking.** Pablo Suarez, Shannon Whirledge\*. *Yale University, New Haven, CT, United States.*

**Introduction:** Race is the strongest risk factor for the development of uterine fibroids, the most common gynecologic tumor affecting women. Racial inequities are also reflected in disease severity and medical treatment, as Black women develop more severe symptoms and undergo hysterectomies at higher rates than white women. Yet, the factors driving racial disparities in uterine fibroids are unclear. Few studies have been designed to identify racially-linked factors, and *in-vitro* models infrequently report the race/ethnicity of the studied fibroid cell lines. The lack of representation in research impedes progress towards the development of effective therapies that will benefit all women with uterine fibroids. Achieving representation in fibroid research relies on the equitable inclusion of all races in clinical studies and biospecimen collection. The objective of this study was to determine demographic data for university-affiliated OB/GYN biobanks in the U.S. and to develop a targeted strategy to address barriers in participation of Black women in biospecimen collection.

**Methods:** We performed a literature search in PubMed with the search terms: "biobank and gynecology," "biobank and gynecology," "gynecologic biorepository," "biobank and uterine fibroids," "biobank and leiomyomas," "biobank and race," "participation barriers and biobank," and "biobank and underrepresented groups." We also independently searched for university-affiliated OB/GYN biobanks. We then conducted a qualitative review of reported barriers and strategies to participation in biospecimen research among racial/ethnic minorities.

**Results:** Our literature search identified only 6 relevant publications describing OB/GYN biobanking. We independently identified 19

university-affiliated OB/GYN biobanks, with only 1 reporting the racial/ethnic composition of their participating subjects and 4 including a statement addressing diversity. The most common barriers to participation among racial/ethnic minorities identified are: misinformation and misconceptions about biobanking, mistrust, and confidentiality concerns. To account for these barriers, tools assessing biobanking literacy, medical trust, and perceptions of justice in research have been validated. Key strategies identified to improve participation of underrepresented groups in biobanking are community-based partnerships, educational structured interviews, multi-layer privacy protections, and accessible research records.

**Conclusion:** We identified a significant deficit in publicly available data on the racial/ethnic demographics of banked OB/GYN biospecimens, a barrier to translational research in uterine fibroids. Considering historic underrepresentation in biospecimen donation and participation barriers, we designed a recruitment model implementing tools and strategies that foster equity in biobanking, ultimately supporting the development of targeted therapeutics for uterine fibroids.

### T-061

**Ultrasonographic Anterior Uterocervical Angle (UCA) and Prediction of Preterm Birth: A Patient Level Meta-Analysis.** Erica K Nicasio†,<sup>1</sup> Zi-Qi Liew†,<sup>1</sup> Jesse Llop,<sup>1</sup> Tiffany Mei†,<sup>1</sup> Lynch Tara,<sup>2</sup> Neil S Seligman\*.<sup>1</sup>  
<sup>1</sup>University of Rochester, Rochester, NY, United States; <sup>2</sup>Albany Medical Center, Albany, NY, United States.

**Introduction:** Currently, cervical length (CL) is the gold standard for prediction of spontaneous preterm birth (sPTB). The anterior UCA, a measurement of the angle between the cervix and the anterior lower uterine segment, has been investigated as an alternative predictor for sPTB, but, results have been inconsistent. A patient-level meta-analysis was performed to evaluate the ability of anterior UCA measurement to predict sPTB.

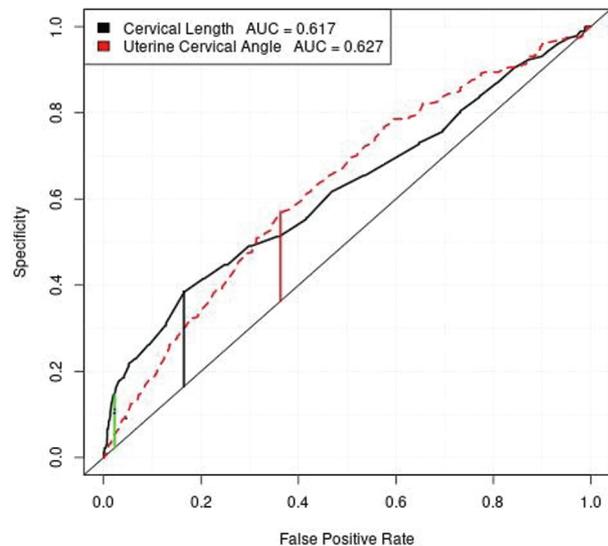
**Methods:** Literature review of search terms “uterocervical angle” and “preterm birth” initially identified 494 studies. A total of 3 studies with available patient level data met inclusion criteria. These were combined with data from a retrospective cohort from 2013-2020 at our hospital. Patients were included if they had a transvaginal ultrasound measurement of CL and UCA between 16+0 to 23+6 weeks. The primary outcome was sPTB < 37 weeks. Secondary outcome was sPTB < 34 weeks. The overall and individual study mean UCA was calculated. A receiver-operator curve (ROC) was created and the UCA and CL most predictive of sPTB were determined by maximizing the Youden’s index.

**Results:** A total of 2,016 women were included in the analysis. The overall mean UCA was 99.5 degrees. Individual study-specific mean UCA ranged from 94.9°-106.1° ( $p < .001$ ). The mean UCA was wider in patients with sPTB < 37 weeks (109.6°) than term (97.9°;  $p < .001$ ) and sPTB < 34 weeks (109.8°) than term (98.6°;  $p < .001$ ). Based on the ROC, a cut point for UCA of 107° was most predictive of sPTB with sensitivity of 57% and specificity of 64%. In this population, the predictive value of UCA was similar to that of CL, which had an area under the curve AUC = 0.62 compared to 0.63 for the anterior UCA.

**Conclusion:** Larger UCA measurement was associated with an increased risk of sPTB < 37 and < 34 weeks. An anterior UCA of 107° had an increased sensitivity for sPTB < 37 weeks. In this population, UCA can be as valuable as CL for prediction of sPTB. Further investigation may identify situations in which UCA and CL together are better predictors than CL alone.



ROC predicting preterm 37 wks



### T-062

**Multi-Omic, Longitudinal Profile of Third-Trimester Pregnancies Identifies a Molecular Switch That Predicts the Onset of Labor.** Ina Stelzer,<sup>1</sup> Mohammad Sajjad Ghaemi,<sup>1,2</sup> Xiaoyuan Han,<sup>1,3</sup> Kazuo Ando,<sup>1</sup> Julien Hedou,<sup>1</sup> Dorien Feyaerts,<sup>1</sup> Laura Peterson,<sup>1</sup> Edward Ganio,<sup>1</sup> Amy Tsai,<sup>1</sup> Eileen Tsai,<sup>1</sup> Kristen Rumer,<sup>1</sup> Natalie Stanley,<sup>1</sup> Ramin Fallazadeh,<sup>1</sup> Martin Becker,<sup>1</sup> Anthony Culos,<sup>1</sup> Dyani Gaudilliere,<sup>1</sup> Ronald Wong,<sup>1</sup> Virginia Winn,<sup>1</sup> Gary Shaw,<sup>1</sup> Michael Snyder,<sup>1</sup> David Stevenson,<sup>1</sup> Kevin Contrepois,<sup>1</sup> Martin Angst\*,<sup>1</sup> Nima Aghaeepour\*,<sup>1</sup> Brice Gaudilliere\*.<sup>1</sup>  
<sup>1</sup>Stanford University, Stanford, CA, United States; <sup>2</sup>National Research Council Canada, Toronto, ON, Canada; <sup>3</sup>University of the Pacific, San Francisco, CA, United States.

**Introduction:** Current conception-based approaches to estimate the due date do not allow to integrate real-time pregnancy progression and thus, remain inaccurate. However, estimating the time of delivery is of high clinical importance as pre- and post-term deviations are associated with complications for the mother and her baby. With approaching labor, major transitions occur in fetomaternal systems that culminate in the delivery of the fetus. We aimed to comprehensively characterize the metabolic, proteomic and immune cell events that precede the spontaneous onset of labor, in order to understand these physiological transitions and identify predictive biomarkers of parturition.

**Methods:** In blood collected during the last 100 days of pregnancy from 63 women, over 7,000 circulating plasma analytes and peripheral immune cell responses were analyzed by mass cytometry, high-throughput mass spectrometry, and an aptamer-based protein assay.

**Results:** An integrated, multi-omic model accurately predicted the time to spontaneous onset of labor ( $R = 0.85$ ,  $p\text{-value} = 1.2\text{e-}40$ , training set;  $R = 0.81$ ,  $p\text{-value} = 3.9\text{e-}7$ , validation set). The most informative features of the multi-variate model included STAT1 signaling responses in CD56<sup>+</sup>CD16<sup>+</sup>NK cells, IL-33-receptor IL1R4, Cystatin C, Angiopoietin-2, Cortisol, and Progesterone. Strikingly, analyte trajectories revealed precisely-timed fluctuations that marked a transition from pregnancy progression to pre-labor biology which occurred 2-4 weeks before delivery.

**Conclusion:** The multi-omic assessment of maternal blood across different biological systems revealed a multivariate model of cellular and molecular events that accurately predicted the time to delivery. Our modeling approach is key to developing blood-based methods for accurately predicting the due date based on common pathways in term, pre- and post-term pregnancies.

### T-063

**Increased Rates of Cesarean and Operative Vaginal Delivery with Extended Second Stage Pushing.** Derek Lee†, Lisa Duong†, Michael G Ross\*. Harbor-UCLA Medical Center, Torrance, CA, United States.

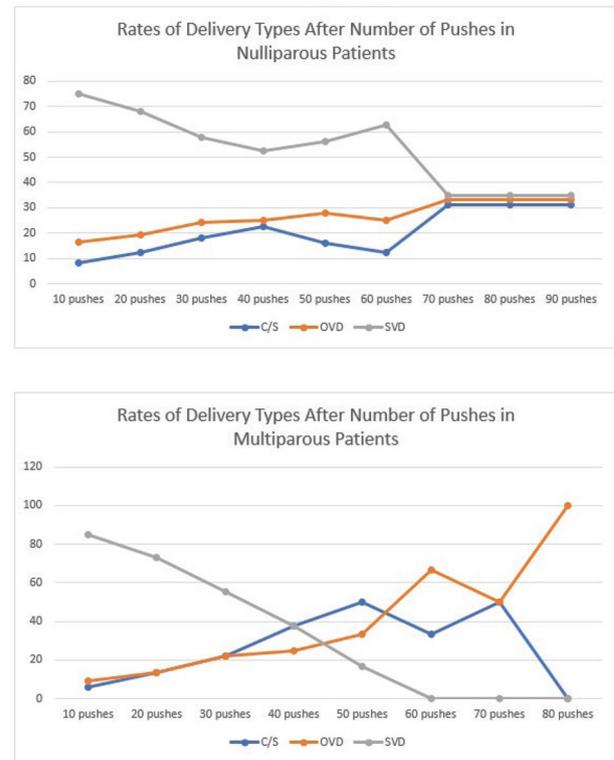
**Introduction:** Debate continues regarding the length of the second stage of labor. We previously demonstrated that second stage can be quantified by number of pushing contractions as an adjunct to time. In this study, we investigated the probabilities of operative delivery and maternal/neonatal morbidities in relation to number of second stage pushing contractions.

**Methods:** This was a retrospective analysis of 865 patients who entered the second stage of labor with singleton, term fetuses at Harbor-UCLA Medical Center from January 1, 2017 to December 31, 2019. Probabilities of spontaneous (SVD), operative vaginal delivery (OVD) and Cesarean were calculated for each decedentile of number of maternal pushing contractions. Maternal (postpartum hemorrhage, chorioamnionitis, endometritis, third or fourth degree lacerations) and neonatal morbidities (shoulder dystocia, UA pH <7.10, 5 minute Apgar <6, neonatal sepsis, neonatal resuscitation, NICU admission) were assessed in relation to second stage (pushes).

**Results:** 439 nulliparas (age 25.1±0.5) and 426 multiparas (age 29.9±0.6) were studied. Nulliparas required significantly more pushing contractions to deliver than multiparas (20.3±1.8 vs 7.8±1.0 pushes,  $p<0.01$ ). Cesarean deliveries were associated with significantly more pushing contractions than SVDs among both nullipara and multipara cohorts (40.1±10.1 vs 17.9±1.9, 32.1±23.4 vs 6.9±0.8, respectively;  $p<0.01$ ). After the 70th percentile of pushing contractions, the probabilities of Cesarean deliveries are at their highest (nulliparas 33%, multiparas 50%) and SVD rates are at their lowest (nulliparas 33%, multiparas 0%) (Figure 1). After the 80th percentile of pushing contractions, the probabilities of OVDs are at their highest (nulliparas 33%, multiparas 100%). There was no significant difference in maternal or neonatal composite morbidities as number of pushing contractions increased ( $p>0.32$ ).

**Conclusion:** These results establish a normative pattern of number of pushing contractions for nulliparous and multiparous patients. Providing patients a goal for expected number of pushing contractions may be of motivational benefit. We recommend that patients be counseled of an increased probability of Cesarean and OVD if SVD does not occur within the 70th to 80th percentile of pushing contractions.

Figure 1 Rates of Delivery Types After Each Decedentile of Pushes



### T-064

**Investigating Differential Effects of Interpregnancy Interval on Pregnancy Complications by Country Developmental Status.** Caitlyn E Flint†, Jasmine M De Giovanni, Jason Phung, Craig E Pennell\*,<sup>1,2,3</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>3</sup>John Hunter Hospital, New South Wales, Australia.

**Introduction:** Preterm birth (PTB) is the leading cause of death in children under the age of five. In 2014, there were approximately 15 million preterm births and of these around 12 million occur in Africa and Asia. A short interpregnancy interval (IPI) is well recognised as a risk factor for PTB, low birth weight (LBW) and small for gestational age babies (SGA). The aim of this study was to examine the association of these adverse birth outcomes with the country of birth economic status and IPI.

**Methods:** A systematic review and meta-analyses were performed. EMBASE and Medline were searched to identify studies assessing IPI and its impact on PTB, LBW and SGA. Studies were included if they had an observational design. Systematic reviews with no new data were excluded. All studies were screened, data extracted, and bias assessed by two independent authors. Discrepancies were resolved by consensus. Studies were stratified by developmental status of the study country as described by the World Economic Situation and Prospects 2019 report. The co-primary outcomes were PTB, LBW and SGA. The Joanna Briggs critical appraisal tool was used to assess methodological quality. Overall summary estimates and 95% CIs with inverse variance weighted random-effects meta-analyses were performed because heterogeneity was high.

**Results:** We screened 1667 abstracts; 75 were eligible for full text review; data were extracted from 43 studies with 8585907 participants. The risk of PTB was increased in women with IPI <12-months when compared to women with IPI > 12-months: developed countries (OR 1.43 95%CI 1.26-1.62), developing countries (OR 1.19, 95%CI 1.08-1.30). For IPI <18-months (compared to IPI >18-months), similar odds of PTB were seen in developed (OR 1.16, 95%CI 1.01-1.32) and developing countries (OR 1.12, 95%CI 1.07-1.18). IPI > 24 months were not associated with PTB risk. The risk of LBW was increased with IPI <12-months

compared to IPI >12-months in developed countries (OR 1.22, 95%CI 1.05-1.42); whereas in developing countries, the risk was increased with IPI <12-months (OR 1.19, 95%CI 1.09-1.31), <18-months (OR 1.23, 95%CI 1.08-1.40) and <24-months (OR 1.09, 95%CI 1.04-1.13) when compared to IPI > 12-months, > 18-months, and >24-months respectively. There was no association between IPI and SGA in developed countries. In contrast, associations were demonstrated in developing countries for IPI <12-months (OR 1.21, 95%CI 1.11-1.31) and <18-months (OR 1.21, 95%CI 1.01-1.46) compared to IPI > 12-months and > 18-months respectively.

**Conclusion:** The risks of PTB, LBW and SGA are influenced by country developmental status. For PTB the effect size is greater in developed than developing countries whereas for LBW and SGA the impact is greatest in developing countries.

**T-065**

**Predictors of Breastfeeding among Women Admitted with Preterm Pre-Labor Rupture of Membranes.** Carmen Maria Avram†, Alice Darling†, Melissa Montoya†, Jennifer Gilner\*, Sarah Wheeler\*, Sarah Dotters-Katz\*. *Duke University, Durham, NC, United States.*

**Introduction:** Initiation of breastmilk feeding (BF) among preterm and low birth weight infants occurs at lower rates compared to term infants, despite clear benefits to breastmilk in this population. Women admitted with preterm pre-labor rupture of membranes (PPROM) are at high risk for preterm delivery and may have limited access to prenatal BF education. We aimed to describe rates of BF initiation and cessation, and to identify risk factors for BF non-initiation among women with PPRM.

**Methods:** Nested case-control study of women with PPRM admitted to a single tertiary center (2013-2019). Women without BF data and deliveries complicated by intrauterine or neonatal demise within 48 hours were excluded in primary analysis. Neonatal demise and those missing postpartum (PP) data excluded from secondary analysis. Demographic, antepartum, and delivery characteristics were evaluated. Primary analysis included rate of any BF initiation (BFI) at maternal discharge, and factors associated with (a/w) non-initiation. Secondary analysis performed for rates of BF continuation (BFC) at 6-weeks PP and factors a/w BFC. Bivariate statistics were used compare characteristics and logistic regression to estimate adjusted odds ratios (aOR).

**Results:** Of 397 women with PPRM, 342 (86.1%) had BFI. Women who didn't initiate BF were more likely to use tobacco in pregnancy (Table 1). In contrast, women with private insurance (aOR 2.53; 95%CI 1.19,5.37) and PPRM latency ≥14 days (aOR 3.02; 95%CI 1.09,8.38) had increased odds of BFI (Table 1). At 6 weeks PP, only 214 (73%) of 297 women had BFC. Age <20 (aOR 0.07; 95%CI 0.01,0.67) and multiparity (aOR 0.54; 95%CI 0.29,0.99) were associated with non-BFC. Women with private insurance were observed to have increased odds of BFC (Table 2).

**Conclusion:** Among women with PPRM, tobacco use was associated with non-initiation of BF prior to discharge, while age <20 and multiparity were associated with BF cessation. BF education and support should be offered to all women admitted for PPRM. Further research is needed how to best support these women in BF.

Table 1: Maternal demographics, antepartum, and delivery characteristics at discharge.

	Breastmilk feeding initiated at discharge			P-value	aOR (95% CI)
	Overall N=397(%)	No N=55(%)	Yes N=342(%)		
Average maternal age, years (SD)	29.8 (6.3)	28.7 (7.3)	30.0 (6.1)	0.16	
Maternal age <20	20 (5.0)	6 (10.9)	14 (4.1)	0.03	0.46 (0.15-1.45)
Limited prenatal care	29 (7.3)	11 (20.0)	18 (5.3)	<0.001	0.42 (0.16-1.07)
Race				0.005	
White	149 (37.5)	17 (30.9)	132 (38.6)		
Black	170 (42.8)	34 (61.8)	136 (39.8)		0.79 (0.38-1.64)
Asian	27 (6.8)	1 (1.8)	26 (7.6)		2.25 (0.27-18.97)
Other	14 (3.5)	3 (5.5)	11 (3.2)		0.60 (0.14-2.65)
Hispanic/Latinx	37 (9.3)	0 (0.0)	37 (10.8)		
Private insurance	212 (53.4)	14 (25.5)	198 (57.9)	<0.001	2.53 (1.19-5.37)
Multiparous	212 (53.4)	33 (60.0)	179 (52.3)	0.29	
History of depression	68 (17.1)	14 (25.5)	54 (15.8)	0.08	
Tobacco use in pregnancy	68 (17.1)	24 (43.6)	44 (12.9)	<0.001	0.32 (0.16-0.64)
Multiple gestation	49 (12.3)	2 (3.6)	47 (13.7)	0.03	2.25 (0.48-10.68)
Average admission gestational age, weeks (SD)	29.2 (3.7)	29.6 (4.1)	29.1 (3.7)	0.36	
ROM <28 weeks	134 (33.8)	14 (25.5)	120 (35.1)	0.16	
Average PPRM latency, weeks (SD)	1.4 (1.8)	1.0 (1.2)	1.4 (1.9)	0.08	
Latency ≥7 days	175 (44.1)	19 (34.5)	156 (45.6)	0.12	
Latency ≥14 days	88 (22.2)	5 (9.1)	83 (24.3)	0.01	3.02 (1.09-8.38)
Average delivery gestational age, weeks (SD)	30.6 (3.3)	30.6 (3.6)	30.6 (3.2)	0.92	
Mode of delivery				0.08	
SVD	215 (54.2)	37 (67.3)	178 (52.0)		
OVD	8 (2.0)	0 (0.0)	8 (2.3)		
CS	174 (43.8)	18 (32.7)	156 (45.6)		
Chorioamnionitis	72 (18.5)	13 (23.6)	59 (17.6)	0.29	
Maternal LOS PP, days (SD)	2.3 (0.8)	2.2 (0.9)	2.4 (0.8)	0.11	

aOR, adjusted odds ratio. SD, standard deviation. ROM, rupture of membranes. PPRM, premature pre-labor rupture of membranes. SVD, spontaneous vaginal delivery. OVD, operative vaginal delivery. CS, cesarean section. LOS, length of stay. PP, postpartum

Table 2: Maternal demographics, antepartum, and delivery characteristics at 6 weeks postpartum.

	Breastmilk feeding continued postpartum			P-value	aOR (95% CI)
	Overall N=293(%)	No N=79(%)	Yes N=214(%)		
Average maternal age, years (SD)	30.3 (5.9)	28.4 (6.5)	31.0 (5.5)	<0.001	
Advanced maternal age	80 (27.3)	15 (19.0)	65 (30.4)	0.05	1.59 (0.79-3.21)
Maternal age <20	7 (2.4)	6 (7.6)	1 (0.5)	<0.001	0.07 (0.01-0.68)
Limited prenatal care	16 (5.5)	9 (11.4)	7 (3.3)	0.007	0.61 (0.18-2.08)
Race				0.006	
White	112 (38.2)	22 (27.8)	90 (42.1)		
Black	115 (39.2)	45 (57.0)	70 (32.7)		0.57 (0.28-1.15)
Asian	24 (8.2)	4 (5.1)	20 (9.3)		1.06 (0.31-3.62)
Other	10 (3.4)	2 (2.5)	8 (3.7)		2.56 (0.37-17.75)
Hispanic/Latinx	32 (10.9)	6 (7.6)	26 (12.1)		2.08 (0.66-6.55)
Private insurance	173 (59.0)	30 (38.0)	143 (66.8)	<0.001	2.10 (1.07-4.12)
Multiparous	153 (52.2)	52 (65.8)	101 (47.2)	0.005	0.54 (2.9-0.99)
Depression	44 (15.0)	15 (19.0)	29 (13.6)	0.25	
Tobacco use in pregnancy	34 (11.6)	16 (20.3)	18 (8.4)	0.005	0.56 (0.25-1.26)
Multiple gestation	42 (14.3)	6 (7.6)	36 (16.8)	0.05	1.67 (0.63-4.41)
Average admission gestational age, weeks (SD)	29.1 (3.7)	28.9 (3.5)	29.2 (3.7)	0.67	
Average PPRM latency, weeks (SD)	1.5 (1.9)	1.4 (1.8)	1.5 (2.0)	0.92	
Average delivery gestational age, weeks (SD)	30.5 (3.2)	30.4 (3.2)	30.6 (3.1)	0.58	
Mode of delivery				0.16	
SVD	144 (49.1)	37 (46.8)	107 (50.0)		
OVD	8 (2.7)	0 (0.0)	8 (3.7)		
CS	141 (48.1)	42 (53.2)	99 (46.3)		
Chorioamnionitis	51 (17.7)	20 (25.3)	31 (14.8)	0.04	0.58 (0.29-1.20)
Neonatal morbidity	156 (53.2)	42 (53.2)	114 (53.3)	0.99	
Postpartum depression	22 (7.5)	6 (7.6)	16 (7.5)	0.97	

aOR, adjusted odds ratio. SD, standard deviation. PPRM, premature pre-labor rupture of membranes. SVD, spontaneous vaginal delivery. OVD, operative vaginal delivery. CS, cesarean section.

**T-066**

**Antibiotic Use during Pregnancy and Preterm Birth.** Brittani Steinberg†, Yanzhi Wang\*, Laura Meints\*. <sup>1</sup>University of Illinois College of Medicine, Peoria, IL, United States; <sup>2</sup>St. Francis Medical Center, OSF HealthCare System, Peoria, IL, United States.

**Introduction:** Antibiotics are one of the most commonly prescribed medication classes during pregnancy. Little is known regarding their impact on pregnancy. Our primary aim is to determine whether antibiotic use during pregnancy is associated with preterm birth (PTB) less than 37

weeks estimated gestational age (EGA). Secondary aims will assess the relationship of antibiotic use with cytokine levels, maternal outcomes, and neonatal outcomes.

**Methods:** This is a secondary analysis of a multicenter, prospective cohort study to evaluate the impact of vaginal infections during pregnancy on PTB. Exposure was any antibiotic use during pregnancy. Primary outcome was PTB < 37 weeks EGA. Secondary outcomes were midtrimester cervical length (CL), vaginal bleeding (VB), inflammatory cytokine levels, and maternal and neonatal outcomes associated with infection. Descriptive statistics were generated. Univariate and multivariable logistic regression were performed to examine potential associations between antibiotic use and the primary outcome. Results were considered statistically significant if  $p < .05$ .

**Results:** There were 3073 women included in the analysis: 1233 with antibiotic use during pregnancy, 1840 without. Women with antibiotic use were younger, had lower BMI, were more likely to have smoked during pregnancy, and were more likely to have less than high-school level education. When controlling for confounders, antibiotic use during pregnancy was not associated with PTB less than 37 weeks EGA (OR = 1.13; 95% CI 0.91-1.40;  $p = 0.28$ ). Women with antibiotic use had greater CL and were more likely to experience first or second trimester VB. Neonates from mothers with antibiotic use during pregnancy had lower birth weights and were more likely to experience necrotizing enterocolitis. Serum IL-10 levels at 24 weeks EGA and TNF receptor type 2 levels at 28 weeks EGA were higher for the antibiotic use group, while C-reactive protein levels at 24 weeks EGA were lower for the antibiotic use group.

**Conclusion:** In this study, antibiotic use during pregnancy was not associated with PTB less than 37 weeks EGA.

#### T-067

**ECM-Associated Long Non-Coding RNAs Inc-ADAM9 and Inc-PCDH10 Regulate Cell Adhesion Pathway in Premature Rupture of Fetal Membrane.** Guixian Wang,<sup>1</sup> Dongxia Hou,<sup>1</sup> Xiaoyan Dong,<sup>2</sup> Nanbert Zhong\*,<sup>2</sup> <sup>1</sup>Inner Mongolia Maternal and Children's Hospital, Hohhot, China; <sup>2</sup>New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States.

**Introduction:** Preterm syndrome (PTS) behaves a pre-labor premature rupture of membrane (pPROM) or a spontaneous premature labor (sPTL) with uterine contraction that may result in a rupture of fetal membrane as the course of parturition initiated. We have determined that intracellular long noncoding RNAs (lncRNAs) were involved in a ubiquitin-proteinase-collagen pathway that may result in down-regulation of collagens in pPROM. We hypothesize that lncRNA may involve in regulation of extracellular matrix (ECM) pathway in PTS.

**Methods:** Two lncRNAs, the Inc-ADAM9 and Inc-PCDH10, and their epigenetic regulatory impact on ECM in the PTS, were studied. Gene expression profiles of these lncRNAs and their overlapped mRNA loci were assessed with a cohort of 120 prebanked fetal membrane tissues, among which 30 cases were in each pPROM, FTB (full-term birth), sPTL, and PROM (full-term premature rupture of membrane), respectively. Over-expression of IncADAM9 and IncPCDH10 followed by RNA-seq, qRT-PCR, and Western blot in cultured HTR8 cell models.

**Results:** Differential expression genes that were enriched in both cell cultures of overexpressing IncADAM9 and IncPCDH10 were enriched in extracellular matrix structural constituent, biological adhesion, extracellular matrix organization, homotypic cell-cell adhesion, and cell-cell adhesion *via* plasma-membrane adhesion molecules (Figure 1). A pathway, the cell adhesion molecules (CAM), was enriched in both over-expression cell cultures. RNAs and proteins in CAM pathway, *in vivo* analyzed with fetal membranes, showing that they presented significantly in distinguishing PTS from controls (Table 1).

**Conclusion:** Our results indicated that overexpressed Inc-ADAM9 and Inc-PCDH10 has a pathogenic impact on extracellular matrix and cell adhesion molecules, which lead a potential of applying these molecules as clinical biomarker for early prediction of PTS.

#### T-068

**Early Childhood Growth after Term NICU Admission by Admission**

**Diagnosis.** Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulski,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>1,5</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pongasachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*.<sup>1,2,5</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute for Basic Research, Staten Island, NY, United States.

**Introduction:** Neonatal intensive care unit (NICU) admission marks potentially at-risk children for developmental programming. We explore patterns of early childhood growth after NICU admission in a low-risk community-based population with universal placental examination.

**Methods:** A community hospital based sample with universal placental examination was searched for those births delivered at a gestation of at least 37 completed weeks and followed to at least age 2 years at our institution. Gross placental examination was performed according to a protocol that recorded trimmed placental weight (PW), major and minor disk axes, minimum and maximum disk thickness and cord eccentricity. NICU admission and admission diagnoses were extracted from the medical record. Infant sex and centiles for weight and length/height at birth, age 1, and age 2 were extracted from medical records. Gross measures and centiles of growth were both analyzed with nonparametric tests due to non-normal distributions.

**Results:** 1054 infants met inclusion criteria, 101 of whom were admitted to the NICU. There were no associations of birth weight, length or head circumference centiles, or centiles of growth at ages 1 or 2 years in this term population. This lack of association held within the NICU population when indications for NICU admission were considered, including neonatal hypoglycemia, abnormal fetal heart rate, hyperbilirubinemia, or newborn complications related to meconium exposure.

**Conclusion:** The data suggest that NICU admission of a term newborn does not correlate with longterm abnormalities of early childhood growth. Term infants admitted to our NICU show no evidence of significant reduction in birth weight or length centiles, which may reflect the nature of our community based hospital sample.

#### T-069

**Investigating the Neuro-Regenerative Potential of MicroRNAs in Wharton's Jelly-Derived Small Extracellular Vesicles (sEV) for Perinatal White Matter Injury Outcomes.** Vera Tscherrig†,<sup>1,2,3</sup> Sophie Cottagnoud†,<sup>1,2</sup> Valérie Haesler,<sup>1,2</sup> Patricia Renz†,<sup>1,2,3</sup> Daniel Surbek,<sup>1,2</sup> Andreina Schoeberlein,<sup>1,2</sup> Marianne Jörger-Messerli.<sup>1,2</sup> <sup>1</sup>Department of Obstetrics and Feto-maternal Medicine, University Women's Hospital, Inselspital, Bern University Hospital, Bern, Switzerland; <sup>2</sup>Prenatal Medicine, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland; <sup>3</sup>Graduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern, Switzerland.

**Introduction:** Perinatal white matter injury (WMI) is one of the most common neurological complications of preterm birth and it is a global health problem resulting in long-term neurodevelopmental and neurobehavioral disabilities. Until now, there is no cure for perinatal WMI. Our research group and others have recently shown promising results towards the use of mesenchymal stromal cell-derived small extracellular vesicles (MSC-sEV) as a therapeutic approach for cerebral injuries. It is known that sEV secreted by MSC carry small non-coding RNAs such as microRNAs (miRNAs). MicroRNAs might interfere with signaling pathways involved in premature WMI. Thus, we hypothesize that miRNAs, released by sEV upon uptake in their target cells, have a key function in the observed beneficial effects of MSC-sEV.

**Methods:** MSC were isolated from the connective tissue of human umbilical cords, the so-called Wharton's jelly. sEV were purified from the cells using ultracentrifugation, followed by size-exclusion chromatography (SEC). The fractions were characterized according to the expression of sEV markers using western blot analysis and miRNAs by quantitative PCR.

**Results:** The SEC fractions with the highest protein content also showed positive signals for the sEV markers CD81 and CD63. No cellular contamination has been observed (no signals for GM130 or Grp94). These sEV fractions contained high amounts of miRNAs, such as miRNA 22-5p, miRNA 27b-3p or let7b-5p.

**Conclusion:** The highly abundant miRNAs in the sEV fractions target specific apoptotic or inflammatory pathways and drive oligodendrocyte differentiation. Therefore, these miRNAs might have positive effects on WMI outcomes. For a better understanding of this hypothesis, the sEV fractions containing the most abundant miRNAs are currently tested for their functionality using dual luciferase assays. For this, the 3' UTR of the target mRNA is cloned into a luciferase reporter vector and transfected into HEK293-cells followed by incubation with sEV fractions containing highly expressed miRNAs. The inhibitory or excitatory effects of miRNAs on the gene expression in the transfected HEK293 cells are monitored by the amount of luciferase activity in the different experiments and deliver further knowledge about the functionality of WJ-MSC-derived sEV.

#### T-070

**Diabetic Environment Affects the Proteomic Profile of Different Populations of Extracellular Vesicles Originating from Placental Cells.** Carlos Palma<sup>†</sup>,<sup>1</sup> Andrew Lai,<sup>1</sup> David McIntyre,<sup>1</sup> Carlos Salomon.<sup>1,2</sup> <sup>1</sup>The University of Queensland, Brisbane, Australia; <sup>2</sup>University of Concepción, Concepcion, Chile.

**Introduction:** Annually, about 15% of pregnant women in Australia suffer from Gestational Diabetes Mellitus (GDM). GDM can lead to several pregnancy complications, and also postpartum effects, such as the development of diabetes. Although there is an increasing body of research on GDM, there are several gaps in understanding. Nonetheless, recent studies have shown a key link between placental etiology and intercellular communication. Extracellular vesicles (EVs) are fundamental mediators of cell-cell communication and have a significant role in both physiology and pathology. Studies have shown that the release of EVs, and their protein profile, can be altered depending on the cellular milieu of the parent cell. Therefore, it is imperative to understand how glycemic changes in the placental microenvironment can affect the expression of proteins in the cell, and extracellular vesicles generated by them. Thus, this study evaluated the proteomic profile of placental cells and different populations of extracellular vesicles released by them, in a placental model under a mimetic condition of hyperglycemia.

**Methods:** BeWo cells were used as a placental model. They were exposed to high glucose concentrations to mimic diabetic microenvironment. Control cells were exposed to 5 mM glucose, whereas 25 mM glucose was used to simulate high glucose. After 48 hours of treatment, the media and lysate were collected. Three different populations of EVs were isolated from the supernatant, based on centrifugation speed (2000 x g, 10000 x g and 100000 x g). Mass spectrometry analysis (IDA/SWATH) was performed in each population of EVs, as well as the lysate, to identify and quantify the proteins.

**Results:** BeWo cells exhibited differential expression of proteins in response to high glucose treatment. 177 proteins significantly changed ( $p < 0.05$ ) as a result of high glucose treatment in the lysate. This change was also observed in 281 proteins identified in the 2000 x g EVs preparation, 238 proteins in the 10000 x g EVs preparation, and 179 proteins in the 100000 x g EVs preparation. Interestingly, there was no evidence of proteins with significant changes in expression that were shared among the four groups. However, a set of uniquely and significantly expressed proteins was identified in each group. Lysate expressed 107 unique proteins, with 195 proteins identified only in the 2000 x g population, 160 proteins in the 10000 x g, and 119 proteins in the 100000 x g.

**Conclusion:** This IDA/SWATH method evidenced the role of placental cells as sensors of the extracellular microenvironment, and revealed that the placental microenvironment can significantly alter the proteomic profile under pathological conditions. The results also provide encouraging data from a biomarker perspective, due to the unique expression of proteins in specific populations of EVs.

#### T-071

**Immediate Pre-Pregnancy Weight Loss Improves Highly Atherogenic Dyslipidemia Throughout Pregnancy.** Robert A. Wild,<sup>1</sup> Rodney K Edwards,<sup>1</sup> David S Wrenn,<sup>2</sup> Daniel Y Zhao,<sup>1</sup> Karl R Hansen.<sup>1</sup> <sup>1</sup>University of Oklahoma HSC, Oklahoma City, OK, United States; <sup>2</sup>Quest Diagnostics, Seacaucus, NJ, United States.

**Introduction:** FIT-PLESE was a Reproductive Medicine Network RCT that assessed whether 16 weeks of pre-pregnancy [orlistat + frozen meals + exercise (D+E) vs. exercise alone (E)] lifestyle interventions, immediately before ovarian stimulation intrauterine insemination (OS-IUI) for conception, could improve live birth rates for obese women with unexplained infertility. Our **OBJECTIVE** was to determine the effects of this 16-week lifestyle intervention on levels of atherogenic lipids from baseline to after lifestyle intervention and during each trimester of PREGNANCY for those with a live-birth (n=76)

**Methods:** Frozen samples were blindly analyzed by Ion Mobility Quest Diagnostics. Linear mixed models with repeat measures compared mean values across the time points.

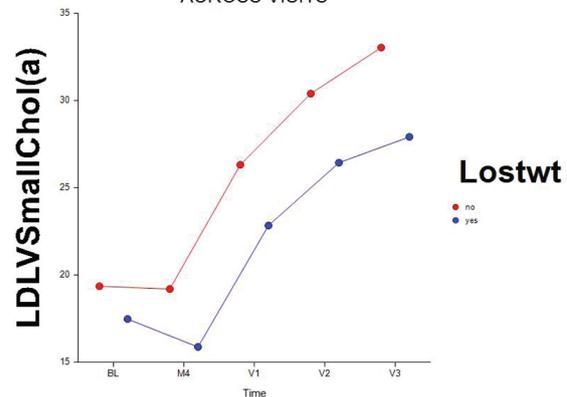
**Results:** Pre-pregnancy weight loss reduced cholesterol, triglycerides, and remnant cholesterol across the visits (Baseline to after D+E or E and at 16,24, and 36 weeks gestation) (all  $p < 0.05$ )-data not shown. For persons who lost any weight 20/39 (51.3%) in the E arm and 31/35 (88.6%) in the D+E arm, LDL cholesterol (not shown) and the highly atherogenic very small ldl cholesterol particles (a,c) shown below were significantly reduced across the visits ( $p < 0.001$ ).

**Conclusion:** Immediate pre-pregnancy lifestyle interventions before OS-IUI for obese women with unexplained infertility can result in weight loss. For those who lost some weight, improvements in atherogenic dyslipidemia continued throughout pregnancy. Highly atherogenic dyslipidemia is a risk factor for short-term (complications during) and long-term (cardiovascular complications later) maternal morbidity/mortality. Improvement in highly atherogenic dyslipidemia may have beneficial implications for epigenetic effects for her offspring.

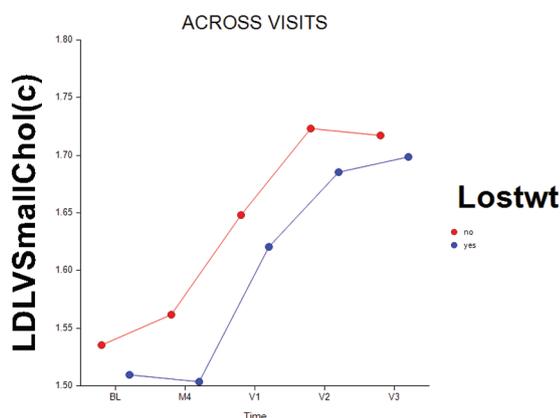
Funded in part NICHD grants U10HD077680 Reproductive Medicine Network, 1 R03 HD101893-01, Quest Diagnostics

#### FIT-PLESE

ACROSS VISITS



## FIT-PLESE



## T-072

**Investigating a Role for the NLRP3 Inflammasome in the Pathophysiology of Gestational Diabetes Mellitus.** Colm J McElwain<sup>†</sup>,<sup>1</sup> Samprikta Manna,<sup>1</sup> Fergus P McCarthy\*,<sup>2</sup> Cathal M McCarthy\*.<sup>1</sup> <sup>1</sup>University College Cork, Cork, Ireland; <sup>2</sup>Cork University Maternity Hospital, Cork, Ireland.

**Introduction:** Aberrant NLRP3 inflammasome activation has been postulated to contribute to the inflammatory phenotype of gestational diabetes mellitus (GDM). This deleterious pathway initiates in maternal placental and adipose tissue, leading to IL-1 $\beta$  and IL-18 secretion and systemic inflammation. Our aim is to investigate NLRP3 inflammasome activation in maternal placental and adipose tissue and examine how direct inflammasome inhibition and mitochondrial antioxidant therapy may ameliorate NLRP3-related inflammation.

**Methods:** Placental and visceral omental tissue explants were collected after caesarean sections from GDM (n=4) and age-matched control uncomplicated pregnancies (n=3). Tissue explants were cultured for 24 hours  $\pm$  treatment with mitochondrial antioxidants (1mM L-Ergothioneine, 1 $\mu$ M MitoQ) and NLRP3 inhibitor (1 $\mu$ M MCC950). IL-1 $\beta$  and IL-18 were measured by ELISA in explant supernatants. Statistical analysis using paired t-tests was performed with GraphPad Prism 8.

**Results:** In placental tissue, IL-1 $\beta$  expression was significantly higher in explant supernatant of GDM vs. control (106.5 pg/ml  $\pm$  21.72 vs. 10.87 pg/ml  $\pm$  2.72; p=0.014). No significant increase in IL-18 expression was noted in GDM vs. control (12.12 pg/ml  $\pm$  1.92 vs. 7.61 pg/ml  $\pm$  0.71; p=0.11). Treatment of GDM placental explants with MCC950 significantly attenuated IL-1 $\beta$  expression compared to untreated explant (26.88 pg/ml  $\pm$  9.94 vs. 106.5 pg/ml  $\pm$  21.72; p=0.027). There was no significant change in IL-1 $\beta$  expression following treatment with L-Ergothioneine (68.83 pg/ml  $\pm$  22.19 vs. 106.5 pg/ml  $\pm$  21.72; p=0.27) or MitoQ (115.6 pg/ml  $\pm$  60.11 vs. 72.68 pg/ml  $\pm$  38.84; p=0.57). No significant change was observed vs. control in IL-18 expression with MCC950 (p=0.07), L-Ergothioneine (p=0.21) or MitoQ (p=0.24) treatment. In visceral omental tissue, IL-1 $\beta$  expression was significantly reduced in explant supernatant of GDM vs. control (22.35 pg/ml  $\pm$  1.79 vs. 53.63 pg/ml  $\pm$  10.07; p=0.038). There was no significant IL-18 expression difference in GDM vs. control (10.91 pg/ml  $\pm$  4.42 vs. 13.18 pg/ml  $\pm$  0.42; p=0.64). In GDM omental explants, no significant change in IL-1 $\beta$  expression was seen with MCC950 (31.6 pg/ml  $\pm$  13.74 vs. 22.35 pg/ml  $\pm$  1.79; p=0.53), L-Ergothioneine (61.72 pg/ml  $\pm$  47.47 vs. 22.35 pg/ml  $\pm$  1.79; p=0.48) or MitoQ (27.75 pg/ml  $\pm$  13.59 vs. 65.25 pg/ml  $\pm$  39.49; p=0.51). No significant change in IL-18 expression was observed vs. control with MCC950 (p=0.28), L-Ergothioneine (p=0.47) or MitoQ (p=0.22) treatment.

**Conclusion:** NLRP3 activity is implicated in both placental and omental tissue dysfunction in GDM pathophysiology and can be attenuated in placental tissue by MCC950 treatment, however the potential therapeutic benefit of mitochondrial antioxidants remains unclear.

## T-073

**Does Preconception Bariatric Surgery Detrimentially Influence Maternal Nutritional Status during Pregnancy, Fetal Growth and Birth Weight?** Katinka Snoek,<sup>1</sup> Régine Steegers-Theunissen\*,<sup>1</sup> Nadia van de Woestijne<sup>†</sup>,<sup>1</sup> Sten Willemsen\*,<sup>1</sup> René Klaassen<sup>†</sup>,<sup>2</sup> Sam Schoenmakers\*.<sup>1</sup> <sup>1</sup>Erasmus MC, Rotterdam, Netherlands; <sup>2</sup>Maasstad Hospital, Rotterdam, Netherlands.

**Introduction:** Due to the worldwide burden of obesity in women of reproductive age, bariatric surgery is increasing. Excessive rapid weight loss and high dose vitamins lead to iatrogenic malnutrition or synthetic 'toxicity' influencing foetal growth, birthweight and pregnancy outcome. We will evaluate the influence of bariatric surgery and supplementation on maternal nutritional status, foetal growth and pregnancy outcome.

**Methods:** A retrospective chart review was performed at a bariatric expertise center (Maasstad Hospital, Rotterdam) in pregnancies between 2009 and 2019. Nutritional status during all trimesters regarding blood samples, estimated foetal weight, 2nd and 3rd trimester ultrasonographic parameters and birth weight were collected.

**Results:** Women had a median BMI >40kg/m<sup>2</sup> before bariatric surgery and 29.0 kg/m<sup>2</sup> at conception. Vitamin deficiencies (table 1) occurred less often in recommended supplement Fit For Me users than in standard multivitamins users (p<0.001), however, vitamin excesses of folate and vitamin B12 occurred more frequently (p<0.001). Compared to a prebariatric group from Lapolla et al. 2010, pregnancy complications were reduced (p<0.001).

A subgroup of 104 pregnancies was included with 2nd and 3rd trimester foetal growth parameters. Estimated foetal weight Z-scores were significantly lower (p<0.001) compared to the Verburg reference curves. Birth weight was significantly reduced compared to the general Dutch population ([18.5% vs 10.4% with a birth weight <p10, p=0.006] and [2.8% vs 10.9%, p=0.007 with a birth weight >p90]).

**Conclusion:** Here we demonstrate that bariatric surgery decreases the risk for pregnancy complications. However, despite supplementation, deficiencies occurred mainly during the first trimester and up to 40%. The reduced foetal growth and birth weight suggests that other nutrients and hormones regulated by the stomach are also influenced by bariatric surgery resulting in an iatrogenic malnutritional, metabolic and endocrine state. This data emphasises the urgent need for periconceptional lifestyle counselling of women after bariatric surgery contemplating pregnancy, extra ultrasounds examinations and regular vitamin level monitoring during pregnancy. Further research on the impact of bariatric surgery on metabolism and endocrinology and the consequences of high dose multivitamins is needed.

Table 1. Nutritional and vitamin deficiencies per trimester.

	Trimester 1 (%)	Trimester 2 (%)	Trimester 3 (%)
Folate	1.5	0	1.1
Vitamin B12	21.0	25.2	30.2
Vitamin D	40.4	9.2	19.3
Calcium	17.1	0	1.1
Iron	34.6	8.3	9.4

## T-074

**Impact of Western/USA Diet on Maternal Metabolism in Vervet Pregnancy.** Sarah Therese Shepard<sup>†</sup>, Jeffrey Denney\*, Kylie Kavanagh\*, Mathew Jorgensen\*, Brian Brost\*. Wake Forest Medical School, Winston-Salem, NC, United States.

**Introduction:** Maternal obesity is a rising complication affecting pregnancy. Maternal weight gain and diet practices are thought to contribute to increasing rates of gestational diabetes and increased maternal and fetal complications. Diet manipulation in pregnancy is limited by patient compliance and concern that inadequate nutrition will affect fetal growth. As in human's, old-world African green vervet non-human primates (vervet) are at increased risk for type II diabetes, obesity and metabolic syndrome. The traditional vervet diet is a soy based high fiber diet that differs significantly from the standard American diet (USA).

We hypothesized that the introduction of a Western diet similar to an USA diet versus a soy based Standard Chow diet in pregnant vervet primates would increase insulin resistance and have a poor lipid profile effect.

**Methods:** 33 pregnant vervet primates were divided into three groups for diet exposure: Standard chow ad libitum (ad lib), Western diet ad lib or restricted Western diet. Each group was exposed to their assigned diet for a minimum of one trimester equivalent (55 of 165 days total in gestation). Maternal body weight was measured every 2 weeks. CBC, HbA1c and clinical chemistry analyses were performed at baseline and 4 wk after the diet change. Plasma lipid concentrations, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glucose, insulin, and were measured at baseline and at 4, 8, and 12 wk after the diet change. Fetal weight, head circumference and outcome were recorded. Univariate and multivariate analyses were used as appropriate.

**Results:** 33 pregnant vervet primates had complete data and were analyzed by group as follows: Standard Chow ad lib (n=15), Western Diet ad lib (n=8), Western Diet Restricted (n=10); see Table 1. While there was not a significant difference in total cholesterol, the Standard Chow (soy-based) ad lib diet was associated with favorable HDL levels (>60) when compared to receiving Western ad lib diet (OR 2.8 95 CI 1.1, 7.1; p=0.025). Western diets demonstrated a nonsignificant increase in measured mean glucose values, but a normal HbA1c (<6.2%) was associated with the soy based Chow ad lib diet as opposed to Western ad lib diet (OR 7.5 CI 2.6, 21.3; p<0.01). Maternal and fetal weight gain were not affected. The restricted Western diet did have a trend toward lower maternal weight gain than the other two diet cohorts.

**Conclusion:** A Western (USA) diet in pregnancy imparts significant variation in maternal metabolism as assessed by HbA1c, triglycerides and HDL levels.

**T-075**

**Diabetes Distress Scores and Perinatal Outcomes among Women with Gestational and Pregestational Diabetes.** Jennifer Jacobson†,<sup>1</sup> Amy Godecker,<sup>1</sup> Jennifer Janik,<sup>1</sup> April Eddy,<sup>2</sup> Jacquelyn Adams\*.<sup>1,1</sup> *University of Wisconsin School of Medicine and Public Health, Madison, WI, United States;* <sup>2</sup>*Unity-Point Health Meriter Hospital, Madison, WI, United States.*

**Introduction:** Living with diabetes is associated with depression and anxiety in women. Little pregnancy data is available despite high prevalence of gestational diabetes (GDM) and pregestational diabetes (preDM). Several clinical assessments have sought to quantify diabetes-related emotional distress and the abbreviated Diabetes Distress Scale (DDS) demonstrates high reliability. We hypothesize that women with high DDS will have higher HbA1c and EPDS scores in pregnancy.

**Methods:** A retrospective cohort study of 295 women with preDM (prediabetes, type 2 DM, type 1 DM, n=121) or GDM (n= 174) between 1/1/2018 and 7/31/2020. Patients were identified using the American Diabetes Association database. Demographic and pregnancy data were obtained from PeriData, a statewide quality improvement database, and the electronic medical record.

**Results:** Demographics were similar between groups, but women with preDM delivered at earlier GA (37.4 vs. 38.5, p=0.000). High DDS was present in 27.5% with no difference between preDM and GDM (30.3% vs. 25.4%, p=0.387). We performed a logistic regression while controlling for DM type and GA at delivery. High DDS score was associated with higher mean 3<sup>rd</sup> trimester A1c (6.0% vs. 5.7%, aOR 0.24, 95% CI [0.05-0.44], p=0.016) vs. low DDS, but not with postpartum (PP) A1c (7.1% vs. 6.2%, 0.22 aOR, 95% CI [-0.63-1.06], p=0.605). High DDS was associated with positive Edinburgh Postpartum Depression Scale (EPDS) screen at 28-32 weeks (32.5% vs. 15.7%, aOR 2.65, 95% CI [1.13-6.18], p=0.024) but not at 6 weeks PP (17.5% vs. 16.1%, aOR 1.12, 95% CI [0.50-2.50], p=0.788). For neonatal outcomes, high DDS was not independently associated with higher rates of NICU admission (30.4% vs. 18%, aOR 1.91, 95% CI [0.92-3.94], p=0.082), neonatal respiratory distress syndrome (RDS) (13% vs. 5.5%, aOR 2.50, 95% CI [0.86-7.29], p=0.092) or neonatal hypoglycemia (45.6% vs. 39%, aOR 1.24, 95% CI [0.70-2.20], p=0.462) vs. low DDS.

**Conclusion:** High DDS is equally prevalent among women with GDM and preDM. High DDS was associated with higher A1c values and EPDS scores in the 3<sup>rd</sup> trimester but not at 6 weeks PP. High DDS alone was

not associated with NICU admission, RDS or hypoglycemia. This study asserts that DDS may be a useful tool for risk stratification of women with diabetes in pregnancy.

Maternal and Neonatal Outcomes	Diabetes Distress Score						Diabetes Distress Score					
	Total	Low DDS	High DDS	p-value	aOR (95% CI)*	p-value	Total	Low DDS	High DDS	p-value	aOR (95% CI)*	
<b>Cholesterol</b>	70(290)	70(290)	70(290)				70(290)	70(290)	70(290)			
<b>Maternal A1c</b>												
Mean	5.7(0.8)	5.7(0.8)	6.0(0.8)	0.05	0.34 (0.05)	0.05	5.7(0.8)	5.7(0.8)	6.0(0.8)	0.05	0.34 (0.05)	
Prevalence	4.2(0.36)	<0.05	6.1(0.36)	0.001	6.45	0.001	4.2(0.36)	<0.05	6.1(0.36)	0.001	6.45	
Gestational	5.4(0.45)	<0.05	6.1(0.45)	0.001	2.32	0.001	5.4(0.45)	<0.05	6.1(0.45)	0.001	2.32	
<b>A1c</b>												
Mean	6.1(1.0)	6.1(1.0)	6.2(1.0)	0.21 (0.04)	0.001	0.21 (0.04)	6.1(1.0)	6.1(1.0)	6.2(1.0)	0.21 (0.04)	0.001	
Prevalence	6.1(1.0)	<0.05	6.2(1.0)	0.001	2.90	0.001	6.1(1.0)	<0.05	6.2(1.0)	0.001	2.90	
<b>Perinatal</b>												
Mean (SD)	28.2 (2.8)	28.2 (2.8)	28.7 (2.8)	0.141		28.2 (2.8)	28.2 (2.8)	28.7 (2.8)	0.141			
Prevalence	38.4 (0.2)	0.006	38.4 (0.2)	0.489		38.4 (0.2)	38.4 (0.2)	38.4 (0.2)	0.489			
Gestational	24.4 (0.4)	0.001	24.2 (0.4)	0.423	2.65 (1.3)	0.001	24.4 (0.4)	24.4 (0.4)	0.423	2.65 (1.3)		
EPDS	19.0 (2.8)	0.305	19.0 (2.8)	0.001	6.18	0.001	19.0 (2.8)	19.0 (2.8)	0.001	6.18		
<b>Weight (kg)</b>												
Mean (SD)	34.9 (4.4)	34.9 (4.4)	35.0 (4.4)	0.921		34.9 (4.4)	34.9 (4.4)	35.0 (4.4)	0.921			
Prevalence	31.1 (7.6)	31.1 (7.6)	31.1 (7.6)	0.911		31.1 (7.6)	31.1 (7.6)	31.1 (7.6)	0.911			
<b>Weight (lb)</b>												
Mean (SD)	77.0 (9.8)	77.0 (9.8)	77.1 (9.8)	0.921		77.0 (9.8)	77.0 (9.8)	77.1 (9.8)	0.921			
Prevalence	70.9 (18.0)	70.9 (18.0)	70.9 (18.0)	0.921		70.9 (18.0)	70.9 (18.0)	70.9 (18.0)	0.921			
<b>NICU admission, n (%)</b>												
Mean (SD)	19.0 (2.8)	19.0 (2.8)	19.0 (2.8)	0.921		19.0 (2.8)	19.0 (2.8)	19.0 (2.8)	0.921			
Prevalence	45.9 (4.5)	<0.05	45.9 (4.5)	0.001		45.9 (4.5)	45.9 (4.5)	0.001				
<b>Hypoglycemia, n (%)</b>												
Mean (SD)	23.0 (2.8)	23.0 (2.8)	23.0 (2.8)	0.921		23.0 (2.8)	23.0 (2.8)	0.921				
Prevalence	42.9 (4.2)	0.001	42.9 (4.2)	0.001	2.00 (0.8)	42.9 (4.2)	42.9 (4.2)	0.001	2.00 (0.8)			

**T-076**

**The Relationships between BMI and Patient-Provider Weight Goals during Pregnancy.** Hannah Dugoni†,<sup>1</sup> Shelby Alsup†,<sup>1</sup> Katherine Elder\*,<sup>1</sup> Olivia Doyle†,<sup>2</sup> Kristen Mackiewicz Seghete\*,<sup>2</sup> Alice Graham\*,<sup>2</sup> *Pacific University, Hillsboro, OR, United States;* <sup>2</sup>*Oregon Health & Science University, Portland, OR, United States.*

**Introduction:** Insufficient and excessive gestational weight gain (GWG) are important predictors of adverse maternal and offspring health outcomes. The Institute of Medicine recommends that all individuals, regardless of pre-pregnancy body mass index (BMI), gain weight during pregnancy. Individuals who receive appropriate GWG recommendations from providers have greater odds of gaining a healthy amount of weight during pregnancy. Underweight and overweight pre-pregnancy BMI are also risk factors for insufficient or excessive GWG, respectively. More research is needed to explore factors related to individuals' GWG and weight goals (WGs) for pregnancy, and factors that influence providers' WG recommendations to their patients. This study examines factors that contribute to pregnant individuals' own WGs for pregnancy, and factors that contribute to providers recommending appropriate WGs to patients.

**Methods:** Participants are pregnant individuals (N = 149) enrolled in an ongoing longitudinal study examining the effects of Mindfulness-Based Cognitive Therapy on maternal functioning and offspring outcomes. The current study examined self-report measures collected at baseline (14-23 weeks gestation). Self-report measures included questionnaires about medical history and prenatal care, which assessed participants' WGs for pregnancy and their providers' WG recommendations. Provider WG recommendation and participant WG for pregnancy were defined as binary dependent variables: staying at pre-pregnancy weight or losing weight (an "inadequate" WG recommendation/participant WG) and gaining weight (an "adequate" WG recommendation/participant WG).

**Results:** A logistic regression was conducted to test the relationship between the outcome variable, adequacy of provider WG recommendation, and predictor variable, BMI, adjusting for ethnicity. The model was statistically significant,  $\chi^2(2) = 25.10, p < .001$ . To examine the relationship between provider WG recommendation and participant WG for pregnancy, a Pearson's chi-square analysis was conducted. There was a significant association between provider WG recommendation and participant WG,  $\chi^2(2) = 39.71, p < .001$ .

**Conclusion:** The findings suggest that maternal BMI affects the adequacy of providers' WG recommendations for pregnancy, such that providers are more likely to recommend inadequate WGs for individuals with higher BMIs. Our results suggest that providers who recommend inadequate WGs to patients may increase the risk of insufficient (or excessive) GWG and subsequent adverse maternal and offspring outcomes, which will be further examined. Future studies should explore the relationship between patients' self-report of their providers' WG recommendation and providers' documented recommendation using medical record data.

Thursday Posters

## T-077

**Enhanced Fatty Acid Binding Protein (FABP)-4 Secretion in Placental Villi with Gestational Diabetes Mellitus: Implication for Impaired Glucose Homeostasis.** Anthony M Kendle†, Nihan Semerci, Asli Ozmen, Xiaofang Guo, Ali Wells†, Ozlem Guzeloglu-Kayisli\*, Umit Kayisli\*, Charles J Lockwood\*. *The University of South Florida, Tampa, FL, United States.*

**Introduction:** Gestational diabetes mellitus (GDM) affects 2-10% of pregnancies annually in the United States, and these mothers and their offspring are at increased risk for metabolic derangement later in life. Fatty acid binding protein 4 (FABP4) is a secreted adipokine that plays a major role in glucose homeostasis, progression of insulin resistance and inflammation. FABP4-deficient mice are highly resistant to developing various metabolic diseases, including diabetes, atherosclerosis, diet-induced obesity, genetic obesity, or hypercholesterolemia. Recent studies have demonstrated increased serum levels of FABP4 in both maternal and umbilical cord serum at time of delivery in pregnancies affected by GDM. We hypothesize that FABP4 expression is increased in placental villous tissues of pregnancies affected by GDM compared to non-GDM, and this differential expression may contribute to the pathogenesis of GDM.

**Methods:** Placentas were collected prospectively from pregnancies with GDM (n=4) and from normal pregnancies (n=3), matching for age, gestational age at delivery, and body mass index. A piece of placental villous tissue was processed for RNA and protein extraction to detect *in situ* FABP4 levels, whereas another portion of placental villi was used for explant cultures for 24 h. Explant cultures and media were then collected for RNA and protein extraction. All samples were then analyzed by qPCR and immunoblotting.

**Results:** Immunoblot analysis revealed that *in situ* FABP4 protein levels were significantly increased (Mean  $\pm$ SEM; 2.34  $\pm$ 0.24 vs. 0.25  $\pm$ 0.05; p=0.001) in placentas from pregnancies with GDM vs. from normal pregnancies. *FABP4* mRNA levels were 5.74-fold higher (p<0.05) in explant cultures of placental villi of pregnancies with GDM vs. normal pregnancies. Moreover, analysis of explant culture media by immunoblotting detected significantly higher FABP4 secretion (129.3  $\pm$  24.8 vs. 11.8  $\pm$  7.8; p=0.011) in placental villi of pregnancies with GDM vs. normal pregnancies.

**Conclusion:** Placentas of patients with GDM display higher amounts of FABP4 protein compared to non-GDM placentas, suggesting placental-derived FABP4 involvement in the pathogenesis of GDM by impairing maternal glucose homeostasis or promoting insulin resistance. Higher levels of FABP4 mRNA expression and protein secretion in explant cultures of GDM placentas also support the hypothesis that increased FABP4 levels in GDM maternal blood may originate from placental FABP4 secretion to impair glucose homeostasis.

## T-078

**Neonatal Outcomes in Preterm Trial of Labor in Women without a Prior Vaginal Delivery.** Sunitha C Suresh†, Annie Dude\*. *University of Chicago, Chicago, IL, United States.*

**Introduction:** Trial of labor (TOL) after cesarean delivery is associated with a low absolute risk of neonatal morbidity. Few studies have examined the effect of TOL on neonatal morbidity in preterm infants, who account for the majority of neonatal morbidity in non-anomalous neonates. Over half of the women studied in a prior study on preterm TOL had a prior successful vaginal delivery, which increases the likelihood of success. The purpose of this study was to characterize neonatal morbidity following preterm TOL in comparison to elective repeat cesarean section (eRCS) specifically among women without a prior vaginal delivery who may have a lower success rate.

**Methods:** This is a secondary analysis of the MFMU Cesarean Section Registry. Analysis was restricted to singleton pregnancies without a prior vaginal delivery who delivered an infant between 24 weeks and 36 weeks 6 days gestation. Patients with an antepartum stillbirth, major congenital malformations, or an indicated cesarean delivery were excluded. Neonatal outcomes were compared using chi squared analysis between those with a TOL and those undergoing eRCS. Logistic regression was used to control for potential confounders including gestational age.

**Results:** 2120 patients were included in analysis. The TOL success rate was 62.7%. Trial of labor was related to gestational age at time of delivery with 83% of women having a trial of labor at < 30 weeks, and only 48.0% of women having a trial of labor at > 30 weeks. Women with a TOL were less likely to have pre-existing medical conditions, but more likely to have conditions such as abruption and chorioamnionitis (Table 1). After adjustment for confounders, women with a TOL had no difference in neonatal outcomes with the exception of a decreased risk of proven/suspected sepsis (adjusted OR 0.69 (0.54, 0.88), Table 1).

**Conclusion:** TOL in women without a prior vaginal delivery delivering in the preterm period is not associated with increased neonatal morbidity, and is in fact associated with decrease risk of sepsis. TOL remains a safe option for this patient population.

Demographic Information and Clinical Characteristics			
	Elective Repeat N = 1050	Trial of Labor N = 1070	p-value
Maternal Age	30 [25,34]	30 [25,34]	<0.001
% Black Race	227 (21.6%)	358 (33.5%)	<0.001
% Married	654 (62.3%)	576 (53.8%)	<0.001
% Prenatal Care	1013 (96.5%)	1010 (94.4%)	0.02
% Tobacco Use	141 (13.4%)	179 (16.7%)	0.04
Pre-pregnancy BMI	25.8 [22.1, 30.9]	25.3 [21.9, 30.4]	0.34
Maternal Diabetes	172 (16.4%)	118 (11.0%)	<0.001
Gestational Age (median)	36.1 [35, 36.7]	35.3 [32.9, 36.4]	<0.0001
Placental Abruption	15 (1.4%)	53 (5.0%)	<0.0001
Chorioamnionitis	26 (2.5%)	69 (6.5%)	<0.001
Hypertensive Disorder of Pregnancy	136 (13.0%)	210 (19.6%)	<0.001
Complete Antenatal Steroids	156 (14.9%)	334 (31.2%)	<0.0001
Uterine Rupture	1 (0.1%)	3 (0.3%)	0.36

Neonatal Outcomes			
	Elective Repeat N=1050	Trial of Labor N= 1070	Adjusted Odds Ratio
NICU Admission	401 (38.2%)	570 (53.3%)	.91 (0.72, 1.14)
Any Neonatal Morbidity	312 (29.7%)	440 (41.1%)	0.85 (0.68, 1.07)
Intrapartum or Neonatal Death	0	1 (0.1%)	
Respiratory Distress Syndrome	148 (14.1%)	242 (22.7%)	0.82 (0.62, 1.1)
Ventilator Use (in first 24 hours)	103 (9.8%)	181 (17.0%)	0.73 (0.52, 1.03)
Proven/ Suspected Sepsis	223 (21.3%)	276 (25.9%)	0.69 (0.54, 0.88)
Necrotizing Enterocolitis	3 (0.3%)	14 (1.3%)	1.10 (0.27, 4.53)
Hypoxic Ischemic Encephalopathy	1 (0.1%)	4 (0.4%)	1.5 (.1, 21.14)
Injuries at Delivery	5 (0.5%)	12 (1.1%)	2.39 (0.79, 7.23)
Any Intraventricular Hemorrhage	15 (1.4%)	59 (5.5%)	1.58 (0.82, 3.04)
Seizures	3 (0.3%)	12 (1.1%)	0.91 (0.2, 3.9)
Transient Tachypnea of the Newborn	94 (9.0%)	95 (8.9%)	0.89 (0.64, 1.24)

**T-079**

**First Trimester Subchorionic Hemorrhage and Impact on Pregnancy Outcomes in the IVF Population.** *Avanthi Sai Ajjarapu*†, *Liubin Yang*†, *William Gibbons*\*. *Baylor College of Medicine, Houston, TX, United States.*

**Introduction:** A first trimester ultrasound finding of subchorionic hemorrhage (SCH) can be alarming to patients, as little data exists on their impact on pregnancy outcomes. As such, this study aims to evaluate the impact of subchorionic hematoma (SCH) on early pregnancy loss (EPL).

**Methods:** A retrospective cohort study was conducted on 205 patients who received in vitro fertilization treatment and underwent embryo transfer between 2014 and 2018 at Texas Children’s Hospital. The primary outcome was EPL and preterm delivery was studied as a secondary outcome. EPL was defined as ultrasound confirmed spontaneous abortion at less than 20 weeks gestation, where gestational age was determined based on date of embryo transfer. Ultrasound imaging completed in the first trimester was reviewed for each patient. Presence, date first noted, size, and largest dimensions of a SCH were recorded. Inclusion criteria were those patients undergoing embryo transfer between 2014-2018. Those without documented first trimester ultrasounds findings were excluded (n=14). Baseline characteristics were compared using chi square and t-test statistics. Multivariate logistic regression was utilized to adjust for baseline characteristics that were statistically significant in the univariate analysis. Adjusted RR were obtained from the regression analyses.

**Results:** The prevalence of SCH was 46.3% in our IVF population between 2014-2018. Of those with subchorionic hemorrhage, 10.5% had early pregnancy loss, compared to 7% in those without SCH (p=0.49, RR 1.38 (0.57-3.36)). Baseline characteristics did not statistically differ between those with and without SCH. No statistically significant relation was found between SCH and rates of EPL or preterm delivery both without and with adjustment for vaginal bleeding (Adjusted RR: 1.15 (0.39-3.50); 0.84 (0.29-2.39)). Largest diameter of SCH was not correlated with EPL (p=0.4). The presence of multiple SCHs, however, did correlate with EPL (p=0.01).

**Conclusion:** Despite high rates of SCH identified in the IVF population, there was no evidence of increased rates of early pregnancy loss or preterm delivery. Further studies must be completed to understand the etiology of IVF and increased rates of SCH to assess for ways in which SCH could impact pregnancy outcomes outside of loss and preterm delivery.

**T-080**

**History of Infant Respiratory Morbidity Predictive of Neonatal Respiratory Morbidity in Subsequent Pregnancy.** *Naima Ross*†, *Sunitha Suresh*†, *Ann Dude*\*. *University of Chicago, Chicago, IL, United States.*

**Introduction:** Prior studies have demonstrated a potential genetic contribution to respiratory distress syndrome (RDS). Delivery in the late preterm period is a known risk factor for respiratory morbidity. It is not well established whether a history of an infant with respiratory problems at birth relates to respiratory morbidity in a subsequent pregnancy.

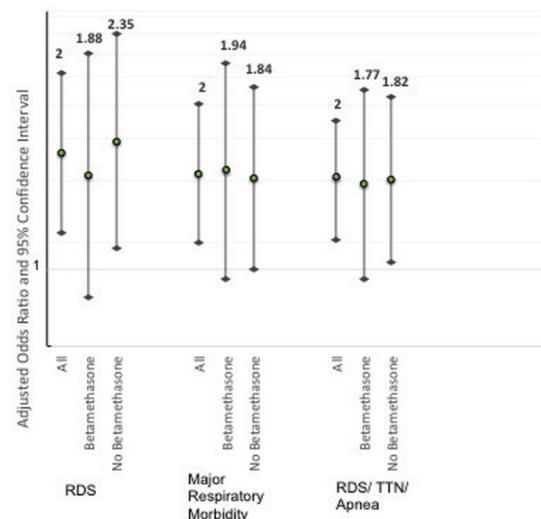
**Methods:** This is a secondary analysis of a randomized control trial of antenatal corticosteroids (ACS) in the late preterm period (34+0 to 36+6 weeks gestation). Multiparous patients were included, and participants with a prior delivery at <34 weeks were excluded. Demographic characteristics were compared between those with a self-reported history of a neonate with respiratory problems and those without. The presence of respiratory morbidity (major respiratory morbidity (MRM), RDS, or transient tachypnea (TTN) of the newborn) was compared by history of a previous infant with respiratory problems. Logistic regression was performed to adjust for confounders, including gestational age and variables significantly different between groups at a p of 0.1. The analysis was repeated stratified by any betamethasone (BMZ) use.

**Results:** We included 1,412 multiparous patients. Those with a prior infant with respiratory problems were more likely to be non-Hispanic, have private insurance, and have infants with higher birth weight (Table 1). MRM was more likely if mothers reported a history of a prior infant with respiratory morbidity (adjusted OR 1.90 95% CI [1.20, 3.02]). Similar results were seen for RDS and a composite of RDS/ TTN/Apnea (Figure

1). When stratified by administration of BMZ, respiratory morbidity was only more likely among those who did not receive BMZ (aOR for MRM in BMZ group 1.92 [.93, 3.96]).

**Conclusion:** A history of a late term/ term infant with respiratory problems at birth is associated with respiratory morbidity in a subsequent late preterm/ term infant. Future work is needed in both determining the underlying pathophysiology of respiratory morbidity in the late preterm/ term period, and the utility of ACS in patients with a history of respiratory morbidity in early term gestation.

Demographic/ Clinical Characteristics by History of Infant with Respiratory Morbidity			
	Prior infant without respiratory morbidity (N=1217)	Prior infant with respiratory morbidity (N= 195)	p-value
Treatment arm	51.19 % (623)	45.64% (89)	0.15
Gestational age at delivery	36.1 [35.4,36.8]	36.3 [35.4, 36.9]	0.52
Maternal age	29 [25,33]	28.5 [24, 33]	0.55
Private insurance	29.3% (357)	41.0 % (80)	0.005
Maternal race white	55.1% (670)	64.1% (125)	0.13
Maternal years of schooling	12 [11,14]	12 [12,16]	0.01
Hispanic/ Latino ethnicity	36.5% (442)	26.7% (52)	0.01
Male sex	50.7% (617)	53.3% (104)	0.49
Severe preeclampsia	14.1 % (172)	15.9% (31)	0.51
Maternal tobacco use	16.1 %(196)	16.4% (32)	0.91
Polyhydramnios	1.1% (13)	2.6% (5)	0.08
Gestational diabetes	12.6% (153)	10.3% (20)	0.36
Cesarean delivery	27.9% (340)	27.7% (54)	0.94
Birthweight (g)	2670 [2365, 3026]	2770 [2435, 3090]	0.02



## T-081

**Identification of Bacterial Metabolic Abundances in the Gut Microbiome of Preterm Infants.** Anujit Sarkar\*,<sup>1</sup> Ji Youn Yoo†,<sup>1</sup> Jean Lim†,<sup>1,2</sup> Samia Dutra†,<sup>1</sup> Larry Dishaw\*,<sup>1</sup> Bradley Kane\*,<sup>1</sup> Maureen Groer Edith Groer\*,<sup>1</sup> Elizabeth Miller\*.<sup>1</sup> <sup>1</sup>University of South Florida, Tampa, FL, United States; <sup>2</sup>University of Tennessee, Knoxville, TN, United States.

**Introduction:** Infants born prematurely experience health challenges and must adapt to extrauterine life with developmentally immature systems. An important regulator of developmental physiology is the gut microbiome. These infants often develop dysbiosis during their NICU stay, which could have potential effects on many developing systems. Apart from determining composition, predicting functional potential of the microbiome may provide important new insights into factors that shape later growth and development.

**Methods:** Stool samples were collected weekly up to eight weeks from 83 preterm infants (mean gestational age 28 weeks). The V4 region of 16S rRNA gene was PCR amplified and sequenced in a paired-end manner on the MiSeq platform (Illumina). The DADA2 pipeline was employed to predict Exact Sequence Variants (ESVs) and deduce bacterial taxonomy. To compare these preterm infants with appropriate controls, publicly available comparable 16S raw data from term infants were accessed from a previous study (Pannaraj et al., 2017) and were analyzed with the preterms. Overall, a total of 375 and 112 specimens from preterm and term infants, respectively, were studied. Metagenomic assemblies and functional potential were achieved by piphillin using the KEGG database (Oct 2018 version).

**Results:** The preterms displayed marked Gammaproteobacteria abundances during this period and alpha diversity was low throughout the study in comparison to term infants. Pathway prediction identified 317 bacterial metabolic pathways in the preterms and 325 in the term infants. There were fifteen pathways predicted in term infants that were not found in preterms. Apart from these, a total of 228 metabolic pathways were significantly different in abundance between the groups. A few of them, which were most significantly lower in preterms, are shown in Figure 1 and include alanine, aspartate and glutamate metabolism (KO00250), Vitamin B6 metabolism (KO00750), Sphingolipid metabolism (KO00600) and peroxisome (KO04146).

**Conclusion:** The present study constitutes one of the first attempts to correlate gut microbiome composition to predicted function, to ultimately predict relationships between early gut microbiome composition to later physical and mental growth and development. However, metagenomic and metatranscriptomic, along with metabolomic follow-up studies, will be essential to help validate these predictions.

## T-082

**Trends in Postpartum Venous Thromboembolism and Chemical Thromboprophylaxis among Insured U.S. Patients.** Ann M Bruno†,<sup>1,2</sup> Amanda A Allshouse,<sup>1</sup> Brett D Einerson,<sup>1,2</sup> Heather M Campbell,<sup>1</sup> D Ware Branch,<sup>1,2</sup> Robert M Silver,<sup>1,2</sup> Torri D Metz\*.<sup>1,2</sup> <sup>1</sup>University of Utah Health, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Murray, UT, United States.

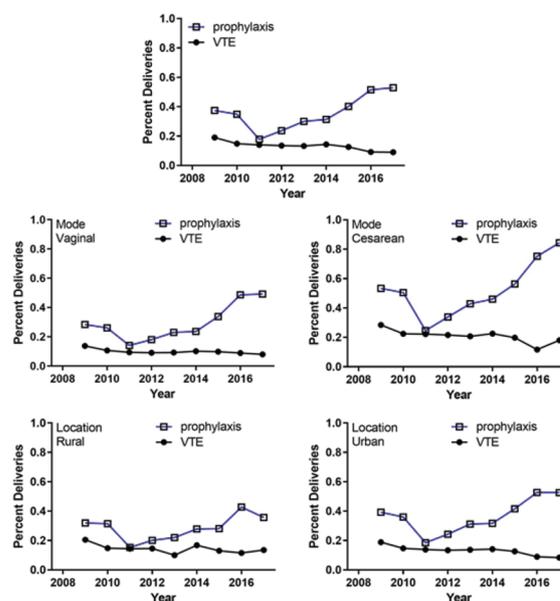
**Introduction:** Postpartum venous thromboembolism (VTE) is a significant contributor to maternal morbidity and mortality. National guidelines have called for postpartum risk-stratification protocols to identify patients at elevated risk of VTE who would benefit from chemical thromboprophylaxis. We aimed to evaluate modern trends in VTE incidence and use of chemical thromboprophylaxis in postpartum patients in a large, insurance-claims database. Secondly, we aimed to evaluate VTE and thromboprophylaxis use by delivery mode and location.

**Methods:** This was a retrospective cohort study of all females aged 16 to 50 years with at least one delivery (>20 weeks gestation) in the IBM MarketScan Research Database between 2008 and 2017. The first delivery from 2009 through the first half of 2017 was included. VTE events were identified by International Classification of Diseases 9<sup>th</sup> and 10<sup>th</sup> Revision codes. Administration of chemical prophylaxis (low molecular weight heparin or unfractionated heparin) was identified using medication codes. Women with a VTE prior to delivery were excluded. The rate of VTE and chemical thromboprophylaxis in the first 12 weeks postpartum

was estimated over the full study period and by year. VTE and chemical thromboprophylaxis use rates were compared across years with a test of trend. Differences by mode of delivery and urban versus rural location were tested with a Cochran-Mantel-Haenszel statistic.

**Results:** Of 3,145,105 individuals included for analysis, 4,480 (0.14%) had a VTE in the first 12 weeks postpartum, and 10,173 received postpartum chemical thromboprophylaxis (0.32%). The rate of VTE decreased over time from 0.19% in 2009 to 0.09% in 2017 ( $p < 0.001$  for test of trend), while the rate of chemical thromboprophylaxis increased from 0.37% to 0.53% over the same period ( $p < 0.001$  for test of trend). One-third (34%) of women delivered by cesarean and the majority (87%) delivered in an urban hospital. The rate of VTE decreased and the rate of thromboprophylaxis increased across all modes of delivery and delivery locations, but significantly more among cesarean deliveries and urban deliveries ( $p < 0.001$  for test of trend).

**Figure.** Trends in venous thromboembolism and chemical thromboprophylaxis in the first 12 weeks postpartum over time, by mode of delivery and delivery location.



**Conclusion:** The incidence of VTE decreased while rates of chemical thromboprophylaxis increased over time in this U.S. cohort. It is unknown whether changes in prophylaxis, or other changes to obstetric care over this time period, reduced VTE events.

## T-083

**Is Antenatal Vaginal Bleeding in Placenta accreta Spectrum a Harbinger of Adverse Outcomes?** Lihong Mo†, Nandini R Nittur†, Zahabiya H Chithiwala†, Herman L Hedriana\*. UC Davis, Sacramento, CA, United States.

**Introduction:** Placenta Accreta Spectrum (PAS) is associated with significant maternal morbidity and mortality (SMM). With the increase of Cesarean deliveries, PAS quadrupled from the 1980s to 2010s. ACOG recommends delivery at a gestational age (GA) of 34 0/7-35 6/7 weeks. However, many women will have vaginal bleeding (VB) before the recommended gestation age of delivery resulting in prolonged antepartum hospitalization, neonatal morbidities due to extreme premature births, and significant healthcare cost. This study is to understand the different clinical characteristics of PAS patients with or without VB.

**Methods:** Retrospective cohort study of patients between 1/1/2015 to 6/30/2020 with ultrasound findings for PAS at a tertiary academic center (N=54) excluding patients who underwent abortion (N=4) and who had preterm prelabor rupture of membrane (N=3). The cohort group included patients who had antepartum VB (N=22), and compared to patients without the same experience (N=25). Student t-test was performed on continuous

variables assuming a normal distribution, Wilcoxon signed-rank test was performed on continuous variables with a skewed distribution, and Chi-square test was performed on dichotomous variables.

**Results:** Demographic characteristics are comparable and presented in table 1. The VB mean episodes were 2.05±2.19 (median of 1). VB group delivered at an earlier GA (33.1 ±3.5 vs 35.4±1.3, p=0.006). Of the VB group, 45.5% delivered before 34 weeks while 20% of control delivered after 35 weeks. The VB group had a higher rate of unplanned delivery (45.5% vs 12%, p=0.01) and tended to have a longer antepartum stay (3.23±5.19 vs 0.85±2.09, p=0.056). The neonates in the VB group had significantly longer length of stay (19.1±17.3 vs 7.96±4.57, p=0.009). Outcomes are presented in table 2.

**Conclusion:** PAS patients presenting with VB have a higher likelihood for unplanned delivery before the recommended GA with an associated longer neonatal length of stay. Patients with no VB may deliver closer to term without significant maternal and neonatal adverse outcomes.

Table 1. Characteristics of Patients with Placenta Accreta Spectrum, 1/1/2015-6/30/2020

	VB positive (N=22)	VB negative (N=25)	p-value
<b>Patient Demographics</b>			
Age (Mean, SD)	33.41 (3.7)	33.00 (4.2)	0.72
Race/Ethnicity			0.17 <sup>b</sup>
White Non Hispanic (%)	18.2 [4/22]	13.6 [3/25]	
Black (%)	9.1 [2/22]	13.6 [3/25]	
Asian (%)	31.8 [7/22]	13.6 [3/25]	
Hispanic/Latino (%)	31.8 [7/22]	72.7 [16/25]	
Native American (%)	4.5 [1/22]	0	
Other (%)	4.5 [1/22]	0	
BMI (Mean, SD)	33.91 (10.48)	34.04 (10.72)	0.97
Smoking (%)	22.7 [5/22]	12.0 [3/25]	0.95
<b>Antepartum characteristics</b>			
Gravida (Mean, SD)	5.5 (2.77)	5.24 (2.07)	0.72
Para (Mean, SD)	3.23 (2.18)	3.2 (1.53)	0.92
Prior Cesarean section	2.36 (1.43)	2.68 (1.41)	0.45
Interpregnancy interval from last Cesarean (months) (Mean, SD)	49.8 (24.24)	54.1 (31.92)	0.91
Prior other uterine surgery	0.3 (0.73)	0.11 (0.32)	0.31
Presence of uterine contractions (%)	31.8 [7/22]	12 [3/25]	0.10
Placenta location			0.39
Anterior (%)	50.0 [11/22]	76.0 [19/25]	
Posterior (%)	18.2 [4/22]	4.0 [2/25]	
Others (%)	9.1 [2/22]	12.0 [3/25]	
Betamethasone doses (Mean, SD)	1.95 (1.07)	1.64 (0.76)	
<b>Labor Characteristics</b>			
Average gestational age at delivery (weeks) (Mean, SD)	33.07 (3.45)	35.41 (1.34)	0.006*
Median gestational age at delivery (weeks)	34	35	0.001*
Unplanned delivery (%)	45.5 [10/22]	12.0 [3/25]	0.01*
Delivery gestational age			0.04 <sup>§</sup>
< 28 weeks (%)	4.5 [1/22]	0	
28 0/7-31 6/7 weeks (%)	9.1 [3/22]	0	
32 0/7-33 6/7 weeks (%)	31.8 [7/22]	8.0 [2/25]	
34 0/7-35 6/7 weeks (%)	50.0 [11/22]	72.0 [18/25]	
>= 36 0/7 weeks (%)	4.5 [1/22]	20.0 [5/25]	

\* p<0.05; § comparison was done in the groups with numbers not equal to 0 in both groups. Student t test was performed on continuous variables assuming a normal distribution. Wilcoxon signed rank test was performed on continuous variables with a skewed distribution. Chi square test was performed on dichotomous variables. SD: standard deviation; BMI: body mass index.

Table 2. Outcomes of Patients with Placenta Accreta Spectrum, 1/1/2015-6/30/2020

	VB positive (N=22)	VB negative (N=25)	p-value
<b>Maternal outcomes</b>			
<b>Hemorrhage</b>			
Average EBL (ml) (Mean, SD)	2887.50 (2676.73)	2227.44 (1934.63)	0.34
Median EBL (ml)	2300	1500	
Transfusion (%)	54.5 [12/22]	52.0 [13/25]	0.86
Average # pRBCs (units) (Mean, SD)	3.45 (4.57)	2.17 (3.47)	0.29
Median # pRBCs (units)	3	1	0.31
Average # combined blood products (Mean, SD)	5.95 (10.11)	3.32 (6.39)	0.30
Median # combined blood products	3	1	0.37
Severe Maternal Morbidity Composite <sup>†</sup>	18.2 [4/22]	12 [3/25]	0.56
Maternal LOS (Mean, SD)	8.27 (5.04)	5.32 (1.75)	0.015*
Maternal antepartum LOS (Mean, SD)	3.23 (5.19)	0.85 (2.09)	0.056
Maternal postpartum LOS (Mean, SD)	4.19 (6.71)	3.80 (3.2)	0.81
<b>Neonatal outcomes</b>			
Fetal/Neonatal Demise (%)	4.5 [1/22]	0	NA
Apgar 5 min <= 7 (%)	18.2 [4/22]	8.0 [2/25]	0.30
Arterial pH (Mean, SD)	7.31 (0.1)	7.3 (0.06)	0.6
Arterial base excess (Mean, SD)	-1.96 (3.27)	-2.02 (1.86)	0.94
NICU admission (%)	90.0 [18/20]	76.0 [19/25]	0.22
Average neonatal LOS (Mean, SD)	19.14 (17.27)	7.96 (4.57)	0.009*
Median neonatal LOS	14	7	0.0007*

\* p<0.05; † Severe Maternal Morbidity Composite: ICU admission, re-operation, DIC, ureteral injury; pRBC, packed red blood cells; EBL, estimated blood loss; LOS: length of stay.

T-084

**Umbilical Artery Abnormalities in Women with OUD: Is a Revision of Cutoffs Appropriate?** Brittany McKinley<sup>†</sup>, Calvin Lee Ward, Katia Vela<sup>†</sup>, Aarthi Srinivasan, Erin MacLeod<sup>†</sup>, Zachary Stanley<sup>†</sup>, Brooke Andrews<sup>†</sup>, Katherine Vignes<sup>†</sup>, Cynthia Cockerham<sup>†</sup>, Leon Su, Arnold Stromber\*, John O'Brien\*. University of Kentucky College of Medicine, Lexington, KY, United States.

**Introduction:** Women with OUD are at increased risk for an abnormal growth profile. Most cutoffs for abnormal Doppler studies assessing fetal placental perfusion utilize a cutoff at the 95<sup>th</sup> percentile for gestational age. The aim of this study was to determine the incidence of fetal or neonatal adverse outcomes when a 90<sup>th</sup> percentile cutoff in patients with opioid use disorder (OUD) and evaluate maternal and neonatal outcomes.

**Methods:** This was a retrospective cohort study of women with OUD in a comprehensive perinatal OUD treatment program between April 2015 and December 2020. Women were included if third trimester ultrasound umbilical artery measurements (UmbA) were available in addition to maternal/neonatal outcomes. The primary outcome was IUGR in women at a 90<sup>th</sup> percentile cutoff. Secondary outcomes included rates of preterm labor (PTL), gestational hypertension (GHTN), oligohydramnios, birthweight, APGARs, and NICU length of stay (LOS).

**Results:** Of the 228 women included, 38 (16.6%) had an abnormal S/D ratio >90<sup>th</sup> percentile. Eleven of these 38 women (29.7%) women were diagnosed with IUGR vs 43 (22.9%) women with values <90<sup>th</sup> percentile (sensitivity 11/54, %). In comparison, seven of 23 (31%) women with UmbA values greater than the 95<sup>th</sup> percentile had IUGR. Secondary outcomes were also concerning in women with OUD and UmbA >90<sup>th</sup> percentile. This subpopulation was more likely to have GHTN (36.1% vs 12.2%), oligohydramnios (13.5% vs 3.7%), and deliver at lower gestational age (37.9 +/- 1.22 vs 38.4 +/- 1.3). Infants of women with elevated UmbA measurements had lower birthweights (2635 +/- 431 gms vs 2993 +/- 503 gms), birthweight percentile (23.4<sup>th</sup> vs 35<sup>th</sup> percentile), and higher rates of respiratory distress (36.8% vs 14.7%).

**Conclusion:** Women with OUD showed a high incidence of elevated UmbA measurements. Women with a cutoff >90<sup>th</sup> percentile were at increased risk of maternal and neonatal adverse events which suggests this value should alert practitioners of an increased risk for potential adverse outcomes.

Table 1: Demographics

	<90 <sup>th</sup> %tile (n=190)	>90 <sup>th</sup> %tile (n=38)	P-value
Age	29.37	30.32	0.277 <sup>a</sup>
BMI	27.59	28.88	0.263 <sup>a</sup>
<b>Race</b>			
Amer.Indian/Alaskan Native	3 (1.6%)	1 (2.6%)	0.520 <sup>b</sup>
White	187 (98.4%)	37 (97.4%)	
<b>Ethnicity</b>			
Hispanic/Latino	3 (1.6%)	0 (0.0%)	1.0 <sup>b</sup>
Non-Hispanic	187 (98.4%)	38 (100.0%)	
<b>Cigarette use</b>			
Current	169 (90.9%)	38 (100.0%)	0.244 <sup>b</sup>
Former	9 (4.8%)	0 (0.0%)	
Never	8 (4.3%)	0 (0.0%)	
OUD	183 (96.3%)	37 (100.0%)	0.602 <sup>b</sup>
Medicaid	181 (97.3%)	34 (100.0%)	1.0 <sup>b</sup>
High School Diploma	115 (80.4%)	19 (82.6%)	1.0 <sup>b</sup>
Full Time Employment	18 (11.8%)	0 (0.0%)	0.227 <sup>b</sup>
Married	21 (11.2%)	11 (28.9%)	0.004 <sup>b</sup>
Housed	184 (98.4%)	4 (10.5%)	0.017 <sup>b</sup>

a: Student's T-test, b: Fisher's Exact, c: chi-squared

Table 2: Maternal and Neonatal Outcomes

	<90 <sup>th</sup> %tile (n=190)	>90 <sup>th</sup> %tile (n=38)	P-value
<b>Mode of Delivery</b>			
Cesarean	74 (39.8%)	19 (54.3%)	0.111 <sup>c</sup>
Vaginal	112 (60.2%)	16 (45.7%)	
<b>Preterm labor</b>	16 (8.4%)	3 (8.1%)	1.0 <sup>b</sup>
PPROM	8 (4.2%)	0 (0.0%)	0.36 <sup>b</sup>
GHTN	23 (12.2%)	13 (36.1%)	0.0004 <sup>c</sup>
IUGR	43 (22.9%)	11 (29.7%)	0.372 <sup>c</sup>
<b>Oligohydramnios</b>	7 (3.7%)	5 (13.5%)	0.030 <sup>b</sup>
<b>Drug Screens</b>	138 (81.7%)	16 (66.7%)	0.087 <sup>c</sup>
Appropriate	31 (18.3%)	8 (33.3%)	
Inappropriate			
<b>Gestational age</b>	38.35	37.88	0.0437 <sup>a</sup>
<b>Meconium screen</b>			0.859 <sup>b</sup>
None	13 (7.1%)	2 (5.7%)	
Amphetamines	5 (2.7%)	1 (2.9%)	
Barbituates	2 (1.1%)	0 (0.0%)	
Benzodiazepines	1 (0.5%)	0 (0.0%)	
Buprenorphine	67 (36.8%)	9 (25.7%)	
Cannabis	2 (1.1%)	0 (0.0%)	
Cocaine	2 (1.1%)	0 (0.0%)	
Fentanyl	7 (3.8%)	3 (8.6%)	
Methadone	1 (0.5%)	0 (0.0%)	
Other Opiates	4 (2.2%)	1 (2.9%)	
Other	1 (0.5%)	0 (0.0%)	
Nonbuprenorphine	77 (42.3%)	19 (54.3%)	
<b>1 Minute APGAR</b>	8.14	7.76	0.09 <sup>a</sup>
<b>Respiratory distress</b>	28 (14.7%)	14 (36.8%)	0.0013 <sup>c</sup>
<b>Birthweight</b>	2993.73	2635.21	<0.0001 <sup>a</sup>
<b>Birthweight %ile</b>	34.95	23.42	0.0141 <sup>a</sup>
<b>Hospital LOS</b>	11.71	13.05	0.361 <sup>a</sup>
<b>NACU LOS</b>	7.08	6.82	0.856 <sup>a</sup>
<b>NICU LOS</b>	4.38	5.94	0.318 <sup>a</sup>

a: Student's T-test, b: Fisher's Exact, c: chi-squared

## T-085

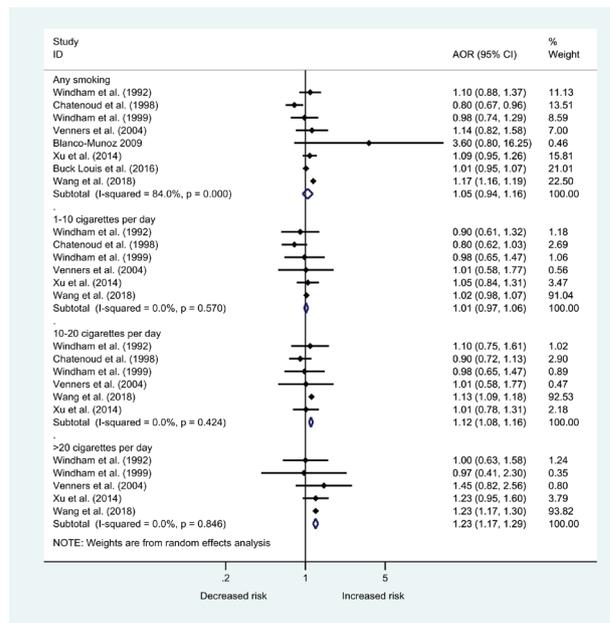
**Paternal Smoking Is Associated with an Increased Risk of Pregnancy Loss in a Dose-Dependent Manner: A Systematic Review and Meta-Analysis.** Nadia A. du Fossé†, Marie-Louise P. van der Hoorn\*, Nina H. Buisman†, Jan M.M. van Lith\*, Saskia le Cessie\*, Eileen E.L.O. Lashley\*. *Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** Although maternal lifestyle risk factors for pregnancy loss are well-established, studies on potentially contributing paternal factors remain sparse. Biological evidence indicates that smoking, excessive alcohol consumption and obesity may lead to sperm oxidative DNA damage, being a known risk factor for pregnancy loss. The aim of this systematic review and meta-analysis was to evaluate the association between paternal lifestyle factors in the preconception period and the risk of pregnancy loss.

**Methods:** PubMed and Embase databases were searched in August 2020. Paternal factors examined were: cigarette smoking, alcohol consumption and Body Mass Index (BMI). A qualitative risk of bias assessment was performed for all included studies. Meta-analysis was performed if sufficient data were available from studies that controlled for maternal factors. PRISMA guidelines for systematic reviews were followed.

**Results:** The systematic search included 3386 articles of which 11 articles met the inclusion criteria. In a meta-analysis (Figure 1) of eight studies, paternal smoking of >10 cigarettes per day in the preconception period was found to be associated with an increased risk of pregnancy loss, after adjustment for maternal smoking status (1-10 cigarettes per day: 1.01, 95% CI 0.97-1.06; 11-20 cigarettes per day: 1.12, 95% CI 1.08-1.16; >20 cigarettes per day: 1.23, 95% CI 1.17-1.29). Based on five available studies, no clear association was found between paternal alcohol consumption and pregnancy loss. No studies were retrieved that evaluated the association between paternal BMI and pregnancy loss.

**Conclusion:** Awareness of the association between paternal smoking in the preconception period and the risk of pregnancy loss should be raised. More well-designed studies are needed to further investigate the effects of paternal lifestyle factors, including obesity and alcohol consumption, on the risk of pregnancy loss.



## T-086

**Association of Abnormal Doppler Evaluation in Suspected Fetal Growth Restriction Near Term with Placental Pathology.** William M Curtin\*,<sup>1</sup> Kristi L Haedrich,<sup>2</sup> Emily O'Brien,<sup>1</sup> Laura H Brubaker,<sup>1</sup> Niamh A Condon,<sup>3</sup> Serdar H Ural,<sup>1</sup> Jaimie L Maines,<sup>1</sup> Karmaine A Millington.<sup>4</sup> <sup>1</sup>Penn State Health, Milton S. Hershey Medical Center, Penn State College of Medicine, Hershey, PA, United States; <sup>2</sup>Penn State Health Milton S. Hershey Medical Center, Hershey, PA, United States; <sup>3</sup>University of Florida Health, Jacksonville, FL, United States; <sup>4</sup>Northwell Health/Long Island Jewish Medical Center and Donald and Barbara Zucker School of Medicine at Hofstra University, New Hyde Park, NY, United States.

**Introduction:** We sought to determine if an abnormal Doppler evaluation at 36 weeks for suspected fetal growth restriction (FGR) was associated with a higher incidence of placental pathology in comparison to normal Doppler evaluation.

**Methods:** This prospective study enrolled pregnant women with singletons suspected of FGR on ultrasound (estimated fetal weight <10th percentile) from 2014-2019. Doppler evaluations were performed on the uterine, umbilical and middle cerebral (MCA) arteries at 36 weeks' gestation. An abnormal umbilical and uterine artery Doppler were defined as a pulsatility index (PI)>95th percentile. An abnormal MCA was defined as a PI and/or cerebroplacental ratio <5th percentile. One or more abnormal Doppler placed the pregnancy into the abnormal Doppler category. Delivery occurred at 37-39 weeks. Placentas with one or more pathologic features based on standard list guidelines as determined by a perinatal pathologist were classified as abnormal. The number of infants classified as small for gestational age (SGA), having a low (<2.2) ponderal index (PI) and experiencing one or more morbidities were compared between Doppler groups. Results were expressed in Odds ratios and 95% confidence intervals, significance was set at p<0.05.

**Results:** During the study period 65 women were recruited and 21 (32.3%) and 44 (67.7%) were in the normal and abnormal Doppler groups respectively. Overall 54 (82.1%) had an abnormal placenta. Having at least one abnormal Doppler from any category was significantly associated with having an abnormal placenta in comparison to having all normal Doppler. Infants in the abnormal Doppler category were significantly more likely to have a low PI, and experience at least one morbidity.

	Abnormal Doppler n= 44	Normal Doppler n=21	Odds Ratio (95%CI)	P value
Placental pathology	40 (91)	14 (67)	5.00 (1.29-19.70)	0.021
SGA	28 (64)	12 (57)	1.32 (0.46-3.8)	0.615
PI<2.2	16 (36)	2 (10)	5.43 (1.12-26.39)	0.024
Composite neonatal morbidity*	35 (80)	9 (43)	5.19 (1.67-16.10)	0.003

**Conclusion:** An abnormal Doppler evaluation in the fetus with suspected FGR was associated with a higher incidence of placental pathology, a lower PI and more neonatal morbidity in comparison to a normal Doppler evaluation. The prevalence of placental pathology was high and a normal Doppler does not rule out placental disease.

**T-087**

**Isolated Fetal Neural Tube Defects Associate with Increased Risk of Placental Pathology.** Marina White†,<sup>1</sup> David Grynspan,<sup>2</sup> Tim Van Mieghem,<sup>3</sup> Kristin L Connor\*.<sup>1</sup> <sup>1</sup>Carleton University, Ottawa, ON, Canada; <sup>2</sup>University of British Columbia, Vancouver, BC, Canada; <sup>3</sup>Mount Sinai Hospital, Toronto, ON, Canada.

**Introduction:** Neural tube defects (NTDs) are among the most common congenital anomalies and are associated with low birthweight and fetal growth restriction. Despite the placenta’s critical role in fetal growth, placental development is poorly defined for fetuses with NTDs. We hypothesised that infants with an isolated NTD (cases; spina bifida, anencephaly or encephalocele) would be at increased risk of placental pathology and suboptimal size at birth compared to those without any congenital anomalies (controls).

**Methods:** We performed a matched case-cohort study using data from the Collaborative Perinatal Project. Cases (n=74) and controls (n=148) were matched in a 1:2 ratio for maternal pre-pregnancy BMI, maternal race, infant sex, gestational age at birth and study site. Data were analysed using adjusted generalized linear and nominal logistic regression models. Results are presented as adjusted β or odds ratio (95% confidence interval).

**Results:** Irrespective of NTD subtype, case placentae had lower weight (-22.2 g [-37.8, -6.6]\*) and surface area (-9.6 cm<sup>2</sup> [-18.3, 1.0]\*), increased odds of having many Hofbauer cells (3.0 [1.2, 7.3]\*) and stromal fibrosis (2.7 [1.4, 7.3]\*) in the placental villi, and hypermaturity (6.8 [3.1, 14.7]\*) compared to controls. Cases had lower birth length z-scores (INTERGROWTH-21<sup>st</sup>; -0.4 [-0.7, -0.001]\*) than controls. In subgroup analysis of NTD subtypes, cases with spina bifida had increased odds of inflammation in the umbilical cord (15.6 [4.2, 58.1]\*) and fetal membranes (3.7 [1.3, 10.8]\*), placental hypermaturity (6.3 [1.6, 25.3]\*) and stromal fibrosis (3.9 [1.4, 10.8]\*) compared to controls. Cases with anencephaly had increased odds of placental hypermaturity (6.3 [1.6, 25.3]\*), many Hofbauer cells (5.0 [1.0, 24.7]\*), and pathological edema (5.0 [1.5, 16.1]\*) compared to controls. Placental hypermaturity was more prevalent in female than male cases (6.5 [1.1, 126]\*). Preterm cases had lower placental weight (-42.1 g [-67.8, -16.3]\*) and birthweight-to-placental weight ratio (-0.7 [-1.3, -0.1]\*) compared to preterm controls. Term case placentae were more likely to have many Hofbauer cells (7.8 [2.5, 24.7]\*), stromal fibrosis (3.0 [1.2, 7.5]\*) and placental hypermaturity (5.5 [2.3, 13.1]\*) than term controls.

**Conclusion:** Fetuses with isolated NTDs may be at increased risk of placental pathology, which could be contributing to suboptimal fetal growth in these pregnancies and subsequent postnatal morbidities.

**T-088**

**Factors Associated with Delivery within Seven Days of Presentation with Self-Limited Suspected Placental Abruption.** Rachel Anne Newman†,<sup>1</sup> Joshua Makhoul†,<sup>2</sup> Jenny Chang\*,<sup>2</sup> Dana Senderoff†,<sup>2</sup> B. Adam Crosland†,<sup>2</sup> Emily Seet\*,<sup>3</sup> Kenneth Chan\*.<sup>3</sup> <sup>1</sup>Cedars Sinai Medical Center, Los Angeles, CA, United States; <sup>2</sup>University of California, Irvine, Orange, CA, United States; <sup>3</sup>Long Beach Memorial Medical Center, Long Beach, CA, United States.

**Introduction:** Placental abruption is a spectrum of clinical significance, ranging from minor bleeding to massive hemorrhage. In the preterm period, there are no guidelines for how long stable patients with suspected abruption need to be admitted for observation and antenatal steroids, if at all.

**Methods:** This is a retrospective chart review performed at a community-academic tertiary care center. Three hundred and twenty-two consecutive charts associated with admission for vaginal bleeding during pregnancy between January 2015- May 2020 were reviewed. One hundred and twenty-six women were included based on singleton gestation, gestational age 24 0/7 - 36 6/7 weeks, self-limited bleeding, vital sign stability, and absence of labor/placenta previa/accreta. Patient demographic and clinical characteristics were compared using Fisher’s exact and two sample t-tests tests when appropriate. Univariate and multivariate logistic regression models were fitted to predict delivery within 7 days.

**Results:** Thirty-four percent of women who presented with mild vaginal bleeding delivered within seven days, with a mean of 2.6 days (n=44/126). Patients with mild vaginal bleeding and SVE >2 cm were 14 times more likely to deliver within 7 days than SVE ≤ 2 cm (AOR 14.49, 95% CI 3.33-63.03); however, 35.2% of women with SVE ≤ 2 cm still delivered in this timeframe (n=12/34). Of the 59 patients who had cervical lengths (CL) performed, those with CL ≤ 2.5 cm were 4.22 times more likely to deliver within 7 days (OR 4.22, 95% CI 1.10-16.20). Seventy-eight percent of the patients who had CL >2.5 cm and SVE 0-1 cm went on to deliver >14 days from their initial bleeds (n=18/23). Finally, patients with regular contractions on admission were 6.26 times more likely to deliver within seven days, yet this was not significant when adjusted for SVE or CL.

**Conclusion:** Patients who present with self-limited vaginal bleeding and SVE >2 cm, regular contractions, or CL ≤ 2.5 cm should be admitted for antenatal steroids and prolonged inpatient observation. The majority patients with CL >2.5 cm and SVE <2 cm delivered >14 days after their initial bleeds; therefore, there may be a subset of patients eligible for shorter observation. This is the first study to comment on factors associated with delivery in patients with “mild” placental abruption.

	Overall (n=126)	0-7 days (n=44, 34.9%)	>7 days (n=82, 65.1%)	p-value *
Gestational age (weeks)	Mean ± SD 31.1 ± 3.9	Mean ± SD 32.2 ± 3.9	Mean ± SD 30.6 ± 3.8	0.03
	N (Column %)	N (Row % <sup>a</sup> )	N (Row % <sup>a</sup> )	
Contracting on arrival				<0.01
No	95 (75.4)	23 (24.2)	72 (75.8)	
Yes	30 (23.8)	20 (66.7)	10 (33.3)	
Among n=91 who had SVE performed at admission				<0.01
SVE on admission (cm)				
0-1	65 (71.4)	12 (18.5)	53 (81.5)	
2-10	26 (28.6)	22 (84.6)	4 (15.4)	
Among n=59 who had cervical length performed				0.05
Cervical length (cm)				
≤2.5	12 (20.3)	6 (50.0)	6 (50.0)	
>2.5	47 (79.7)	9 (19.1)	38 (80.9)	

Abbreviation: SD, standard deviation; SVE, sterile vaginal exam  
 \* P values from two sample t test or chi-square test or Fisher’s exact test to test the difference between delivery within 7 days and more than 7 days  
<sup>a</sup> Row percentage can also be interpreted as absolute risk for the event

**T-089**

**Pregnancy and Delivery Outcomes in Solid Organ Transplant Recipients: A Modern Cohort.** Jenny Yang Mei†, Ophelia Yin†, Yalda Afshar\*. UCLA, Los Angeles, CA, United States.

**Introduction:** Pregnancy in kidney and liver transplant recipients incur transplant-related risks combined with maternal physiological changes and have been associated with high-risk pregnancies. We aimed to investigate obstetrical outcomes in these transplant recipients in a modern cohort.

**Methods:** A retrospective case-control study was performed of women with history of either kidney or liver transplant compared to a selected

group of control patients who delivered at a large academic referral center over eight years from 2012 through 2019. All previously transplanted patients who delivered a liveborn neonate during the study period were included. Controls were matched by maternal age, parity, and month and year of delivery. Patients with major maternal or fetal co-morbidities were excluded. Demographic and outcome data were chart abstracted. Independent sample t-test and one-way ANOVA was used to compare means across groups. Chi-square was used to analyze differences between groups.

**Results:** The transplant cohort had 37 pregnancy episodes with two sets of twins for total 39 neonates; a similar control group was matched. Average maternal age was 30 years; 80% were nulliparous. Transplant recipients were more likely to have hypertensive disease (56.8% vs 2.7%,  $p<0.001$ ) and lower gestational weight gain (23.8 vs 34.1 lbs,  $p=0.02$ ). Being a transplant recipient resulted in significantly increased risk of preterm delivery (16.2% vs 0%,  $p=0.011$ ), cesarean delivery (67.6% vs 29.7%,  $p=0.005$ ), antepartum admission (64.9% vs 10.8%,  $p<0.001$ ), preeclampsia (45.9% vs 5.4%,  $p<0.001$ ), daytime delivery (70.3% vs 45.9%,  $p=0.028$ ), delivery blood loss (657 vs 438mL,  $p=0.03$ ), and maternal length of stay (6.6 vs 2.6d,  $p<0.001$ ). Neonates of transplant recipients had higher rates of NICU admission (56.4% vs 7.7%,  $p<0.001$ ), lower birthweight (2483 vs 3249g,  $p<0.001$ ), lower 1 and 5-minute APGAR scores (7.4 vs 8.2, 8.4 vs 9.0, respectively;  $p<0.001$ ), longer length of stay (9.7 vs 2.5d,  $p<0.001$ ), and higher rates of neonatal morbidity (17.9% vs 0%,  $p=0.006$ ). In trimodal comparisons, there were differences in rates of hypertensive disease (70% renal (R), 52.6% liver (L), 5% control (C);  $p<0.001$ ), preeclampsia (45% R, 42.1% L, 5% C;  $p=0.009$ ), preterm labor (25% R, 5.3% L, 0% C;  $p=0.023$ ), and antepartum admission (65% R, 42.1% L, 5% C;  $p<0.001$ ).

**Conclusion:** Pregnancy in kidney or liver transplant recipients is associated with significant obstetrical complications, with greater risk noted in the kidney cohort. These are important to discuss with patients for informed decision making and appropriate antepartum and delivery management. IRB #18-000872

## T-090

**Advanced Maternal Age and Obstetric Outcome.** Anna Maria Marconi\*. *University of Milano, Milano, Italy.*

**Introduction:** Advanced maternal age (AMA) is defined as being  $\geq 35$  years at the time of delivery and is associated with a number of adverse perinatal outcomes. The last Europeristat report has shown that in 2015, Italy presented the second highest percentage (36.3%) of AMA mothers in Europe. Moreover, in Italy, mother's age at first birth is also, with Spain and Greece, the highest in Europe, being 30 or older. It has already been shown that AMA is an independent risk factor for cesarean delivery, however, the correlation between this increased risk and parity has not been fully explored. The present study was undertaken to evaluate the impact of AMA on the caesarean delivery (CD) rate according to the Robson classification.

**Methods:** We retrospectively evaluated the obstetric outcome of 30865 pregnancies who delivered in our Institution between January 1<sup>o</sup> 1996 and December 31<sup>o</sup> 2018, with maternal age and Robson group available. Data are presented as mean  $\pm$  SD. Differences among groups were assessed with the chi squared test;  $p<0.05$  was considered significant.

**Results:** Table 1 presents the maternal and obstetric characteristics. (GDM= gestational diabetes; GH= gestational hypertension; SB= stillbirth) In this cohort the overall CD rate was 17.4%. Figure 1 presents the CD rates, according to maternal age, in Robson groups 1, 3, 2A and 4A and shows that the rate increased with maternal age for all groups. However, AMA multiparous at term, either in spontaneous (group 3) or induced labor (group 4A) showed a significant ( $p<0.001$  vs all younger women) decreased risk of undergoing a cesarean delivery than a primigravida (group 1 and 2A) of the same age or even younger.

**Conclusion:** Compared to non AMA women, women at or beyond 35 years, experience significantly higher cesarean section rates only if nulliparous.

## T-091

**The Effects of Maternal In Utero Poly-Drug Exposure on Neonatal Abstinence Syndrome Outcomes.** Brooke Charlton Andrews†, Erin L. Macleod†, Zachary D. Stanley†, Brittany M McKinley†, Katia V Vela†, Katherine Vignes†, Cynthia Cockerham, Leon Su†, Arnold J Stromberg\*, John O'Brien\*. *University of Kentucky, Lexington, KY, United States.*

**Introduction:** Despite the successes of medication assisted therapy (MAT), continued illicit drug abuse in conjunction with MAT is still common. The objective of this study is to evaluate rates of poly-drug exposure in utero on neonatal outcomes and assess possible differences based on this profile.

**Methods:** A retrospective cohort study was conducted on all patients enrolled in the University of Kentucky's Perinatal Assessment and Treatment Home (PATHways) program that delivered between 2015-2020 with a UDS result at delivery. Patients with an early preterm birth, multiple gestation, or fetal anomaly were excluded.

**Results:** 205 patients were included: 57 (27.8%) had evidence of polysubstance use, with 13 (6.3%) testing positive for amphetamines or methamphetamines, 13 (6.3%) for cannabinoids, 19 (9.3%) for additional opioids, 7 (3.4%) for multiple additional substances, and 148 (72.2%) were positive only for prescribed MAT. Demographics between the MAT only group and the poly-drug group noted no significant differences between age, race, Medicaid insurance, educational status, employment status, marital status, or homelessness (Table 1). The gestational age at delivery was significantly earlier ( $37.9\pm 0.18$  vs  $38.5\pm 0.11$ ,  $P=.009$ ) in patients with poly-drug exposure at delivery; however, other obstetrical outcomes including birth weight, Apgar scores were not significantly different between groups. Both hospital length of stay (LOS), and NICU LOS were longer in the poly-drug exposure group, but not significantly. Sub-analyses of particular combinations of illicit substances showed no statistical significance in neonatal outcomes (Table 2).

**Conclusion:** Women with delivery UDS's positive for polysubstances deliver earlier than women with appropriate UDS's at delivery. Neonates also have longer hospital and NICU LOS. Although in utero exposure to polysubstances did not show differences in neonatal outcomes by type of substance of fetal exposure, our sample size is limited and further study is appropriate.

**Table 1. Demographics and fetal outcomes comparing urine drug screens appropriate at delivery to those positive for polysubstances.**

	Variable	Appropriate Delivery UDS (n=148)	Delivery UDS + for Polysubstances (n=57)	P Value
Demographics	Age at Enrollment	29.5 $\pm$ 0.42	29.8 $\pm$ 0.73	0.689 <sup>a</sup>
	White	146 (98.6%)	57 (100.0%)	1.000 <sup>b</sup>
	Medicaid	140 (97.2%)	51 (91.1%)	0.120 <sup>b</sup>
	High school diploma	80 (81.6%)	35 (81.4%)	0.973 <sup>c</sup>
	Not employed Full time	85 (88.5%)	43 (91.5%)	0.774 <sup>b</sup>
	Not Married	120 (81.6%)	50 (87.7%)	0.295 <sup>c</sup>
	Housed	146 (99.3%)	54 (94.7%)	0.067 <sup>b</sup>
	Rent/Own Apt/Room/House	69 (46.9%)	22 (38.6%)	0.282 <sup>c</sup>
Fetal Outcomes	Gestational age (weeks)	38.5 $\pm$ 0.11	37.9 $\pm$ 0.18	0.009 <sup>a</sup>
	APGAR at 5 minutes	8.7 $\pm$ 0.06	8.6 $\pm$ 0.11	0.374 <sup>a</sup>
	APGAR at 1 minute	8.2 $\pm$ 0.1	7.95 $\pm$ 0.2	0.198 <sup>a</sup>
	Average Birthweight (grams)	3000.8 $\pm$ 37.3	2956.8 $\pm$ 62.9	0.540 <sup>a</sup>
	Birthweight %tile	34.9 $\pm$ 2.04	39.3 $\pm$ 3.3	0.254 <sup>a</sup>
	Hospital LOS (days)	12 $\pm$ 0.73	14 $\pm$ 1.22	0.158 <sup>a</sup>
	NICU LOS (days)	5.7 $\pm$ 0.91	7.5 $\pm$ 1.51	0.287 <sup>a</sup>

<sup>a</sup> Student's T-Test, <sup>b</sup>Fischer's Exact test, <sup>c</sup> chi squared

Delivery UDS result by substance:	Gestational Age (Weeks)	APGAR @ 1 minute	APGAR @ 5 minutes	Birthweight (grams)	Hospital LOS (days)	NICU LOS (days)
MAT+ amphetamines (n=13)	37.7 ± 0.3	7.6 ± 0.3	8.7 ± 0.2	3025.5 ± 138.8	17 ± 3	10 ± 4
MAT+ cannabis (n=13)	38.1 ± 0.4	8.0 ± 0.6	8.5 ± 0.3	2995.3 ± 163.4	14 ± 3	8 ± 4
MAT+ more than one illicit substance (n=7)	37.6 ± 0.2	7.7 ± 0.8	8.4 ± 0.4	2924.3 ± 105.3	20 ± 3	4 ± 2
MAT only (n=138)	38.5 ± 0.1	8.2 ± 0.1	8.7 ± 0.1	3007.4 ± 38.3	12 ± 1	6 ± 1
MAT+ opioids (n=19)	37.7 ± 0.4	8.4 ± 0.2	8.8 ± 0.2	2857.1 ± 110.3	11 ± 2	7 ± 2
P Value	0.0225	0.4292	0.7609	0.7386	0.1001	0.5663

Table 2. Fetal outcomes by delivery UDS results by substance combinations.

T-092

**Performance of Urinalysis as a Screen for Urinary Tract Infection in Symptomatic Patients Presenting to Triage.** Amanda M Wang†, Sara Jacobs, George Saade, Antonio F Saad. *University of Texas Medical Branch, Galveston, TX, United States.*

**Introduction:** Urinalysis (UA) is routinely performed to screen for urinary tract infection (UTI) in triage, particularly in women presenting with preterm contractions or symptoms of UTI. Our objective was to evaluate the performance of UA as a screen for UTI in these patients.

**Methods:** Retrospective medical record data review for 932 women with singleton pregnancies who presented with preterm contractions or UTI symptoms between January 2017-December 2019.

Urinalysis component	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
Nitrite positive	19.6	99.6	85.7	91.9	54.8	0.81
1+ blood	23.91	87.41	17.2	91.3	1.26	0.77
2+ blood	14.1	95.0	23.6	91.0	2.83	0.90
3+ blood	5.4	96.8	15.6	90.3	1.69	0.98
LE >250 + nitrite positive	13.0	99.8	85.7	91.3	54.78	0.87
LE, nitrite, and blood positive	2.2	100	100	90.3	-	0.93
LE >250, positive nitrite, 1+ blood	10.9	99.9	90	91.1	91.3	0.89

UA components measured using automated chemical reactions in an in-house laboratory. Blood was measured on a scale from 0 to 3+ in increments of 1+. LE is measured to a maximum of >500. Nitrites are reported as either positive or negative.

The performance parameters of UA components to predict UTI in urine culture were calculated. Primary outcome was UTI. Secondary outcomes included preterm delivery, maternal and neonatal outcomes.

**Results:** 92 were initially treated for UTI, and 59% were treated after an asymptomatic bacteriuria diagnosis. Of those treated based on UA, 33% were found to have a confirmed UTI on urine culture. Among UA components, positive nitrite was the most specific (99.6%), however the most sensitive component was presence of blood (43.5%). Positive nitrites, 3+ blood, or any amount of all three components were also found to be very specific (99.6, 96.8, 99.9%).

Table 1: Characteristics of cohort

Characteristic	No UTI (n=840)	UTI* (n=92)
Age	26 (14-46)	27 (18-46)
Gravida	3 (1-22)	2 (1-13)
Gestational age at triage visit	34 (11-37)	32 (10-37)
Gestational age at delivery	38 (11-41)	37 (24-40)
Ethnicity		
Hispanic	468 (56)	60 (65)
Non-Hispanic	372 (44)	32 (35)
Race		
White	679 (79)	77 (83)
Asian	20 (2)	1 (1)
African American	148 (18)	12 (13)
Other	3 (0.4)	2 (2)

\* Defined by urine culture with >100,000 colonies by clean catch method or 1000 colonies by catheterization method of uropathogenic bacteria

Data is shown as median (range) or n (%)

Leukocyte esterase >250 was predictive of delivery before 34 weeks (sensitivity 58.3%, specificity 82.4%) and 2-3+ blood was predictive of delivery before 34 weeks (sensitivity 20.8%, specificity 91.3%). UTI was associated with increase in neonatal intracranial or subgaleal hemorrhage (RR: 4.56; 95%CI 1.16-17.95), and neonatal hypotension (RR: 5.48; 95%CI 1.33-22.56).

**Conclusion:** The presence of >250 leukocyte esterase and/or nitrites in UA are best predictors of UTI. UTI on presentation to triage is a risk factor for neonatal hypotension and intracranial hemorrhage despite treatment.

T-093

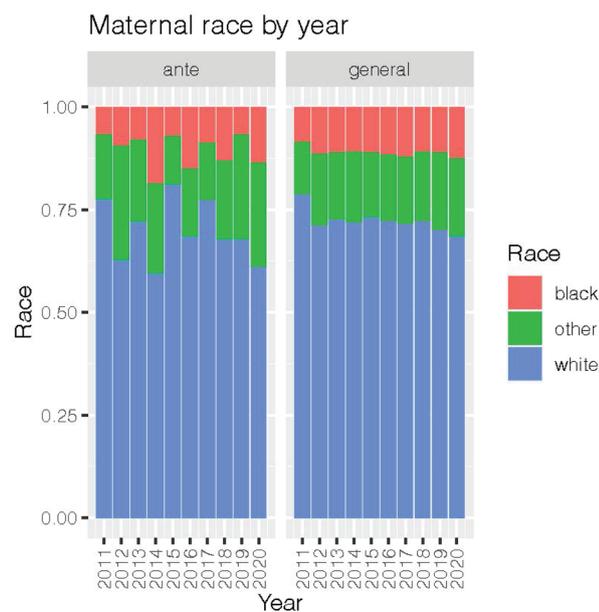
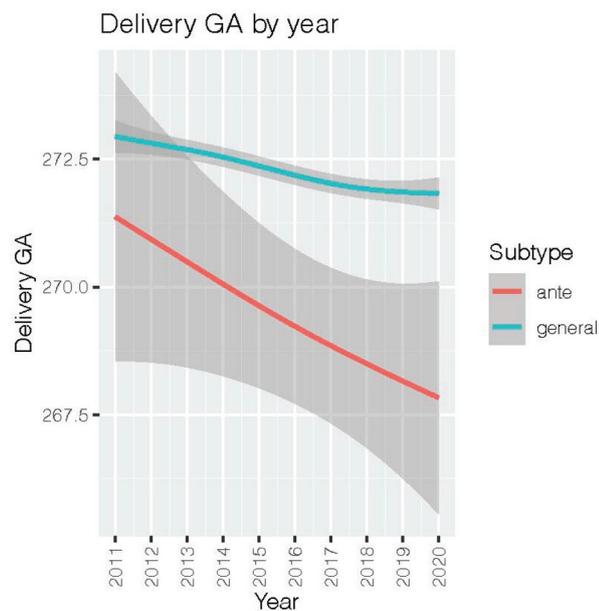
**Changes in the Antepartum Population Over Time.** Anna J Rujan†, Ashley Hesson\*, Deborah R Berman\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** The characteristics of the modern antepartum population are under researched. Existing data from the 1990s indicate that antepartum patients are more likely to be young, black, and without private insurance, but the modern population has not been studied. Furthermore, this population makeup may be quite dynamic as maternal interventions, neonatal resuscitative capabilities, and fetal therapies continue to progress, changing the limits of viability, interventional thresholds, and indications for long-term admissions. We characterize the antepartum hospitalizations of a large tertiary care center and report trends in the composition of the antepartum population over a nine-year retrospective period.

**Methods:** All births occurring at a single center between the years 2011 and 2019 were identified, as were patients admitted for at least 96 hours to the antepartum service. Chart review was conducted to obtain demographic, pregnancy, and neonatal outcome information. Descriptive statistics, Student T tests, Chi-squared tests, linear modeling, and Pearson product-moment correlations were used as appropriate to characterize underlying differences between the antepartum and general obstetric populations and changes in this relationship over time.

**Results:** When evaluated comprehensively over the study period, the antepartum group was younger (29.5 vs 30.5 years, P<0.01), delivered earlier (269 vs 272 days, P<0.01), and had more prior deliveries (0.61 vs 0.50 gestations, P<0.01) than the general obstetric population. The distribution of patient race between the antepartum group and the general group was similar (P=0.18) and the proportion of non-white patients has not significantly increased over the study period as a whole (P=0.28). Notably however, the proportion of non-white patients is increasing at a greater rate in the antepartum population than in the general population over the last three years (beta=0.046 vs 0.011).

**Conclusion:** The antepartum population is an at-risk population at baseline. This analysis suggests they are becoming even more vulnerable as gestational age at delivery continues to decrease and race disparities (percent of non-white antepartum patients) are trending upwards.



#### T-094

**Racial and Ethnic Disparities in Mode of Delivery during Labor Induction.** [Christina Ackerman](#)<sup>†</sup>,<sup>1</sup> Masaru Negi,<sup>2</sup> Uma Reddy,<sup>1</sup> Lisbet Lundsberg,<sup>1</sup> Audrey Merriam,<sup>1</sup> Jessica Greenberg,<sup>1</sup> Sarah Meller,<sup>1</sup> Anna Sfakianaki\*,<sup>3</sup> <sup>1</sup>*Yale New Haven Hospital, New Haven, CT, United States;* <sup>2</sup>*UCLA, Los Angeles, CA, United States;* <sup>3</sup>*University of Miami, Miami, FL, United States.*

**Introduction:** We sought to examine whether racial and ethnic differences were associated with mode of delivery for women undergoing an induction of labor.

**Methods:** We conducted a secondary analysis of a retrospective cohort study of all women for whom a labor induction was performed at Yale-New Haven Hospital from February 1, 2013 to August 1, 2014. Data including maternal age, race/ethnicity, body mass index (BMI), history of cesarean delivery, indication and length of induction, gestational age, and maternal morbidities such as diabetes and hypertension were abstracted from electronic medical records. The primary outcome was

mode of delivery. Adjusted logistic regression analyses were performed to determine the association between the probability of cesarean delivery during an induction of labor and race/ethnicity.

**Results:** 1002 patient records were reviewed and 176 patients were excluded since they only received cervical ripening. Among 826 patients, 24.4% underwent cesarean delivery. Cesarean delivery was significantly more common among Asian, Hispanic and non-Hispanic Black women than among non-Hispanic White women; this association persisted for all racial/ethnic groups even after adjustment for other sociodemographic variables and maternal characteristics, including BMI, parity, bishop score, use of epidural analgesia, use of maternal antibiotics, length of induction, prior vaginal delivery, any hypertensive or diabetes diagnosis, gestational age, and medical insurance status. Non-Hispanic black and Asian women have the highest increased odds of a cesarean delivery during induction of labor compared to non-Hispanic white women with adjusted OR 2.65 (95% CI 1.54-4.56) and aOR 2.69 (95% 1.32-5.48), respectively.

**Conclusion:** All non-white racial/ethnic categories were found to have a 2-3 fold increased risk of cesarean delivery during induction of labor. The underlying reasons for this finding should be further examined in order to optimize maternal health by decreasing the morbidity associated with cesarean delivery and to eliminate the prevalent racial disparities in obstetric care.

#### T-095

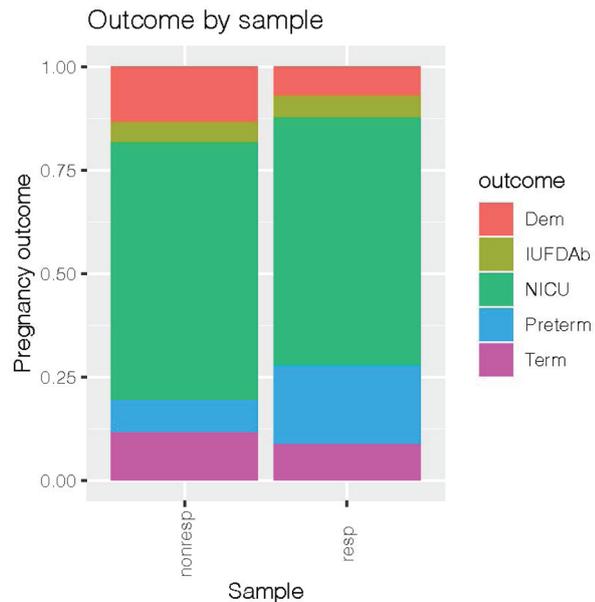
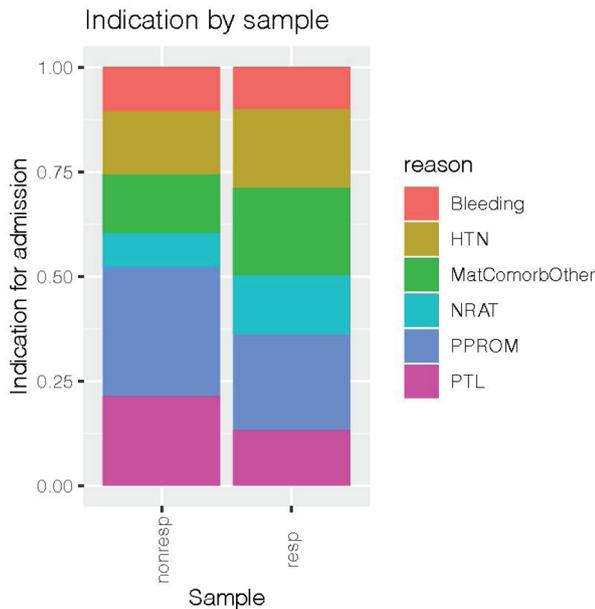
**A Characterization of a Modern Antepartum Inpatient Unit.** [Anna J Rujan](#)<sup>†</sup>, Ashley Hesson\*, Deborah R Berman\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** Prior cohort studies suggest the population of women requiring extended antepartum hospitalization is more likely to be young, black, and without private insurance, thus pointing to potential disparities in the burden of long-term inpatient management. The present study incorporates demographic and outcomes data along with a subset of survey responses to generate a nine-year retrospective snapshot of the modern antepartum population at a large university hospital.

**Methods:** A survey-based, longitudinal cohort study evaluated the antepartum inpatient experience by surveying women, admitted for at least 96 hours to the antepartum service between 2011 and 2019, on demographics, pregnancy outcomes, provider interactions, perceived needs, resource use, and satisfaction. A chart review was conducted on non-responders. Descriptive statistics, Student T tests, and Chi-squared tests were used to characterize underlying differences between the antepartum/general obstetric population, and the survey responder/non-responder groups.

**Results:** Between 2011 and 2020 there were 68,010 deliveries. Of these, 504 (0.74%) were born to antepartum patients. The antepartum group delivered earlier (269 vs 272 days,  $P < 0.01$ ), was younger (29.5 vs 30.5 years,  $P < 0.01$ ), and had more prior deliveries (0.61 vs 0.50,  $P < 0.01$ ) than the general obstetric population. Within the antepartum group, survey responders ( $N = 297$ , 58.9%) and non-responders ( $N = 207$ , 41.1%) were similar in maternal age (30.7 vs 31.2 years,  $P = 0.82$ ), race ( $P = 0.17$ ), gestational age (224.9 vs 225.4 days,  $P = 0.88$ ), length of stay (14.8 vs 16.7 days,  $P = 0.14$ ), and parity (1.03 vs 2.78 gestations,  $P = 0.06$ ). Responders are more likely to be married ( $P < 0.01$ ). Non-responders are more likely to be admitted for preterm labor or non-reassuring antenatal testing ( $P < 0.01$ ). Responders are more likely to deliver prematurely without requiring NICU care and are less likely to have a neonatal demise ( $P < 0.01$ ).

**Conclusion:** Antepartum patients are more likely to be multiparous and younger compared to the general obstetric population. Surveys of this population may underrepresent unmarried individuals and those experiencing very premature deliveries and/or neonatal demises.



### T-096

**A History of Spontaneous Preterm Birth Does Not Increase Cardiovascular Risk among Women in the Fifth Decade of Life.** [Laura E Janssen](#)<sup>†</sup>, [Marjon A de Boer](#)<sup>\*</sup>, [Eline C.E von Königsłow](#)<sup>†</sup>, [Martijn A Oudijk](#)<sup>\*</sup>, [Christianne J.M de Groot](#)<sup>\*,1,2</sup> <sup>1</sup>Amsterdam UMC, VU Medical Center, Amsterdam, Netherlands; <sup>2</sup>Amsterdam UMC, Amsterdam Medical Center, Amsterdam, Netherlands.

**Introduction:** Cardiovascular disease (CVD) is the number one cause of death among women and defining cardiovascular risk (CVR) factors is necessary to reduce its prevalence. A history of preeclampsia or preterm birth has been correlated with increased risk of maternal cardiovascular disease later in life. However, whether a history of spontaneous preterm birth (SPTB), about two thirds of all preterm births, also predisposes to higher CVR is unknown.

**Methods:** We prospectively included 350 women with a history of SPTB between 22 and 37 weeks (cases) and matched them with 166 women with a history of a term birth (controls). In both groups women with pregnancy

complications that are known to be associated with CVD, mainly hypertensive disorders of pregnancy and gestational diabetes in any of their pregnancies, were excluded. Both groups underwent cardiovascular risk assessment 9 to 16 years after pregnancy. We performed a subgroup analysis based upon the severity of SPTB defined as extreme preterm (GA 22+0 - 27+6 weeks), very preterm (GA 28+0 - 31+6 weeks) and moderate preterm (GA 32+0 - 36+6 weeks) and compared spontaneous preterm delivery of a girl with spontaneous preterm delivery of a boy.

**Results:** We found no significant differences in hypertension, blood and urine fasting lipids, Framingham Risk Score (FRS) and Systematic Coronary Risk Evaluation (SCORE). Women with a history of SPTB only had significantly higher diastolic pressure ( $74.6 \pm 9.7$  mmHg vs.  $72.5 \pm 9.7$  mmHg,  $p=.023$ ) and were significantly more often diagnosed with abdominal obesity ( $n=163$ , 46.6% vs.  $n=54$ , 32.5%,  $p=.003$ ). Abdominal obesity was more pronounced with more severe preterm birth. The number of boys was higher in the cases. With cases preterm birth of a boy was associated with a lower CVR compared to women who had a preterm birth of a girl.

**Conclusion:** Cardiovascular risk was comparable between women with a history of SPTB compared to women with a history of uncomplicated term birth 9 to 16 years after pregnancy, in this prospective study in which women with other complications of pregnancy were excluded.

### T-097

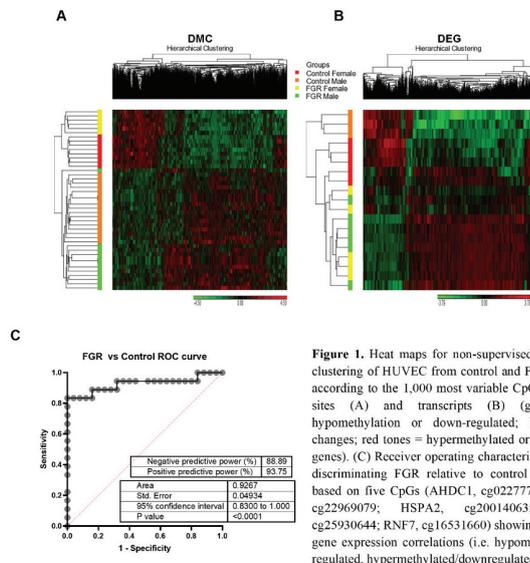
**Transcriptomic and Epigenomic Sex Dimorphisms in Endothelial Cells Converge in a Prediction Model of Vascular Aging in Fetal Growth Restriction.** [Bernardo J. Krause](#)<sup>\*</sup>, [Estefania Peñaloza](#)<sup>†</sup>, [Titia Lely](#)<sup>\*,2</sup>, [Fieke Terstappen](#)<sup>\*,2</sup> <sup>1</sup>Universidad de O'Higgins, Rancagua, Chile; <sup>2</sup>University Medical Center Utrecht, Utrecht, Netherlands.

**Introduction:** Fetal growth restriction (FGR) results in sex-specific epigenomic changes in circulating stem cells, suggesting sexual dimorphism in responses to adverse intrauterine conditions. Conversely, gene-specific epigenetic changes in the umbilical endothelium have been related with vascular dysfunction following FGR. However, scarce studies have addressed if these epigenetic changes in endothelial cells involve coordinated programming of genes related with cardiovascular risk later in life, and if these changes occur in a sex-specific manner.

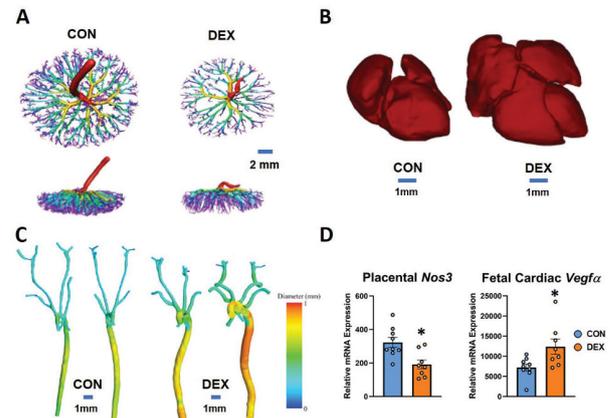
**Methods:** DNA methylation profiling of control ( $n = 25$ ) and FGR ( $n = 18$ ) HUVEC were derived from 450K Illumina array GEO datasets (GSE103253). Transcriptome data was derived from Terstappen et al., (Clin Epigenetics 2020) (control = 8, FGR = 11). Biomarkers of aging were evaluated by comparing CpG probes and transcripts previously described. Analyses were performed considering cut-off values of  $p < 0.05$  and  $FDR < 0.1$  for differentially methylated CpG sites (DMC) and differentially expressed genes (DEG), using sex and FGR as factors. Highly concordant DMC and DEG, based on an inverse correlation of transcripts vs. methylation levels, were used to identify markers of endothelial programming by multivariable logistic analysis.

**Results:** Few DMC were found in females (164 CpG) and males (84 CpG), but comparison of top 1,000 sites (non-adjusted  $p < 0.01$ ) resulted in FGR- and sex-specific clustering (Figure 1A). Top-1,000 DEG resulted in FGR-specific, but not sex-specific clustering (Figure 1B). FGR samples presented epigenetic (12 DMC) and transcriptional (8 DEG) biomarkers of aging compared to controls. Conversely, five DMC selected according to their inverse relation with DEG, allowed to discriminate between FGR and control (AUC, 0.93, Figure 1C), and this model was improved when sex-specific DMC were selected.

**Conclusion:** Vascular dysfunction related with FGR may result from accelerated aging, as shown at epigenomic and transcriptional level in the umbilical endothelium. Furthermore, this profile allows to discriminate between growth restricted or normal growth intrauterine conditions, in a sex-specific manner. Additional studies are required to address the applicability of these markers to diagnose postnatally when impaired fetal growth has been missed during pregnancy. Funded by Fondecyt 1181341.



**Figure 1.** Heat maps for non-supervised hierarchical clustering of HUVEC from control and FGR neonates according to the 1,000 most variable CpG methylated sites (A) and transcripts (B) (green tones, hypomethylation or down-regulated; black = no changes; red tones = hypermethylated or up-regulated genes). (C) Receiver operating characteristic curve for discriminating FGR relative to control endothelium based on five CpGs (AHD1, cg02277710; ARRB1, cg22969079; HSPA2, cg20014063; MYH10, cg25930644; KNF7, cg16531660) showing concordant gene expression correlations (i.e. hypomethylated/up-regulated, hypermethylated/down-regulated).



**Figure 1.** Representative images of reconstructed casts of the (A) placenta (B) fetal heart and (C) fetal aorta from control (CON) and dexamethasone (DEX) exposed pregnancies, with corresponding gene expression profiles (D) in placental and fetal cardiac tissues. Gene expression data are the mean  $\pm$  SEM, n=8-9 per group. \* $P$ <0.05 CON vs. DEX comparison; post-hoc LSD comparison following significant treatment effect in two-way ANOVA. No sex effect (three-way ANOVA) so male and female values are combined for n=1 per litter.

## T-098

**Exploring the Fetal-Placental Vascular Axis in Growth-Restricted Pregnancy: New Perspectives on an Established Model.** Rachael C Crew<sup>†</sup>,<sup>1</sup> Yutthapong Tongpob,<sup>1,2</sup> Nikhilesh Bappoo,<sup>3</sup> Georgia Khinsoe,<sup>3</sup> Barry Doyle,<sup>3</sup> Caitlin S Wyrwoll\*.<sup>1</sup> <sup>1</sup>The University of Western Australia, Perth, Australia; <sup>2</sup>Naresuan University, Bangkok, Thailand; <sup>3</sup>Harry Perkins Institute of Medical Research, Perth, Australia.

**Introduction:** Since the developing fetal heart beats directly against placental vascular bed resistance, optimal placental vascular function is likely vital for healthy fetal cardiovascular development. Fetal growth restriction (FGR) is strongly associated with diminished placental vasculature, but the exact mechanisms involved and specific implications for the fetal cardiovascular system are unknown. Here, we use a rat model of glucocorticoid excess to examine the fetoplacental vasculature in FGR, using a novel, integrative approach encompassing structural and molecular analysis techniques.

**Methods:** Pregnant Wistar rats were administered dexamethasone acetate (DEX) from embryonic day (E)13 to E21 (term is E22). 3D fetoplacental vascular casts were obtained via perfusion of radiopaque contrast medium and assessed with  $\mu$ -CT scans. Expression of angiogenic markers and mechanoreceptors involved in endothelial sensing of blood flow were quantified in the placenta and fetal heart by RT-qPCR.

**Results:** DEX animals exhibited fetal (20%;  $P$ <0.001) and placental (34%;  $P$ <0.01) growth restriction by E21. 3D casts showed that the placental arterial network volume was 50% lower in DEX ( $P$ <0.01); attributed to decreased arterial length (45%;  $P$ <0.01) and branching (58%;  $P$ <0.05) (Fig 1A). This was accompanied by a 40% reduction in *Nos3* expression ( $P$ <0.05) and trends for increased expression of the mechanoreceptor *Piezo1* ( $P$ =0.053) but reduced *Itgb1* ( $P$ =0.055) in DEX placentas. DEX treatment increased fetal heart volume relative to body weight by 50% ( $P$ <0.01; Fig 1B), and substantially increased the volume and diameter of the fetal aorta (Fig 1C). This corresponded to elevated fetal cardiac *Vegfa* (68%;  $P$ <0.05; Fig 1D) but no change to cardiac mechanoreceptor expression.

**Conclusion:** While placental vascular structure was severely compromised by DEX, fetal cardiac sparing occurred, potentially mediated via *Vegfa*. Further, placental nitric oxide sensitivity may contribute to vascular disturbances in this model. Ultrasound analysis and computational modelling of blood flow are currently underway to define the fetoplacental haemodynamic environment. This may drive the development of more accurate diagnostic criteria for at-risk pregnancies to prevent adverse fetal cardiovascular development and corresponding health complications in offspring.

## T-099

**Diastolic Left Ventricular Dysfunction on the Fifth Decade of Life in Women That Had a Spontaneous Preterm Birth: A Prospective Study.** Laura E Janssen<sup>†</sup>,<sup>1</sup> Marjon A Boer\*,<sup>1</sup> Eline C.E von Königslöw<sup>†</sup>,<sup>1</sup> Elisa Dal Canto<sup>†</sup>,<sup>2</sup> Martijn A Oudijk\*,<sup>3</sup> Walter J Paulus\*,<sup>2</sup> Christianne J.M de Groot\*,<sup>1,3</sup> <sup>1</sup>Amsterdam UMC, VU Medical Center, Amsterdam, Netherlands; <sup>2</sup>Amsterdam Vascular Sciences, Amsterdam, Netherlands; <sup>3</sup>Amsterdam UMC, Amsterdam Medical Center, Amsterdam, Netherlands.

**Introduction:** Cardiovascular disease (CVD) is the number one cause of death among women. Defining risk factors among women that predispose for CVD are necessary to reduce the prevalence of CVD. Heart failure with preserved ejection fraction (HFpEF) is especially prevalent in elderly women and preceded in middle age by preclinical left ventricular (LV) diastolic dysfunction. It has been shown that a history of preeclampsia (PE) is associated with preclinical left ventricular diastolic dysfunction. There is an overlap in mechanism of disease between PE and spontaneous preterm birth (SPTB) in terms of vascular disorders found in the histology in the placenta. Therefore, we aimed to determine whether SPTB, one of the most prevalent complications in pregnancy, also predisposes to HFpEF.

**Methods:** Cases were women with a history of SPTB, who were matched to a control group consisting of women with uncomplicated term pregnancies. In both groups women with other pregnancy complications in any pregnancy, including hypertensive disorders and gestational diabetes, were excluded. Both groups underwent echocardiographic investigation 9 to 16 years after pregnancy. We performed a subgroup analysis for the echocardiographic findings based upon the severity of SPTB defined as extreme preterm (GA 22+0 - 27+6 weeks), very preterm (GA 28+0 - 31+6 weeks) and moderate preterm (GA 32+0 - 36+6 weeks).

**Results:** A total of 94 cases and 94 controls were included, on average 13 years after the index pregnancy. Echocardiographic measures of LV diastolic dysfunction did not differ between the groups. Diastolic dysfunction was diagnosed in 2.1% of the cases and 4.3% of the controls ( $p$ =.682). No differences in the subgroup analyses were found.

**Conclusion:** This study is the first that investigated LV diastolic dysfunction in women with a SPTB as an early parameter of CVD. We conclude that women with a history of SPTB have no increased risk on LV diastolic dysfunction later in life. Therefore, the increased risk of CVD after SPTB may result from other mechanisms in vascular injury.

**T-100**

**Oxidative Stress Mediates the Potentiation of Adipogenesis by Exposure to the Bisphenol A (BPA) Analogue, BPS.** Radha Dutt Singh†, Anna Mikolajczak†, Sarah Easson†, Liam Connor†, Jennifer Thompson\*. *University of Calgary, Calgary, Alberta, Canada, Calgary, AB, Canada.*

**Introduction:** Bisphenol S (BPS) is a synthetic plasticizer commonly used to substitute BPA in “BPA-free” products. Bisphenol exposure is ubiquitous and continuous, with detectable levels reported in 70-92% of adult urine samples. While developmental effects of BPA have been well-studied, less is known regarding its analogues, which have been marketed as safer alternatives. Our data show that BPS mimics the pro-adipogenic effects of BPA. Herein, we determined the role of oxidative stress in mediating the BPS-induced increase in adipogenesis in adipocyte progenitors.

**Methods:** The stromal vascular fraction (SVF) was isolated from inguinal SAT (iSAT) of male C57BL6 mice. Prior to differentiation, adipocyte progenitors of the SVF were cultured and treated with vehicle or variable doses of BPS [2.5nM, 250nM (permissible limit), 2.5µM and 25µM] with or without inhibitors of reactive oxygen species (ROS). Separate cells were treated with ROS generators, DMNQ (2,3-dimethoxy-1,4-naphthalenedione) or menadione. Differentiation capacity was determined by staining lipid droplets with Oil-Red O or BODIPY and by measuring protein or mRNA expression of adipogenic mediators. After treatment with BPS or ROS generators, ROS production was measured with DCFDA, MitoSOX and CellROX. Differences between groups were determined by One-way ANOVA.

**Results:** Staining of ROS was increased with increasing concentrations of BPS (all  $p < 0.01$ ). Reduction of ROS via Tempol (ROS scavenger), MitoQ (mitochondria-targeted antioxidant) or Apocynin (inhibitor of NADPH oxidase) abolished the effect of BPS on adipogenesis at all doses (all  $p < 0.01$ ). Increased ROS production in progenitors by treatment with DMNQ (0.1, 1 and 10 µM dose) or menadione (0.1, 1 and 10 µM dose), resulted in a non-monotonic adipogenic response (both  $p < 0.01$ ), similar to that of BPS-induced adipogenesis.

**Conclusion:** Physiological levels of ROS augment differentiation of adipocyte progenitors. BPS exposure increases ROS production, while scavenging ROS abolishes the potentiation of adipogenesis by BPS. Thus, BPS-induced ROS mediates the effect of BPS on adipogenesis. Current studies are underway to determine if changes in the methylation status of adipogenic regulators are responsible for the effect of BPS-induced ROS on adipogenesis.

**T-101**

**Maternal Food Restriction Programs Neonatal Cerebrovascular, Neurobehavioral and Glucocorticoid Responses to Mild Hypoxic-Ischemic Injury.** Naomi Franco†, Lara M Durrant, Coleen Doan, Alejandra Beltran†, William J Pearce\*. *Loma Linda University, Loma Linda, CA, United States.*

**Introduction:** Prenatal undernutrition alters adult cerebrovasculature. How these effects are manifested in the neonatal cerebral circulation, however, remain unstudied. This study explored the hypothesis that prenatal maternal food restriction (MFR) programs the neonatal cerebrovasculature and thereby alters vulnerability to mild hypoxic-ischemic (HI) injury. This study also examined the corollary hypothesis that altered corticosteroids (CSs) help mediate the effects of MFR on the immature cerebrovasculature.

**Methods:** At day 10 of gestation, pair-fed Sprague-Dawley rats experienced 50% caloric restriction. Metyrapone (MET), a CS synthesis inhibitor, was given via drinking water from prenatal day 11 to term. To test cerebrovascular function, we employed a model of mild HI injury in P9 pups. These pups underwent unilateral carotid ligation or sham surgery, followed 24h later by 8% or 21% O<sub>2</sub> for 90 min in a Bell jar. These procedures yielded 4 groups of MFR neonates: 1) Sham-Control; 2) Sham-MET; 3) HI-Control; 4) HI-MET. Plasma corticosterone (GC) levels were measured just before surgery, 2h after surgery, and at 2h and 24h after Bell jar exposure. Behavioral measures of the Negative Geotaxis Reflex, Open Field assessment, and the Righting Reflex also were collected 24h

after Bell jar exposure. At this time point, middle cerebral arteries (MCA) were harvested for vessel myography studies that provided measurements of compliance and pressure-dependent contractility.

**Results:** In Control pups 2h after hypoxia, HI increased GC levels in females only. In MET pups 2h after hypoxia, HI had no significant effect on GC levels in either sex. MET increased passive diameters in both Sham and HI pups. In Control pups 24h after hypoxia, HI had no significant effects on contractility. In contrast in MET pups, HI significantly increased the magnitude of K<sup>+</sup>-induced decreases in diameter, possibly due to a parallel significant increase in myofilament Ca<sup>2+</sup> sensitivity. In Control pups 24h after hypoxia, HI had no significant effects on neurobehavior, but in MET pups, HI significantly worsened Negative Geotaxis times. In addition, relative to HI-Control pups, HI-MET pups exhibited worsened Negative Geotaxis times and Open Field Exploration. Statistical significance implies  $P < 0.05$  (ANOVA).

**Conclusion:** In MFR neonates, mild HI increased GC levels only in females, but did not alter other endpoints significantly, implying successful neonatal adaptation to undernutrition. This adaptation appeared to require CSs, because MET reduction of CS levels produced detrimental changes in MCA structure and function, along with worsened neurobehavior. These results show that CSs are essential for homeostatic adaptation to mild HI, particularly in the immature cerebrovasculature.

**T-102**

**Specific Changes in 3rd Trimester Maternal Fatty Acids Correlate with Maternal % Body Fat, Cytokines, and Neonatal Adiposity.** Stephanie Pierce,<sup>1</sup> David Fields,<sup>1</sup> Martin-Paul Agbaga,<sup>2</sup> Ravindu Gunatilake,<sup>3</sup> Jacob Friedman,<sup>1</sup> Dean Myers\*. <sup>1</sup> *Univ. of Oklahoma HSC, OKC, OK, United States;* <sup>2</sup> *D. McGee Eye Inst., OKC, OK, United States;* <sup>3</sup> *Valley Perinatal, Phoenix, AZ, United States.*

**Introduction:** Long chain ω-3 fatty acids (ω-3 FA; e.g. docosahexaenoic acid [DHA] and docosapentaenoic acid [DPA]) exhibit potent anti-inflammatory activity, while certain saturated fatty acids (SFA; e.g. 14:0, 16:0, 18:0) exhibit pro-inflammatory activity. However, in human pregnancy the relationships of FAs to maternal systemic inflammation and maternal and newborn body composition are not well characterized. Erythrocyte membrane FAs (eFA) provide a stable index of dietary FA intake over time. In the present study, we address relationships between eFAs, plasma cytokines, % maternal fat (%MF), and % neonatal body fat (%NF).

**Methods:** Pregnant women (n=71), evenly distributed between body mass index classes, were recruited in the 1<sup>st</sup> trimester. %MF was estimated using air displacement plethysmography (ADP) during the 1<sup>st</sup> and 2<sup>nd</sup> trimesters. Maternal plasma cytokines (ELISA) and eFA (GC-MS/MS and GC-FID) were determined during the 3<sup>rd</sup> trimester. %NF was obtained by ADP for 44 of the infants within 24-72 hours post-delivery. Relationships between variables were examined using Pearson's r correlation.

**Results:** The maternal n-6/n-3 ratio was significantly increased in women with higher %MF (p=0.01), but was not associated with maternal cytokines nor with %NF (Table). ω-3 FAs were negatively associated with %MF (DHA p=0.008; DPA p=0.001) and IL-6 (DHA p=0.017; DPA p=0.006) and positively associated with adiponectin (DHA p=0.001; DPA p=0.002). The sum of 14, 16, 18:0 SFA (ΣSFA) positively correlated with IL-6, %MF, and %NF (p=0.007, 0.026, and 0.02, respectively) and negatively correlated with adiponectin (p=0.003).

**Conclusion:** Specific pro-inflammatory maternal eSFAs were related to %MF and cytokines, suggesting that a Western-style diet is associated with increased maternal adiposity and inflammation. Since only ΣSFA correlated with %NF, maternal SFAs during gestation may play a role in mediating neonatal adiposity. Further investigation is warranted into mechanisms by which specific maternal SFAs may mediate neonatal adiposity.

Table. Correlations between maternal red blood cell fatty acids, maternal cytokines, maternal % body fat, and neonatal % body fat.

		Correlation		
		r <sup>2</sup>	Pearson r	p value
Ratio n-6/n-3	%MF	0.09	0.299	0.011*
	%NF	0.0001	-0.01	0.9
	IL-6	0.003	0.059	0.63
	CRP	0.009	0.096	0.43
	ADP	0.171	-0.4132	0.0003*
DHA	%MF	0.152	-0.39	0.008*
	%NF	0.004	-0.06	0.65
	IL-6	0.085	-0.292	0.017*
	CRP	0.04	-0.201	0.09
	HMW ADP	0.2	0.443	0.001*
DPA	%MF	0.141	-0.376	0.001*
	%NF	0.76	-0.275	0.06
	IL-6	0.11	-0.331	0.006*
	CRP	0.034	-0.184	0.13
	HMW ADP	0.185	0.43	0.002*
ΣSFA	%MF	0.085	0.271	0.026*
	%NF	0.101	0.32	0.02*
	IL-6	0.106	0.326	0.007*
	CRP	0.05	0.225	0.06
	HMW ADP	0.123	-0.350	0.003*
%MF	%NF	0.084	0.291	0.0502
	IL-6	0.09	0.3	0.014*
	CRP	0.153	0.392	0.0007*
	ADP	0.166	-0.407	0.0004*

MF=maternal fat; NF=neonatal fat; IL-6=interleukin-6; CRP=c-reactive protein; ADP=adiponectin; HMW ADP=high molecular weight adiponectin; DHA=docosahexaenoic acid; DPA=docosapentaenoic acid; SFA=saturated fatty acids  
\*Denotes statistical significance

### T-103

**Maternal Western-Style Diet Drives Glycolytic Programming in Hematopoietic Stem and Progenitor Cells and Underlies a Pro-Fibrotic Liver Response in Non-Human Primate Offspring.** Michael J Nash<sup>†</sup>,<sup>1</sup> Evgenia Dobrinskikh,<sup>1</sup> Taylor Soderborg<sup>†</sup>,<sup>1</sup> Oleg Varlamov,<sup>2</sup> Diana Takahashi,<sup>2</sup> Richard Stouffer,<sup>2</sup> Kjersti Aagaard,<sup>3</sup> Carrie McCurdy,<sup>4</sup> Maureen Gannon,<sup>5</sup> Eric Pietras,<sup>1</sup> Stephanie Wesolowski\*,<sup>1</sup> Jacob Friedman\*,<sup>6</sup> <sup>1</sup>University of Colorado, Anschutz, Aurora, CO, United States; <sup>2</sup>Oregon Health & Science University, Beaverton, OR, United States; <sup>3</sup>Baylor College of Medicine, Houston, TX, United States; <sup>4</sup>University of Oregon, Eugene, OR, United States; <sup>5</sup>Vanderbilt University, Nashville, TN, United States; <sup>6</sup>University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States.

**Introduction:** Early life exposures are key determinants of newborn health and may underlie inflammation-mediated diseases in later life including non-alcoholic fatty liver disease (NAFLD). Hematopoietic stem and progenitor cells (HSPCs) develop in the fetal liver then migrate to the bone marrow (BM), seeding both tissues with hematopoietic cells that will last a lifetime. However, the impact of maternal WSD (mWSD) on fetal HSPC development and postnatal function of HSPCs and macrophage (Mφ) is unclear.

**Methods:** We studied HSPC from the BM and liver of early third trimester non-human primate (NHP) fetuses exposed to mWSD, and BM HSPCs and BM derived Mφ (BMDM) from 3-year-old (3YO) NHP exposed to mWSD and weaned onto a chow diet (CD) at 7 months of age. We used fluorescence lifetime imaging (FLIM) of NADH and FAD to measure glycolysis and oxidative phosphorylation (oxphos) in HSPCs, and metabolome profiling in bone marrow and liver immune cells. Bulk RNA-sequencing was used to measure HSPC gene expression in fetal BM and a nanostring panel of immune response genes was used to assess BMDM gene expression in 3YO. Ingenuity pathway analysis was used to identify pathways and regulators in HSPCs and BMDM. Hepatic collagen content was measured using second harmonic generation imaging. Data were analyzed by t-test.

**Results:** FLIM and metabolite profiling demonstrated that BM and liver HSPCs from mWSD fetuses have increased glycolysis and decreased oxphos, and a similar signature was found in BM HSPCs from mWSD 3YO. Bulk RNA-sequencing of mWSD fetal BM HSPCs showed gene expression consistent with activation of inflammatory pathways. The top predicted regulators were CEBPA and CD38, which both regulate hematopoiesis and response to inflammatory signals. Gene expression in BMDM from mWSD 3YO demonstrated increased NFκB activation, increased glycolysis, decreased oxphos, impaired anti-inflammatory

response to IL4, and a pro-inflammatory response to LPS. Finally, livers from fetal and 3YO mWSD offspring had increased collagen deposition, a feature of fibrosis and NAFLD.

**Conclusion:** Our findings demonstrate that mWSD drives fetal metabolic programming at the level of HSPCs and Mφ, which suggests that fetal rewiring of immunity may precede obesity and plays a formative role in the development of a pro-inflammatory, pro-fibrotic disease pattern in the fetal liver that persists at 3YO.

### T-104

**Sex-Specific Differences in Immune Dysregulation Following Exposure to Maternal Inflammation.** Jin Liu<sup>†</sup>, Yang Liu<sup>†</sup>, Anguo Liu<sup>†</sup>, Jun Lei\*, Irina Burd\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Inflammation during pregnancy can disrupt regulatory/effector immune response balance, which may result in long-term sequelae in the neonates such as allergy and other immune disorders. We have previously found a dysregulation in maternal adaptive immune following intrauterine inflammation exposure. Here, we extend our studies to determine whether maternal inflammation may contribute to fetal immune programming dysregulation and whether there are any sex-specific differences.

**Methods:** At embryonic day (E)14, CD1 dams (n=16, 8/treatment group) were randomly allocated into two groups: intraperitoneal injection (IP) of 100uL phosphate-buffered saline (PBS) or 0.5ug/100uL PBS of murine recombinant IL-1β (rIL-1β). The dams were injected for four consecutive days to simulate maternal sub-chronic inflammation (MI). 26 pups (13 dams/2 pups per dam) were randomly selected to be euthanized by CO2 exposure at post-natal day (PND) 12. Furthermore, to investigate how MI contributed to the immune response when the pups encountered the challenges during their postnatal days, pups received IP rIL-1β treatment at PND12. 32 pups were randomly selected to receive 0.125ug/50uL rIL-1β or 50uL PBS. Splenic immune cells were characterized using flow cytometry after 24 hours (24hpi). Forkhead box P3 (Foxp3) expression was evaluated by intranuclear staining and analyzed by flow cytometry. One-way-ANOVA and unpaired-t test were used for data analysis.

**Results:** Splenic CD8+ T cells (Teff) in MI pups decreased (p<0.05) with no changes in splenic Tregs. The ratio of Treg/Teff were higher (p<0.05) in MI pups than in control. Notably, Treg/Teff of MI female pups was 1.5 times higher than that in male pups (p<0.01). After pups received rIL-1β, we found that Teff cells in control pups decreased (p<0.001), Tregs in MI pups increased (p<0.001), and Treg/Teff increased in control pups. Male pups had a higher ratio of Treg/Teff than that in female pups in MI group (p<0.01).

**Conclusion:** Perinatal exposure to maternal inflammation exhibited sex-specific differences. Compared to female pups exposed to maternal inflammation, male pups had decreased Treg/Teff, which indicated that male pups were more sensitive to inflammation exposure. Furthermore, following maternal inflammation exposure, males encountering post-natal inflammation challenge, showed more immune dysregulation compared to females.

### T-105

**A Life Course Approach to the Relationship between Fetal Growth and HPA-Axis Function.** Wriyu M Martin<sup>†</sup>,<sup>1,2</sup> Carol A Wang,<sup>1,3</sup> Stephen J Lye,<sup>4</sup> Rebecca M Reynolds,<sup>5</sup> Stephen G Matthews,<sup>4,6</sup> Carly E McLaughlin,<sup>7</sup> Roger Smith,<sup>1,3</sup> Craig E Pennell\*,<sup>1,3</sup> <sup>1</sup>University of Newcastle, New South Wales, Australia; <sup>2</sup>Hunter New England Local Health District, New South Wales, Australia; <sup>3</sup>Hunter Medical Research Institute, New South Wales, Australia; <sup>4</sup>Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada; <sup>5</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>6</sup>University of Toronto, Toronto, ON, Canada; <sup>7</sup>Curtin University, Western Australia, Australia.

**Introduction:** Human and animal studies suggest that hypothalamic-pituitary-adrenal axis (HPA-A) function may be programmed in utero; however, these findings are inconsistent. Given the powerful metabolic actions of cortisol, it is vital to clarify the influence of early life on adult HPA-A function.

**Objective:** To determine the relationship between fetal growth and HPA-A stress response to a psychosocial stressor in a large population of young adults.

**Methods: Participants:** A total of 917 participants aged 18 were sourced from Gen 2 of the Raine Study.

**Measurements:** Detailed obstetric and neonatal biometric data were collected. Plasma and salivary measures of cortisol and ACTH were measured before and after the Trier Social Stress Test (TSST).

**Results:** An inverse linear relationship was observed between fetal growth and basal cortisol, peak cortisol, area under the curve above ground (C-AUCg) of plasma cortisol and adrenal sensitivity (C-AUCg/ACTH-AUCg). Similar results were demonstrated for salivary cortisol. Further, participants born in the upper tertile of birthweight had an attenuated and delayed cortisol response to the TSST compared to those in the middle and lower tertile. No quadratic relationships were identified. No associations were found between measures of fetal adiposity and HPA-A function at age 18. Removal of anticipatory responders from the models substantially attenuated the observed relationships.

**Conclusion:** Using a gold standard HPA-A assessment, we confirmed an inverse linear relationship between fetal growth and HPA-A function at age 18. This differs from the inverse parabolic relationship (inverted J curve) reported in adults of advanced age. Our data suggest that decreased adrenal sensitivity may play a role in the relationship between birthweight and HPA-A function in later life.

### T-106

**Longitudinal Assessment of Exosomal SVATs in Preterm, Preeclampsia, and Gestational Diabetes Mellitus.** Nanbert Zhong\*,<sup>1</sup> Jing Pan†,<sup>2</sup> Yong Wang\*,<sup>3</sup> Weina Ju†.<sup>1</sup> *New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, United States;* <sup>2</sup>*Southern Medical University, Guangzhou, China;* <sup>3</sup>*Washington University School of Medicine, St. Louis, MO, United States.*

**Introduction:** Synapses connect neurons into vast networks during intrauterine development of brain. During which, formation and function of synaptic vesicle (SV) are the fundamental condition. Lacking advanced technology, particularly the longitudinal real-time monitoring of the SV-associated transcripts (SVATs) during the intrauterine period, has limited the knowledge-acquiring of dynamic gene expression profile (GEP) of SVATs.

**Methods:** Based on our success of analyzing exosomal GEP of SVATs in peripheral plasmas of young children with autism spectrum disorder (ASD), from which a statistical significance of GEP for SVAT-associated lncRNAs at genetic loci of *STX8*, *SYT9*, *SYT15*, *SV2C*, *SLC18A2*, and *SYP*, and four of SVAT-associated mRNAs at *SYT9*, *SYT15*, *SV2C*, and *SYP* were differentially expressed, we developed a real-time assessment, on a weekly basis during pregnancy from the 11th to the 40th gestational week (GW), for longitudinal dynamic monitoring GEP of SVAT molecules within exosomes that are circulated in maternal plasmas.

**Results:** Our results showed that SVAT-associated lncRNAs/mRNAs were differentially expressed from the first trimester of pregnancy to the term of birth (Figure 1), suggesting that GEP of SVAT-mRNAs has been epigenetically regulated by SVAT-lncRNAs during the period of intrauterine development of fetal neural circuits. Comparing pathological pregnancies, including spontaneous preterm birth (sPTB), preeclampsia (PE), and gestational diabetes mellitus (GDM), to normal pregnancies, we established specific correlations of SVAT-lncRNA and SVAT-mRNA of *STX8*, *SLC18A2*, and *SYP* with sPTB; SVAT-lncRNA and SVAT-mRNA of *STX8* with PE; and SVAT-lncRNA and SVAT-mRNA of *SV2C* as well as SVAT-mRNA of *SYP* with GDM (Figure 2).

**Conclusion:** Our study concluded that variant complication in pathological pregnancies may alter GEP of the SVATs, which is likely to affect the intrauterine development of neural circuits and consequently influence the fetal brain development.

### T-107

**Integrated Multi-Omics Analyses of High-Risk Myometrial Progenitor/Stem Cells: Implication for Uterine Fibroid Pathogenesis.** Qiwei Yang, Ayman Al-Hendy. *University of Chicago, Chicago, IL, United States.*

**Introduction:** The period during which tissue and organ development occurs is a time of exquisite sensitivity to environmental exposure. However, the mechanisms by which the epigenome is disrupted in response to developmental insult leading to an increased risk of hormone-dependent diseases such as uterine fibroids remain unclear. In recent years, high-throughput sequencing technologies provide unprecedented opportunities to depict the development of diseases at multiple molecular levels. The integration and analysis of these multi-omics datasets allow us to yield a better understanding and a clearer picture of the system under study.

**Methods:** Female newborn Eker rats (5 animals per group) were treated subcutaneously with vehicle (VEH) or 10 µg of diethylstilbestrol (DES, a tool compound for environmental EDCs) on postnatal days 10-12, a critical period of uterine development. Myometrial stem cells (MMSCs) were isolated from 5 month-old myometrium tissue (N=5 for each group) using Stro-1 and CD44 surface markers. The multiple high-throughput omics sequencing including RNA-seq, CHIP-seq, and Reduced Representation Bisulfite Sequencing (RRBS) was performed to determine the transcriptome and epigenome landscapes of DES-exposed MMSCs (high-risk) and VEH-exposed MMSCs (low-risk). Fisher's exact and Student's T-test were used for statistical analysis.

**Results:** RNA-seq and CHIP-seq (H3K4me3) revealed 2922 and 6473 differentially expressed and H3K4me3 mark differentially enriched genes respectively. Integration analysis of RRBS, CHIP-seq, and RNA-seq demonstrated that among 1474 EDC-upregulated genes with expected direction, 47.1% of genes were correlated with H3K4me3 enrichment. 25.6% of genes with DNA hypomethylation were correlated with EDC-induced upregulation of RNA expression. Among 1448 EDC-down regulated genes, 33.5% of genes were correlated with H3K4me3 reduction and 10.2% of genes were correlated with DNA hypermethylation. The co-regulation analysis demonstrated that 23.1% and 11.5% of EDC-upregulated and -downregulated genes exhibited overlap between H3K4me3 and DNA methylation. Considering expected direction analysis, the status of H3K4me3 (enrichment or reduction) plays a dominant role in regulating gene expression in MMSCs genome-widely in response to EDC exposure. There is a significant difference between the number of genes with H3K4me3 enrichment+ hypomethylation verse H3K4me3 reduction+ hypermethylation (p<0.001).

**Conclusion:** Together, these data set the stage to study MMSCs as the myometrial stem/progenitor cells that are the target for environmental exposure. Moreover, these studies will be helpful to develop novel interventions to prevent and/or decrease morbidity associated with uterine fibroids.

### T-108

**The Impact of Anticoagulation Use on Cell-Free DNA Metrics for Women without Autoimmune Disease.** H MacKinnon†, T Kolarova†, J Hedge†, E Vinopal†, S Delaney\*, C Lockwood\*, R Shree\*. *University of Washington Medical Center, Seattle, WA, United States.*

**Introduction:** Previous studies showing an association between anticoagulation and indeterminate cell-free DNA (cfDNA) results have included women with autoimmune disease, which alone is associated with indeterminate results likely from low fetal fraction (FF). We sought to evaluate differences in cfDNA test metrics for women on anticoagulation without autoimmune disease compared to controls.

**Methods:** Retrospective, single institution cohort study of singleton pregnancies with a previously validated cfDNA assay using whole genome sequencing. Women with autoimmune disease and pregnancies with suspected aneuploidy were excluded. Anticoagulation included unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH). An indeterminate result was defined as FF<4%. We evaluated the association between maternal anticoagulation use and

FF, indeterminate rate, and total cfDNA concentration using univariate and multivariate analyses, controlling for body mass index (BMI) and gestational age (GA) at sample collection.

**Results:** Of 1,283 patients, 22 were on anticoagulation (2 on UFH; 20 on LMWH). In those on anticoagulation, FF was significantly lower (9% vs 12%,  $p=0.01$ ), the indeterminate rate was significantly higher (18% vs 4%,  $p<0.001$ ), and the total cfDNA concentration was significantly higher (371.1 pg/uL vs 101.9 pg/uL,  $p<0.001$ ) compared to controls. After controlling for maternal BMI and GA at sample collection, anticoagulation was associated with a 6-fold increase in the likelihood of an indeterminate result ( $p<0.01$ ) [Table 1]. In linear regression, anticoagulation was significantly associated with decreasing FF ( $p<0.01$ ) and increasing total cfDNA concentration ( $p<0.001$ ) [Figure 1].

**Conclusion:** Women on anticoagulation without autoimmune disease have lower FF, higher rates of indeterminate cfDNA results, and higher total cfDNA concentration compared to controls. This suggests a possible dilutional effect of anticoagulation on cfDNA assays used for non-invasive prenatal screening and warrants further studies to elucidate precise mechanisms.

**Table 1: Demographics, anticoagulation use and cfDNA test metrics**

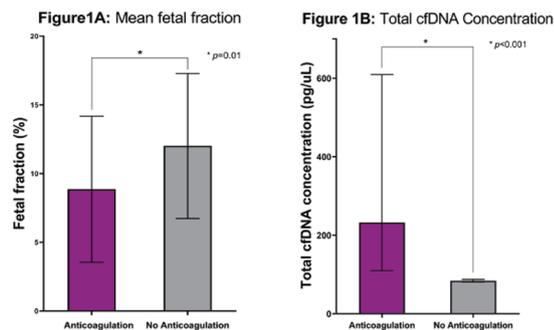
	Anticoagulation N = 22	No Anticoagulation N = 1261
Age at delivery (years)	36.6 +/- 4.9	35.1 +/- 4.6
BMI (kg/m <sup>2</sup> )	27.0 +/- 6.4	26.9 +/- 6.3
Parity	2 (0-6)	1 (0-8)
Gestational age (weeks)	13.5 +/- 3.3	13.2 +/- 2.6
Female fetal sex	10 (45.5%)	493 (45.6%)
Indication		
Advanced maternal age	17 (77.3%)	775 (62.6%)
First line screening	3 (13.6%)	297 (24.0%)
Abnormal serum screening	1 (4.5%)	68 (5.5%)
Ultrasound abnormality	1 (4.5%)	84 (6.8%)
Other	0 (0%)	13 (1.0%)
Total cfDNA (pg/uL)*	371.1 (105.5 – 527)	101.9 (59.5 – 117)
Fetal Fraction (%)*	9.0 +/- 4.8	11.6 +/- 5.0
Indeterminate result*	4 (18.2%)	51 (4.0%)
<b>Indeterminate Result OR (95% CI)</b>		
Unadjusted	5.3 (1.7 – 16.1)*	Reference
Adjusted <sup>†</sup>	6.0 (1.9 – 19.0)*	Reference
<b>Fetal Fraction % <math>\beta</math> (95% CI)</b>		
Unadjusted	-2.6 (-4.7, -0.6)*	Reference
Adjusted <sup>†</sup>	-2.7 (-4.6, -0.8)*	Reference
<b>DNA Concentration (pg/uL) <math>\beta</math> (95% CI)</b>		
Unadjusted	269.3 (222.0, 316.7)*	Reference
Adjusted <sup>†</sup>	269.4 (222.6, 316.2)*	Reference

Demographics presented as mean (SD), median (range), or N (%)

BMI, body mass index; cfDNA, cell-free DNA

\* P value  $\leq 0.01$

<sup>†</sup> Adjusted for gestational age and BMI at blood draw



## T-109

**Fetal Membrane Cells and Their Exosomes A Gateway for Drug Transportation during Pregnancy.** Ananth Kumar Kammala, Lauren Richardson, Enkhtuya Radnaa. *The University of Texas Medical Branch, Galveston, TX, United States.*

**Introduction:** During pregnancy, the drug transport mechanism has been well established in placental tissue utilizing several transporters proteins (TPs). Fetal membranes (FM) or amniochorionic membranes are one of the most intriguing tissues in the intrauterine cavity that is essential for the protection of the fetus, maintenance of pregnancy. However, its role in

drug transport is not studied. We hypothesize that human fetal membranes and exosomes derived from the membrane cells express drug TPs and can function as a gateway for drug transport during pregnancy. This study determined the differential expression of TPs in fetal membrane cells and exosomes derived from them.

**Methods:** Immortalized human amnion epithelial cells (AECs), amnion mesenchymal cells (AMCs), and chorion trophoblast cells (CTCs), were cultured in normal conditions. Exosomes were isolated from media from each cell type using differential ultracentrifugation followed by size exclusion chromatography. Cells and their respective exosomes were screened for the expression of four distinct TPs: P-glycoprotein (P-gp), breast cancer resistance protein (BCRP-1), organic anion/cation transporters (OAT), and Serotonin Transporter protein (SERT) by western blot, and immunofluorescence staining. The human placental cell line (BeWo) was used as control.

**Results:** The efflux TP, P-gp expression was significantly higher in amnion cells (both AECs and AMCs) compared to CTCs (both  $P<0.01$ ). In contrast, the expression levels of BCRP-1, another efflux TP, and OAT-1, an influx TP, were significantly higher in CTCs compared to amnion cells. SERT, a serotonin uptake TP, was expressed in all FM cell types but its expression was significantly lower in AECs compared to the other FM cells (all  $P<0.05$ ). We further examined TPs in exosomes from membrane cells. Significantly higher expression of P-gp was seen in CTC-derived exosomes compared to AEC and AMC-derived exosomes ( $p<0.001$ ) whereas BCRP-1, OAT-1, and SERT were expressed in significantly higher amounts in AMC-derived exosomes than in CTC-derived exosomes ( $p<0.05$ ). The results were summarized in Table -1.

**Conclusion:** For the first time, we report the presence of both influx and efflux drug TP in human FM cells and their exosomes. The expression of TPs in FM cells and exosomes showed an inverse correlation and this was also seen in placental cells. Our ongoing studies indicate that fetal membranes can act as a gateway for drug transport during pregnancy and exosomal TPs can modify the environment of drug transport.

**Table 1. Expression levels of Transporter proteins in Fetal membrane cell lines**

Transporter proteins	Cells			Exosomes		
	AEC	AMC	CTC	AEC	AMC	CTC
P-gp	+++	+++	+	+	+	+++
BCRP-1	++	++	+++	++	+++	+
OAT-1	+	+	+++	++	++	+
SERT	+	+++	+++	+++	++	+

Inverse relationship of transporter proteins expressions were shown in Fetal membrane cells and their derived exosomes. "+++" indicates high expression, "++" indicates moderate expression, "+" indicates lower expression.

## T-110

**Copy Number Changes and Fetal Malformations in Stillborn Fetuses.** Tsegelassie Workalemahu,<sup>1</sup> Susan Dalton<sup>†</sup>,<sup>1</sup> Amanda Allshouse,<sup>1</sup> Jessica M Page,<sup>1,2</sup> Robert M Silver\*.<sup>1</sup> *<sup>1</sup>University of Utah, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States.*

**Introduction:** Genetic and fetal structural abnormalities were identified as the potential cause of death in 13.7% stillborn fetuses (SB), while chromosomal microarray (CMA) identified copy number changes (CNCs) in 44 (9.5%) SB. However, the relationship between specific CNCs, fetal malformations and SB remains uncertain. Thus, we evaluated the associations between specific CNCs and fetal structural anomalies among SB.

**Methods:** We conducted a secondary analysis of the Stillbirth Collaborative Research Network study among SB (n=388) with CMA and postmortem examinations of the fetus and placenta. Major anomalies were grouped by anatomic system and specific anomaly type. To detect CNCs, we used single-nucleotide polymorphism array with  $\geq 500$  kb. We classified CMA into two groups: normal defined as no CNCs or benign CNCs, and abnormal defined as pathogenic CNCs (including aneuploidy) or variants of unknown clinical significance (VOUS). We compared proportions of abnormal and normal CNCs between SB with and without structural anomalies using the Wald Chi-squared test.

**Results:** The proportion of anomalous SB with pathogenic/VOUS CNCs (46.7%) was higher in comparison to those with benign CNCs (19.6%;  $p < 0.001$ ). Anatomic system malformations including cystic hygroma, hydrops, and gastrointestinal, cardiac, skeletal and cranial defects were significantly associated with pathogenic/VOUS CNCs. We identified a pathogenic deletion CNC associated with a skeletal defect involving forty-six genes and a pathogenic duplication CNC associated with a cardiac defect involving four genes.

**Conclusion:** Findings reported herein provide evidence for specific CNCs associated with fetal malformations among SB and warrant further investigation of the identified genes. Knowledge of the specific pathogenic chromosomal abnormalities associated with fetal anomalies may improve fetal genotype-phenotype databases and assist practitioners in designing care plans for anomalous fetuses with increased risk for SB.

**Table 1.** Associations of CNCs with fetal structural malformations in SB

Structural Malformations	Pathogenic/VOUS CNC <sup>a</sup>	Benign CNC <sup>a</sup>	P-value <sup>b</sup>
<b>Total SB, N (%)</b>	<b>58 (100)</b>	<b>326 (100)</b>	
Any Anomaly	27 (46.7)	64 (19.6)	<.001
Cystic Hygroma	10 (16.7)	4 (1.3)	0.003
Central Nervous System	5 (8.5)	10 (3.0)	0.169
Thorax Defects	4 (6.6)	6 (1.8)	0.152
Cardiac Defects	15 (26.3)	14 (4.3)	<.001
Gastrointestinal defects	9 (15.8)	11 (3.3)	0.011
Genitourinary defects	6 (10.8)	11 (3.5)	0.102
Skeletal defects	11 (19.2)	13 (4.0)	0.005
Umbilical cord abnormalities	4 (7.1)	20 (6.0)	0.768
Cranial	13 (21.8)	8 (2.3)	<.001
Hydrops	11 (19.8)	12 (3.8)	0.004
Other anomaly	3 (5.3)	27 (8.2)	0.401

<sup>a</sup> Weighted frequency and column percentage were calculated to reflect the Stillbirth Collaborative Research Network study sampling design.

<sup>b</sup> P-values were based on a Wald chi-squared test.

**Table 2.** Specific pathogenic CNCs associated with fetal structural malformations in SB

Structural Malformation	Type of CNC	Gestational Age at SB	ISCN <sup>a</sup> Nomenclature	Genes
Skeletal defect	Deletion	34w6d	arr 1q21.1(43,845,72-146,838,707)x1	LOC645166, NUDT17, HFE2, GPR89C, FMO5, GNRHR2, NBPFF11, PDE4DIP, CHD1L, POLR3GL, ANKRD35, LOC200030, HFE2, CD160, ITGA10, NOTCH2NL, PDZK1P1, PEX11B, NBPFF14, GPR89A, PRKAB2, BCL9, RBM8A, PPIAL4A, NBPFF10, PPIAL4E, SEC22B, ACP6, LOC728989, LIX1L, RNF115, NBPFF20, LOC728875, PPIAL4E, TXNIP, PDE4DIP, C1orf152, GPR89A, GJA5, GJAR, POLR3C, ANKRD34A, FLJ39739, PDZK1, PIAS3, PDIA3P, NBPFF16, GPR89B, NBPFF15
			arr 21q22.13(36,685,848-37,185,921)x3	CLDN14, SIM2, CHAF1B, HLCS

<sup>a</sup> International System for Human Cytogenetic Nomenclature

**T-111**

**Maternal Exposure to an Environmentally Relevant Mixture of Per- and Polyfluoroalkyl Substances (PFAS) Leads to Adverse Pregnancy Outcomes in a New Zealand White Rabbit Model.** Christine E Crute†, Samantha Hall†, Chelsea Landon, Angela Garner, Susan K. Murphy, Liping Feng\*. *Duke University, Durham, NC, United States.*

**Introduction:** Per- and polyfluoroalkyl substances (PFAS), a class of synthetic chemicals present in commercial products, have been detected in the blood of >95% of the United States population. Human exposure is associated with adverse health effects including endocrine disruption, liver and immune toxicity, and developmental effects. Increasing evidence raises concerns that maternal PFAS exposure is associated with adverse pregnancy and birth outcomes, including maternal hypertensive disorders, thyroid hormone disruption, and fetal growth restriction. We recently found that PFAS levels are two to four times higher in the blood of Pittsboro, NC residents than the U.S. population as a whole, likely due to highly contaminated drinking water drawn from the Haw River.

**Methods:** To understand if maternal PFAS exposure to Pittsboro, NC residents during pregnancy may lead to adverse health outcomes, we evaluated relevant pregnancy endpoints using an *in vivo* rabbit model. Female New Zealand White rabbits were supplied control (no detectable PFAS levels) or PFAS-contaminated drinking water, which was formulated

with PFAS to resemble levels measured in tap water collected from Pittsboro, NC (10 PFAS compounds; total PFAS load = 758.6 ng/L). Dams were bred one week after drinking water exposure began and sacrificed on gestational day 25. All data was analyzed with each dam representing a biological replicate (control, n=5; PFAS-mixture, n=6), with kits as repeated measures when applicable.

**Results:** Maternal PFAS exposure led to increased body weight corrected for gravid uterus weight ( $p=0.002$ ) and liver weight ( $p=0.001$ ) and decreased fetus number ( $p=0.03$ ). PFAS-exposed dams were anecdotally observed to have small lesions across the uterus and two dams had late fetal resorptions, which was not observed in controls. Placenta weight from exposed dams trended heavier than control placentas ( $p=0.06$ ). There was no change in fetal body weight, crown rump length, and brain weight (absolute and adjusted;  $p > 0.05$ ). Maternal blood pressure, taken via indirect doppler arterial measurements at baseline and GD25, showed PFAS-exposed dams with increased mean arterial blood pressure as compared to control dams ( $p=0.01$ ). Blood chemistry analyses showed PFAS-exposed dams had increased blood area nitrogen: creatinine ratio ( $p=0.0008$ ), calcium ( $p=0.02$ ), and glucose ( $p=0.04$ ).

**Conclusion:** Maternal PFAS exposure in an *in vivo* model leads to maternal health effects, including hypertension, increased maternal body and liver weight, poor kidney function, and adverse pregnancy outcomes like decreased kit number. Future work will investigate physiology and function of target organs (placenta, uterus, liver, kidney) through pathology, serum biomarker assays, hormone measurements, and related gene and protein expression.

**T-112**

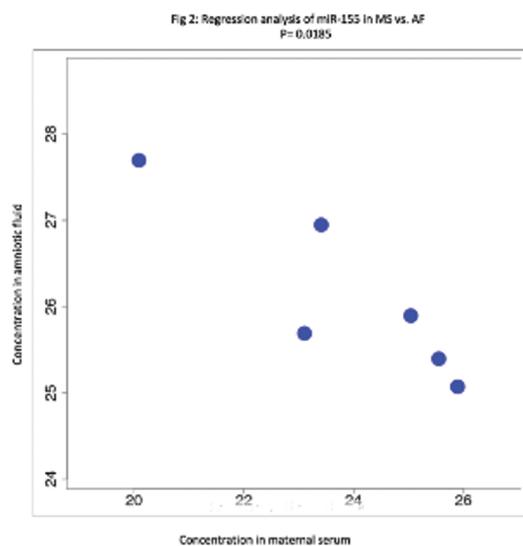
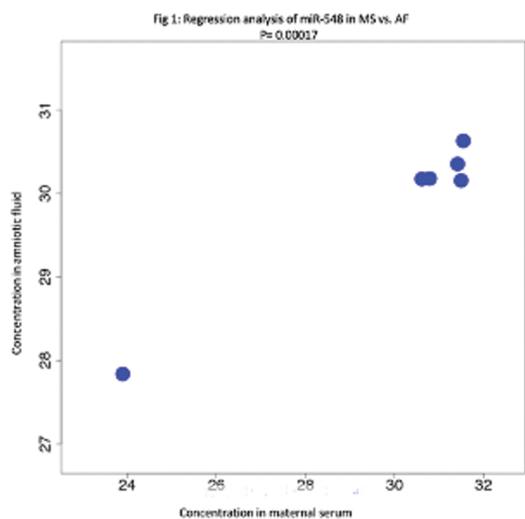
**Maternal Serum Micro RNA Correlates with Amniotic Fluid Micro RNA in Pregnancies Complicated by TTTS: A Potential Prenatal Marker for TTTS.** Chloe Nielsen†, Henry Galan\*, Hilary Hoffman\*, Bettina Cuneo\*, Shelley Miyamoto\*, Carmen Sucharov\*. <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>Childrens Hospital Colorado, Aurora, CO, United States.

**Introduction:** TTTS beyond Stage III carries very high rates of morbidity and mortality. There is no ability to predict which Mo/Di twin pregnancy will progress to TTTS, thus current recommendations are to survey these pregnancies every two weeks via ultrasound. Preliminary data show differences in miRNAs between mild and severe TTTS stages. MicroRNAs (miRs) are small RNAs that regulate gene expression and are associated with many obstetric disease processes, including preeclampsia, fetal growth restriction, and pregnancy loss. To date no studies have evaluated the relationship between amniotic fluid (AF) miR and maternal serum (MS) miR in pregnancies complicated by TTTS. The primary objective of this study is to determine whether there is a correlation between the miRNA profiles in the AF and the maternal serum in TTTS cases undergoing laser treatment.

**Methods:** This is a prospective cohort study. AF and MS were collected at the time of selective fetoscopic laser photocoagulation from monochorionic diamniotic twin pregnancies with TTTS at any stage and those samples were stored at -80° C. RNA was extracted from the AF and MS samples using the miRNeasy Mini Kit (Qiagen) and miR arrays were performed using the TaqMan Open Array miR panel (ThermoFisher). Several candidate miRs were identified from prior studies that differed between pregnancies affected by TTTS and singleton pregnancies (miR-19, miR-155, miR-548, and miR-483). These miRs were then quantified in both MS and AF samples. A regression analysis was performed and a p-value of <0.05 was used to determine whether the candidate MS miRs were significantly correlated with AF miRs.

**Results:** A total of 6 MS and AF sample pairs from pregnancies with TTTS were analyzed. There were 2 MS miR that were either positively or negative correlated with AF miR. MS miR-548 was positively correlated with AF miR with a cor of 0.99 and a p-value of 0.00017 (Fig 1). MS miR-155 was negatively correlated with AF miR, with a cor of -0.89 and a p-value of 0.019 (Fig2). There was a trend towards negative correlation between MS and AF samples for miR-19a and miR-483.

**Conclusion:** MS miR correlates with AF miR profiles in pregnancies complicated by TTTS. This presents a potentially useful and readily-available biomarker for pregnancies at risk of TTTS. Further studies are needed to confirm this and to assess miR profiles across pregnancy.



### T-113

**A Two-Week Insulin Infusion in IUGR Fetal Sheep at 70% Gestation Increases Myoblast Proliferation but Not Total Myofibers.** Eileen L. Chang<sup>†</sup>,<sup>1</sup> Byron Hetrick,<sup>2</sup> Stephanie R. Wesolowski,<sup>1</sup> Paul J. Rozance,<sup>1</sup> Carrie E. McCurdy,<sup>2</sup> Laura D. Brown\*.<sup>1</sup> <sup>1</sup>University of Colorado School of Medicine, Aurora, CO, United States; <sup>2</sup>University of Oregon, Eugene, OR, United States.

**Introduction:** Intrauterine growth restricted (IUGR) fetuses are born with lower skeletal muscle mass, fewer proliferating myoblasts, and fewer myofibers compared to normally growing fetuses. Plasma concentrations of insulin, a myogenic growth factor, are lower in IUGR fetuses. At 70% gestation (GA), the skeletal muscle is still undergoing myogenesis, or myoblast proliferation and fusion to form myofibers; however, the total

myofiber number is set shortly before birth. We hypothesized that a two-week insulin infusion at 70% GA would increase myoblast proliferation and fiber number in IUGR fetal sheep *in vivo*.

**Methods:** Catheters were placed in the fetal jugular vein and femoral artery (106±1 dGA; term=147 d). IUGR fetuses were randomly assigned to receive insulin (IUGR-I, n=8; 0.014±0.001 units/kg/hr) or saline (IUGR-S, n=7) infusion for 14 d and were compared to normally growing controls who received saline (CON; n=6). Dextrose was infused (2.6±0.1 mg/min) into IUGR-I to maintain euglycemia. Blood gases and plasma hormone concentrations were measured at baseline and during the infusion. At necropsy (125±3 dGA) biceps femoris (BF) muscle was digested, and CD56<sup>+</sup> myoblasts were stained with propidium iodide to determine cell cycle stage by flow cytometry. Flexor digitorum superficialis (FDS) and BF were cryosectioned (10 μm) and stained with anti-laminin, anti-dystrophin, and DAPI to quantify fiber and myonuclear number, respectively. Data were analyzed by two-way ANOVA with Tukey's *post-hoc* test with effects of group (CON, IUGR-S, IUGR-I), time (days), and interaction.

**Results:** Fetal P<sub>a</sub>O<sub>2</sub>, O<sub>2</sub> content, and glucose concentrations were lower and P<sub>a</sub>CO<sub>2</sub> was higher in both IUGR-I and IUGR-S vs. CON (P<0.01, group). Insulin concentrations in IUGR-I (0.25±0.02 ng/mL) and IUGR-S (0.21±0.02 ng/mL) were lower vs. CON (0.35±0.02 ng/mL; P<0.0001, group). IGF-1 concentrations tended to be higher in IUGR-I (87.3±6.5 ng/mL vs. IUGR-S (69.8±6.7 ng/mL; P=0.06), but both were lower vs. CON (121.8±7.3 ng/mL; P<0.0001, group). More myoblasts were in S/G2 cell cycle stage in IUGR-I vs. both IUGR-S and CON (145% and 113%, respectively, P<0.01). IUGR-I FDS muscle weighed 40% less and had 40% lower fiber number vs. CON (P<0.05) but were not different from IUGR-S. Myonuclear number per fiber was not different among groups.

**Conclusion:** A two-week insulin infusion at 70% GA promoted myoblast proliferation in the IUGR fetus but did not increase fiber or myonuclear number. Myoblasts in the IUGR fetus retain the capacity to respond to mitogenic stimuli *in vivo*, but we speculate that insulin infusion may suppress differentiation and fusion of myoblasts into myofibers. Alternatively, intrinsic defects in the fetal myoblast may limit the capacity to restore fiber number by 70% GA of an IUGR fetus.

### T-114

**Late Gestation Fetal Hyperglucagonemia Lowers Pancreas Weight, Beta- and Alpha-Cell Proliferation, Islet Area, and Basal Insulin Concentrations.** Sarah N Cilvik,<sup>1</sup> Brit Boehmer,<sup>2</sup> Stephanie R Wesolowski,<sup>2</sup> Laura D Brown,<sup>2</sup> Paul J Rozance\*.<sup>2</sup> <sup>1</sup>Wake Forest University Health Sciences, Winston-Salem, NC, United States; <sup>2</sup>University of Colorado Anschutz Medical Campus, Aurora, CO, United States.

**Introduction:** Overexpression of the glucagon receptor in the mouse beta-cell results in increased insulin secretion and beta-cell mass, while glucagon receptor knockout impairs islet development. Fetal glucagon concentrations are elevated in response to stress, but little is known about the effects of glucagon on insulin secretion and islet development in the late gestation fetus. We hypothesized that hyperglucagonemia would enhance insulin secretion and beta-cell proliferation.

**Methods:** Late gestation fetal sheep received an intravenous infusion of low- or high-dose glucagon (5 or 50ng/kg/min) or vehicle control. Fetal glucose-stimulated insulin secretion (GSIS) was measured in response to acute (3 hour) and chronic (8-10 days) hyperglucagonemia. After 8-10 days of infusion, fetal and organ weights were measured, in addition to islet structure and cell proliferation using immunofluorescent staining. Statistical analysis was performed using one- or two-way repeated measures/mixed-models ANOVA with individual means comparisons.

**Results:** Acute high-dose glucagon infusion increased basal insulin concentrations 3-fold (n=4) and enhanced GSIS with 2.5-fold higher insulin secretion compared to control (n=12) and 3.1-fold higher than acute low-dose glucagon infusion (n=10; p<0.01). In contrast, chronic high-dose glucagon infusion blunted GSIS (n=5), while chronic low-dose glucagon infusion enhanced GSIS (n=7) compared to control (n=7; p<0.05). Both low- and high-dose chronic glucagon exposure resulted in a 2-3-fold reduction in basal plasma insulin concentrations by study end (p<0.0001). In all hyperglucagonemic fetuses, pancreatic weight, alpha-cell mass,

alpha- and beta-cell proliferation, and islet area were lower ( $p < 0.05$ ). There was a trend toward lower beta-cell mass ( $p = 0.063$ ). The relative proportion of alpha- and beta-cells within the islet remained the same.

**Conclusion:** These findings demonstrate a differential beta-cell response to acute vs. chronic and low- vs. high-dose glucagon with regard to GSIS. Despite this difference in GSIS, chronic hyperglucagonemia reduced basal insulin concentrations, alpha- and beta-cell proliferation, islet area, and overall pancreas weight, regardless of dose. This implicates an inhibitory role for glucagon in the regulation of pancreatic growth.

### T-115

**Sheep-Specific IGF-1 Promotes Anabolic Growth in Fetal Sheep.** Jane Stremming,<sup>1</sup> Alicia White†,<sup>1</sup> Pamela A Doerner Barbour,<sup>1</sup> Eileen I Chang†,<sup>1</sup> Stephanie R Wesolowski,<sup>1</sup> Matt Seefeldt,<sup>1</sup> Byron Hetrick,<sup>2</sup> Carrie E McCurdy,<sup>2</sup> Paul J Rozance,<sup>1</sup> Laura D Brown\*.<sup>1</sup> <sup>1</sup>University of Colorado, Aurora, CO, United States; <sup>2</sup>University of Oregon, Eugene, OR, United States.

**Introduction:** IGF-1 is a critical fetal growth hormone; intrauterine growth restriction and fetal macrosomia are associated with low and high cord blood concentrations of IGF-1, respectively. Infusion of IGF-1 LR3, an analogue of IGF-1 designed to have low affinity for the IGF binding proteins, increases organ weight and myoblast proliferation in fetal sheep. We hypothesized that a sheep-specific recombinant IGF-1 (oIGF-1) in its native environment with accessibility to binding proteins will similarly demonstrate anabolic effects in fetal sheep.

**Methods:** oIGF-1 was made using bacterial transformation, fermentation, inclusion body separation, chemical refolding, and purification by reverse phase and ion exchange chromatography. A three-pronged approach was used to test the anabolic effects of oIGF-1, including myoblast proliferation, downstream signaling, and organ growth. First, myoblasts ( $n = 4$ ) harvested from fetal sheep were exposed to oIGF-1 (1, 10, and 100 ng/ml). Cell proliferation was measured by MTT assay. Second, anesthetized and catheterized fetal sheep were infused with oIGF-1 (22 or 220 µg/hr;  $n = 4$ ) or saline ( $n = 3$ ) for 2 hr. Serial biceps femoris muscle biopsies were taken to measure signaling response to oIGF-1 by western blot, including phosphorylated Akt (Ser473), Erk 1/2 (Thr202/Tyr204), and p70 S6 Kinase (S6K, Thr389 and Thr421/Ser424). Finally, in catheterized pregnant sheep carrying twins, one fetus received oIGF-1 infusion ( $n = 6$ ) and the other received saline ( $n = 6$ ) for 1 week. EdU was infused to measure myoblast proliferation *in vivo* using flow cytometry. Fetal and organ weights were obtained.

**Results:** oIGF-1 (100 ng/ml) increased myoblast proliferation ( $P = 0.03$ ). The difference in phosphorylation of Akt, Erk 1/2, and S6K in muscle biopsies taken at multiple time points during infusion compared to baseline was similar in oIGF-1 and saline-infused fetuses. After a one-week infusion of oIGF-1 compared to saline, fetal body and skeletal muscle weights were similar. Adrenal glands were larger ( $P < 0.05$ ), and heart, kidneys, and spleen tended to be larger ( $P \leq 0.06$ ) in oIGF-1-infused fetuses. The percentage of EdU positive myoblasts tended to be higher in oIGF-1 ( $n = 3$ /group,  $P = 0.09$ ).

**Conclusion:** Similar to IGF-1 LR3, oIGF-1 increased fetal organ weights including the adrenal glands, heart, kidneys, and spleen. Anabolic effects were further demonstrated by increased myoblast proliferation *in vitro* and a trend towards increased myoblast proliferation *in vivo*. Infusion of oIGF-1 for a week did not increase skeletal muscle weight potentially due to lack of an increase in IGF-1 signaling. More research is needed to determine the specific role of IGF binding proteins in regulating the effects of IGF-1 on fetal growth.

### T-116

**Associations of Maternal Bisphenol Urine Concentrations during Pregnancy with Neonatal Metabolomic Profiles.** Sophia Maria Blaauwendraad†,<sup>1</sup> Ellis Voerman,<sup>1</sup> Leonardo Trasande,<sup>2</sup> Kurunthachalam Kannan,<sup>2</sup> Susana Santos,<sup>1</sup> George Ruijter,<sup>1</sup> Chalana Sol,<sup>1</sup> Linda Marchioro,<sup>3</sup> Engy Shokry,<sup>3</sup> Berthold Koletzko,<sup>4</sup> Vincent Jaddoe,<sup>1</sup> Romy Gaillard.<sup>1</sup> <sup>1</sup>Erasmus Medical Center, Rotterdam, Netherlands; <sup>2</sup>New York University School of Medicine, New York, NY, United States; <sup>3</sup>Dr. von Hauners Children's Hospital, LMU München, Munich, Germany; <sup>4</sup>Dr. von Hauners Children's Hospital, LMU München, Munich, Netherlands.

**Introduction:** Fetal exposure to bisphenols is associated with altered fetal growth, adverse birth outcomes and childhood cardio-metabolic risk factors. Metabolomics may serve as a tool to identify the mechanisms underlying these associations. We examined the associations of maternal bisphenol urinary concentrations in pregnancy with neonatal metabolite profiles from cord blood.

**Methods:** In a population-based prospective cohort study among 225 mother-child pairs, maternal urinary bisphenol A, S and F concentrations in first, second and third trimester were measured. LC-MS/MS was used to determine neonatal concentrations of amino acids, non-esterified fatty acids, phospholipids, and carnitines in cord blood.

**Results:** No associations of maternal total bisphenol concentrations with neonatal metabolite profiles were present. Higher maternal average BPA concentrations were associated with higher neonatal mono-unsaturated alkyl-lysophosphatidylcholine concentrations, whereas higher maternal average BPS was associated with lower neonatal overall and saturated alkyl-lysophosphatidylcholine ( $p$ -values  $< 0.05$ ). Trimester-specific analyses showed that higher maternal BPA, BPS and BPF were associated with alterations in neonatal non-esterified fatty acids, diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, alkyl-lysophosphatidylcholine, sphingomyelins and acyl-carnitines, with the strongest effects for third trimester maternal bisphenol and neonatal diacyl-phosphatidylcholine, sphingomyelin and acyl-carnitine metabolites ( $p$ -values  $< 0.05$ ). Associations were not explained by maternal socio-demographic and lifestyle characteristics or birth characteristics.

**Conclusion:** Higher maternal bisphenol A, F and S concentrations in pregnancy are associated with alterations in neonatal metabolite profile, mainly in non-esterified fatty acids, phospholipids and carnitines concentrations. These findings provide novel insight into potential mechanisms underlying associations of maternal bisphenol exposure during pregnancy with adverse offspring outcomes but need to be replicated among larger, diverse populations.

### T-117

**Fetal Growth Restriction: Isolated Abdominal Circumference and Perinatal Outcomes.**

Maria Andrikopoulou†,<sup>1</sup> Natalie Bello,<sup>1</sup> Shai Bejerano,<sup>1</sup> Karin Fuchs,<sup>1</sup> Russell Miller,<sup>1</sup> Eliza Miller,<sup>1</sup> David M Haas,<sup>2</sup> William Grobman,<sup>3</sup> Brian M Mercer,<sup>4</sup> Samuel Parry,<sup>5</sup> Robert M Silver,<sup>6</sup> Ronald Wapner,<sup>1</sup> Deborah Wing,<sup>7</sup> George R Saade,<sup>8</sup> Uma Reddy,<sup>9</sup> Hyagriv Simhan,<sup>10</sup> Corette Parker,<sup>11</sup> Cynthia Gyamfi-Bannerman\*.<sup>1</sup> <sup>1</sup>Columbia University Irving Medical Center, New York, NY, United States; <sup>2</sup>Indiana University School of Medicine, Indianapolis, IN, United States; <sup>3</sup>Northwestern University Feinberg School of Medicine, Chicago, IL, United States; <sup>4</sup>MetroHealth Medical Cent Case Western Reserve University, Cleveland, OH, United States; <sup>5</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>6</sup>University of Utah, Salt Lake City, UT, United States; <sup>7</sup>University of California, Irvine, CA, United States; <sup>8</sup>University of Texas Medical Branch, Galveston, TX, United States; <sup>9</sup>Yale School of Medicine, New Haven, CT, United States; <sup>10</sup>University of Pittsburgh, Pittsburgh, PA, United States; <sup>11</sup>RTI International, Research Triangle Park, NC, United States.

**Introduction:** Objective: To examine the frequency of isolated fetal abdominal circumference (AC  $< 10\%$  with estimated fetal weight EFW  $> 10\%$ ) and to compare perinatal outcomes in those with isolated AC  $< 10\%$  to those with EFW  $< 10\%$  (regardless of AC%)

**Methods:** This was a secondary analysis of a prospective longitudinal cohort of nulliparous women. Women with singleton pregnancies

who underwent ultrasound between 22weeks0days-29 weeks6days were included. Participants were divided into 3 groups according to ultrasound visit measurements: 1) EFW<10% 2) AC<10% with EFW≥10% (isolated AC<10%) and 3) EFW≥10% and AC≥10% (normal growth). Primary outcome was a neonatal composite: perinatal death, small for GA (birthweight<10%) or NICU admission. Secondary outcomes included birthweight, gestational age at delivery, hypertensive disorder of pregnancy and Apgar scores. We excluded women with missing data, incomplete ascertainment of primary outcome, fetal anomalies or EFW>90%. We fit a logistic regression model to adjust for cofounders. **Results:** Of 10,038 women, 7,284 participants met inclusion criteria. 1.3% (n=100) had EFW<10%, 1.6% (n=120) had isolated AC<10% and 96.9% (n=7064) had normal growth. Baseline characteristics were similar except for maternal age, race/ethnicity, education, smoking and alcohol use. The primary outcome was significantly more common in women with EFW<10% (67%) compared to women with either normal growth (22%) or isolated AC<10% (42%), p<0.01. In adjusted analysis, women with EFW<10% had higher rates of primary outcome compared to isolated AC<10% (aOR 3.09, 95%CI 1.42,6.69), while women with isolated AC<10% had higher rates compared to those with normal growth (aOR 2.13, 95%CI 1.27,3.57). Other outcomes were similar between women with isolated AC<10% and women with EFW<10%. **Conclusion:** Compared to normal fetal growth, FGR defined by isolated AC<10% was associated with increased risk of adverse perinatal outcomes but not to the extent of women with EFW<10%.

#### T-118

**Intrauterine Hypoxia (HPX) Dysregulates Mitochondrial Respiratory Complex Expression and Activity in Fetal Guinea Pig (GP) Hearts.**  
 Hong Song, Loren P. Thompson\*. *Univ. of Maryland SOM, Baltimore, MD, United States.*

**Introduction:** Chronic intrauterine HPX is a major cause of fetal growth restriction and altered fetal heart maturation. Regulation of energy metabolism is important for both the metabolic switch and transition to cellular hypertrophy that occurs in the fetal heart prior to birth. We hypothesized that intrauterine HPX targets the fetal cardiac mitochondria in disrupting respiration, contributing to developmental programming of the offspring heart. We measured the effects of HPX on indices of mitochondrial respiratory function in fetal GP heart ventricles.

**Methods:** Pregnant GPs were exposed to either normoxia (NMX, N=7) or early- (25d gestation, 40d duration, N=7) and late-onset (50d gestation, 14d duration, N=7) HPX (10.5%O<sub>2</sub>) (term ~65d) to determine the effects of timing of HPX exposure. Near-term (64d gestation) male and female fetuses (N=7 each group, 2 per litter) were obtained by cesarean section of anesthetized sows and fetal body, placenta, and organ weights measured. Left cardiac ventricles were excised and frozen in liquid N<sub>2</sub> and stored at -80C. Cardiac mitochondria were isolated and respiratory chain complex (CI-V) subunit protein levels were measured by Western blot and CI and CIV catalytic rates by colorimetric assays.

**Results:** Chronic HPX slightly decreased fetal body weight and increased relative placenta weight. Early-onset HPX increased (P<0.05) CI, CIII, and CV protein levels by 15.2%, 17.4%, and 34.9%, respectively, increased (P<0.05) CI activity (2.83±0.41 vs 3.74±0.25 ΔOD/min, NMX vs HPX) but decreased (P<0.05) CIV activity (1.32±0.27 vs 0.62±0.08) in male but not female hearts. Late-onset HPX also increased CI and decreased CIV activities in males but not females. However, in contrast to early-onset, late-onset HPX decreased (P<0.05) CI, CIII, and CIV levels in both males and females.

**Conclusion:** Chronic HPX reduces fetal growth in the presence of compensated placenta growth. Yet, HPX differentially disrupts fetal cardiac protein expression and enzyme activities of select complexes of the respiratory chain. Functionally, the increase in CI and decrease in CIV activities with HPX could generate excessive electron build up along the chain, reducing respiratory efficiency and generating free radicals. The sexual dimorphic response of protein levels reflects both compensation with early onset and decompensation with late onset

HPX in cardiac mitochondria. Thus, chronic HPX could dysregulate mitochondrial function in the fetal GP heart, which may be a causal factor in developmental programming. (NIH HL 126859).

#### T-119

**Translatable In Vivo Fetal Cardiac Geometry and Function in Adverse Pregnancy: A Comparison between Human Fetal Growth Restriction and Progressive Hypoxic Pregnancy in Sheep.**

Olga V Patey†,<sup>1,2</sup> Kimberley L Botting,<sup>1</sup> Youguo Niu,<sup>1</sup> Lin Zhang,<sup>1</sup> Sage G Ford,<sup>1</sup> Wen Tong,<sup>1</sup> Conrado M Coutinho,<sup>2</sup> Basky Thilaganathan,<sup>2</sup> Dino A Giussani\*.<sup>1,1</sup> *University of Cambridge, Cambridge, United Kingdom; <sup>2</sup>St. George's University NHS Foundation Trust, London, United Kingdom.*

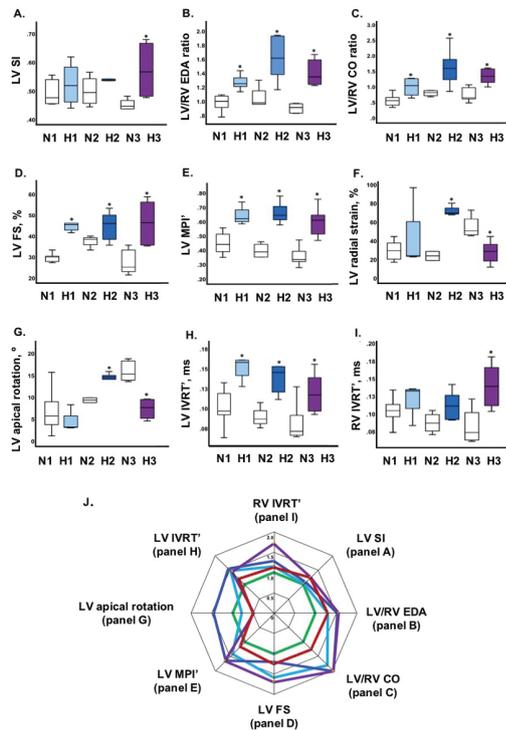
**Introduction:** Pregnancy complicated by FGR and hypoxia both trigger fetal cardiac dysfunction (Crispi et al. *Fet Diag Ther* 47:337, 2020). However, whether fetal heart changes in FGR pregnancy reflect *in utero* chronic hypoxia remains unclear. Compared with conventional tools, modern Tissue Doppler Imaging (TDI) and Speckle Tracking Echocardiography (STE) can better predict cardiac dysfunction. Here, we compare fetal heart changes using novel *in vivo* imaging modalities between pregnant sheep undergoing progressive hypoxia and a human cohort of FGR pregnancy.

**Methods:** We studied 40 sheep and 87 human singleton pregnancies. Ewes experienced hypoxia (10%O<sub>2</sub>) in isobaric chambers for 11±1 (H1, n=5), 17±2 (H2, n=6) or 32±2 (H3, n=6) days. Bi-ventricular fetal comprehensive echocardiography at the end of exposure was compared with gestation-matched normoxic sheep pregnancies: 117±0.9 days gestation (dGA; term is ~150 days; N1, n=12), 124±1 dGA (N2, n=5) and 133±3 dGA (N3 (n=6). In clinic, the same protocol, ultrasound system and software (Vivid iq, EchoPAC) were applied to 54 control (39±0.5 weeks) and 33 FGR (38±1 weeks) human pregnancies (Fetal Medicine Unit, St George's, London).

**Results:** Restricted to controls with expected RV dominance, hypoxic fetal sheep showed a progressive switch to LV dominance (increased LV sphericity, LV/RV size and LV/RV output, Fig.1A-C). LV shortening fraction and the LV myocardial performance index were greater for all H1-H3 groups (Fig.1D&E). While myocardial deformation intensified in H2, these indices were lower in H3 relative to control fetuses (Fig.1F&G). Hypoxic fetal sheep showed bi-ventricular diastolic dysfunction, with LV impairment preceding RV diastolic function (Fig.1H&I). Human FGR pregnancy showed fetal LV preponderance and cardiac dysfunction similar to hypoxic fetal sheep. However, alterations in human FGR best corresponded to fetal sheep between H1 and H2 groups (Fig.1J-spidergram).

**Conclusion:** Progressive hypoxia in fetal sheep switches RV to LV dominance, with consequent fetal cardiac remodelling and function, triggered by fetal brain sparing. Human FGR pregnancy shows striking similarity, suggesting a primary role for chronic hypoxia in triggering fetal cardiac remodelling and dysfunction in FGR pregnancy.

*Support: British Heart Foundation*



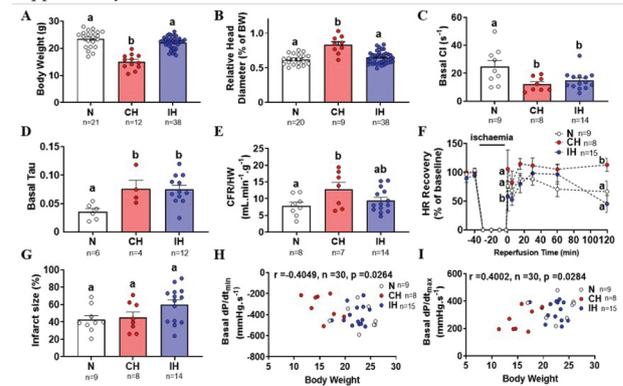
**Figure 1.** Comparison of echocardiographic data derived from hypoxic fetal sheep and human growth restricted fetuses. **Panel A-I:** Box plots. Alterations in cardiac variables in fetal sheep after 11 days (H1 – light blue), 17 days (H2 – dark blue) or 32 days (H3 – purple) of chronic hypoxia compared to gestation-matched normoxic controls (sheep N1, N2, and N3 – white). Significant ( $P < 0.001$ ) differences are: \* vs. normoxia, two-way ANOVA. **Panel J:** The spider plot shows similar patterns in significant ( $P < 0.001$ ) alterations of cardiac variables in FGR human fetuses (red) compared with 11-day (light blue), 17-day (dark blue), and 32-day (purple) hypoxic fetal sheep relative to control human and sheep pregnancies (green). Healthy controls in both cases are presented as a value of 1. CO, cardiac output; EDA, end-diastolic area; FS, fraction shortening; IVRT', isovolumetric relaxation time; LV, left ventricle; MPI', myocardial performance index; RV, right ventricle; SI, sphericity index. Myocardial performance index = (isovolumetric contraction time' + isovolumetric relaxation time')/ejection time' derived by pulsed wave tissue Doppler technique. Shortening fraction = (ventricular end-diastolic dimension-end-systolic dimension)/end-diastolic dimension  $\times 100$ . Sphericity Index = ventricular end-diastolic dimension/ventricular end-diastolic length. Myocardial time-intervals were normalized by cardiac cycle length adjusting for heart rate.

size analysis. Statistical comparisons were made using one- or two-way RM ANOVA and correlations determined using the Pearson correlation. n numbers are shown in the Figure.

**Results:** Relative to N, CH but not IH promoted asymmetric growth restriction (Fig.1 A & B). However, both CH and IH induced similar cardiac systolic and diastolic dysfunction (Fig.1 C & D). Only CH, but not IH, increased basal CFR and improved cardiac recovery to IR. However, no treatment affected infarct size (Fig. 1 E, F, G). Across groups, embryo weight was directly related to systolic function and inversely related to diastolic function (Fig. 1 H & I).

**Conclusion:** The data show that IH for half a day for the duration of the incubation period has a direct negative impact on cardiac systolic and diastolic function in the chicken embryo, in similar fashion to development under CH. However, unlike CH, IH does not reduce growth and it does not improve cardiac recovery post IR in the chicken embryo. Thus, OSA in pregnancy may adversely affect fetal cardiac function.

Supported by The Lister Institute and The British Heart Foundation



**Figure 1.** The data show the mean  $\pm$  SEM for A, body weight (BW); B, relative head diameter (as a % of BW); C, basal contractility index (CI), calculated as the maximum first derivative of left ventricular pressure ( $dp/dt_{max}$ ) divided by pressure at this time point; D, basal time constant of left ventricular relaxation (Tau); E, coronary flow rate as a proportion of heart weight (CFR/HW); F, heart rate (HR) recovery to IR and H, infarct size. Different letters are significantly different ( $P < 0.05$ ; one- or two-way RM ANOVA). Correlations (Pearson) between BW and basal  $dp/dt_{max}$  and basal minimum first derivative of left ventricular pressure ( $dp/dt_{min}$ ) are shown in H and I, respectively.

T-120

**Modelling Obstructive Sleep Apnoea in Pregnancy: Direct Effects of Intermittent Hypoxia on Embryonic Cardiac Function and Growth.**

Anna L K Cochrane†, Youguo Niu, Sage G Ford, Dino A Giussani\*. University of Cambridge, Cambridge, United Kingdom.

**Introduction:** Obstructive sleep apnoea (OSA) increases during pregnancy (Fung et al. *J Perinatol*. 32(6):399, 2012). However, the effects of maternal intermittent hypoxia (IH) on the fetal heart are completely unknown. This is important because IH is a much greater stimulus of oxidative stress, and it is known that chronic hypoxia (CH), secondary to oxidative stress, induces a fetal origin of heart disease (Giussani & Davidge, *J DOHaD* 4(5):328, 2013). Here, we have used a chicken embryo model to isolate the direct effects of IH on the developing heart, independent of effect on the mother and/or placenta. The study tested the hypothesis that IH, compared with CH, has greater direct detrimental effects on the developing heart.

**Methods:** Fertilised chicken eggs were incubated under normoxia (N; 21% O<sub>2</sub>), CH (13.5% O<sub>2</sub>) or IH (15 cycles/h between 21 and 13.5% O<sub>2</sub> for 12h/day) from day 1 (term is 21 days). On day 19, following biometry, embryonic hearts were isolated and mounted on a Langendorff preparation to determine cardiac function during baseline and following an ischaemia/reperfusion (I/R) challenge (30 min ischaemia, 120 min reperfusion). Coronary flow rate (CFR) was calculated gravimetrically. Hearts were then sectioned and stained (triphenyltetrazolium) for infarct

T-121

**Impaired Expression of Mechanosensing Genes in Human Pregnancy Complicated by Fetal Growth Restriction and Chronic Hypoxia.**

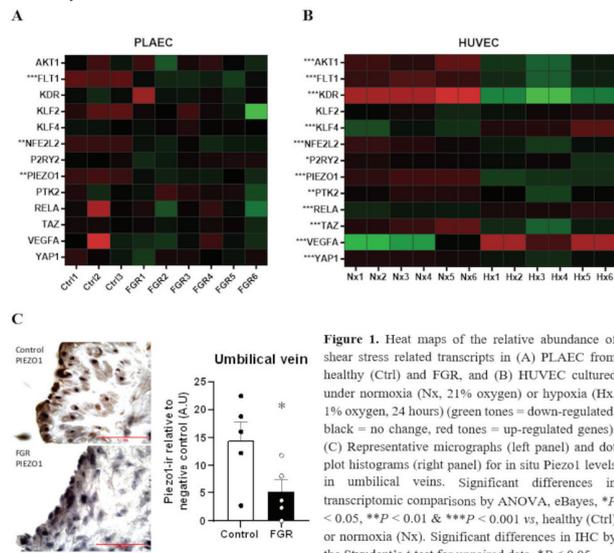
German Arenas,<sup>1</sup> Estefania Peñaloza†,<sup>2</sup> Dino A Giussani\*,<sup>3,3,3</sup> Bernardo J Krause\*,<sup>2</sup> Pontificia Univ. Católica de Chile, Santiago, Chile; <sup>2</sup>Universidad de O'Higgins, Rancagua, Chile; <sup>3</sup>University of Cambridge, Cambridge, United Kingdom.

**Introduction:** The mechanosensitive cation channel Piezo1, a shear stress sensor, has been implicated in eNOS activation and endothelial-dependent relaxation (Wang et al., *J Clin Invest*. 2016). Placental vascular tone is tightly regulated by local cues, such as endothelial-derived factors released in response to changes in blood flow and shear stress. However, nothing is known about the role of Piezo1 in control pregnancy or development complicated by fetal growth restriction (FGR) or chronic hypoxia. Here, we tested the hypothesis that the expression of Piezo1 among other mechanosensing genes is decreased by FGR and hypoxia in the umbilical and placental endothelium in human pregnancy.

**Methods:** Transcriptome datasets from placental endothelial cells (PLAEC) from healthy and FGR pregnancies, and from umbilical vein endothelial cells (HUVEC) exposed to hypoxia *in vitro* were analyzed using the Transcriptome Analysis Console 4.0. Additionally, *in situ* levels of Piezo1 protein were determined by immunohistochemistry of paraffin-embedded sections of human umbilical cords from healthy and FGR pregnancies.

**Results:** Transcriptome analysis of FGR PLAEC showed downregulation of several genes [71 out of 78,  $p < 0.01$ , False discovery rate (FDR)  $< 0.05$ ] related to mechanosensing pathways (i.e. focal adhesion-P13K-Akt). This effect was also observed in HUVEC exposed to hypoxia *in vitro* 83 out of 146,  $p < 0.01$ , FDR  $< 0.05$ ). Further analysis showed that both FGR

(PLAEC) and hypoxia (HUVEC) were associated with decreased levels of Piezo1 and other key shear stress-related transcripts (Fig. 1 A&B). Analysis of Piezo1 protein in the endothelium of umbilical vessels showed decreased levels in FGR umbilical veins compared with control (Fig. 1C). **Conclusion:** Reduced Piezo1 expression provides a molecular link between impaired endothelial-dependent relaxation and human pregnancy complicated by FGR and chronic fetal hypoxia. Piezo-1 is a clear therapeutic target in human complicated pregnancy. *Supported by Fondecyt 1181341, The Wellcome Trust and The British Heart Foundation*



## T-122

### Dexamethasone Reduces 11 $\beta$ -Hydroxysteroid Dehydrogenase 1 Expression the Fetal Lung Following Hypoxic Pregnancy.

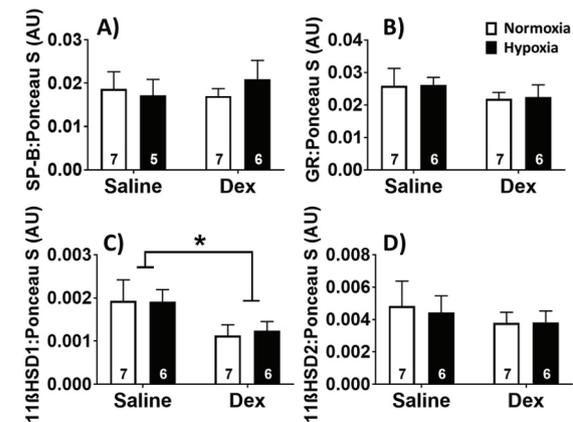
Mitchell C Lock,<sup>1</sup> Kimberley J Botting,<sup>2</sup> Youguo Niu,<sup>2</sup> Sage G Ford,<sup>2</sup> Sandra Orgeig,<sup>1</sup> Dino A Giussani,<sup>2</sup> Janna L Morrison\*.<sup>1,1</sup> *University of South Australia, Adelaide, Australia; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.*

**Introduction:** Chronic fetal hypoxia is commonly associated with intrauterine growth restriction (IUGR), defined as a birth weight below the 10th percentile for gestational age. IUGR babies are at increased risk of premature delivery and thus also to antenatal exposure to dexamethasone, a synthetic glucocorticoid used clinically to promote lung maturation during late gestation. Though the use of glucocorticoids in premature birth has been well studied, their effect on fetal lungs that have experienced chronic hypoxia has not yet been fully explored.

**Methods:** Under general anaesthesia, sheep bearing a singleton male fetus were surgically instrumented with femoral catheters at 100d gestation (term, 145d). Ewes were then randomly assigned to Normoxia Saline (21% O<sub>2</sub>, 2ml vehicle), Hypoxia Saline (10-11% O<sub>2</sub>), Normoxia Dexamethasone (2 i.m. injection containing 12mg of dexamethasone 24h apart at 115 and 116d gestation) or Hypoxia Dexamethasone groups. Chronic hypoxia for 1 month was induced in isobaric chambers (Brain et al. *Physiol Rep* 3(12): e12614, 2015). At 138d gestation animals were humanely killed (sodium pentobarbitone). Fetal lungs were frozen in liquid N<sub>2</sub> for total protein extraction. Western blot was used to determine the expression of proteins involved in surfactant maturation and glucocorticoid regulation within the fetal lung. Data were analysed by Two-way ANOVA.

**Results:** Hypoxia did not significantly alter the expression of surfactant protein B involved in surfactant maturation in fetal lungs [*SFTPB* (Fig. 1A)]. There was no effect of hypoxia or dexamethasone treatment on the expression of Glucocorticoid receptor [GR (Fig. 1B)]. However, fetal lungs from dexamethasone treated pregnancy showed decreased expression of 11 $\beta$ -Hydroxysteroid dehydrogenase 1 [11 $\beta$ HSD1 (Fig. 1C)], the enzyme responsible for conversion of inactive cortisone to cortisol.

**Conclusion:** We show that maternal hypoxia in the late gestation fetal lung did not affect fetal lung maturation regardless of dexamethasone treatment. Although there was no change in the expression of Glucocorticoid receptor, there was a reduction in the enzyme responsible for conversion of cortisone to cortisol in the lung due to dexamethasone treatment, likely due to reduced reliance on endogenous glucocorticoid.



**Figure 1.** Expression of Surfactant Protein -B (*SFTPB*; A), Glucocorticoid Receptor (*GR*; B) 11 $\beta$ -Hydroxysteroid dehydrogenase 1 (11 $\beta$ HSD1; C) and 2 (11 $\beta$ HSD2; D) in the fetal lung following normoxic or hypoxic pregnancy with and without dexamethasone treatment. Data analysed by Two-way ANOVA (Numbers within columns; sample size, \*;  $P < 0.05$ ).

## T-123

### Neuroinflammatory Pathways Investigated Using Neural Stem Cells.

Keith A Kwan Cheung<sup>†</sup>, Pevindu H Abeyasinghe<sup>†</sup>, James M Bassett<sup>†</sup>, Murray D Mitchell\*. *Queensland University of Technology, Brisbane, Australia.*

**Introduction:** Recently, neuroinflammation has been posited to be a major mechanism in the aetiology and progression of many neurodevelopmental disorders (such as epilepsy and autism), especially during the gestation and early development of newborns. However, investigating human neuroinflammatory pathways in newborns is very challenging *in vivo*. To better investigate the complex relationships behind neuroinflammation and neurodevelopment, human immortalised neural stem cells can be used as viable experimental models. Cytokines and prostaglandins are crucial components of inflammation, but prostaglandins' roles in neuroinflammation are still ill-defined. Therefore, developing a valid *in vitro* model using neural stem cells can provide a way to better understand the interactions between prostaglandins, cytokines and neuroinflammation during gestation and early-stage development.

**Methods:** ReNcells CX and VM (cortical and ventral mesencephalon fetal neural stem cell lines, respectively) were acquired from Merck. Cells were seeded in 24-well plates (70 000 cells per well) for 24 hours and treated with 10 ng/mL IL-1 $\beta$  for 24 hours, after which the media was collected from each treated well. PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub>  and IL-6 production (pg/mL/well) was calculated using measurements in medium by ELISA. A sigmoidal equation (4-parameter logistic and X is concentration) was used to interpolate the unknown from the standard curve, following the manufacturer's instructions.

**Results:** ReNcell CX production of IL-6 (29.71  $\pm$  0.01428 pg/well/24hr) was significantly increased by 353.33  $\pm$  1.023412 percent in response to 10 ng/mL IL-1 $\beta$ , compared to untreated control. In contrast, PGE<sub>2</sub> production (29.7  $\pm$  4.4 pg/well/24hr) was decreased by 19.83  $\pm$  26.98 percent and PGF<sub>2 $\alpha$</sub>  production (6.9  $\pm$  4.76 pg/well/24hr) was decreased by 62.25  $\pm$  31.14 percent in response to 10 ng/mL IL-1 $\beta$ , compared to untreated control. ReNcell VM production of IL-6 (92.58  $\pm$  0.01428 pg/well/24hr) was increased by 1977.731  $\pm$  6.76 percent in response to 10 ng/mL IL-1 $\beta$ , compared to untreated control. However, the same treatment showed that production of PGE<sub>2</sub> (62.45  $\pm$  9.595pg/well/24hr) was decreased by

33.9168 ± 25.40 percent and  $\text{PGF}_{2\alpha}$  ( $14.25 \pm 5.34 \text{ pg/well/24hr}$ ) production was increased by 88.43921 ± 47.10 percent in response to 10 ng/mL IL-1 $\beta$ , compared to untreated control. Data are represented as mean ± SEM, N=3.

**Conclusion:** Our results show that 10 ng/mL IL-1 $\beta$  treatment for 24 h substantially increased production of IL-6 in ReNcell CX and VM. In contrast,  $\text{PGE}_2$  shows decreased production by both ReNcell CX and VM in response to 10 ng/mL IL-1 $\beta$  treatment for 24 h. These results provide a basis for greater insights into inflammatory mediator regulation by neural stem cells.

### T-124

#### IFN $\gamma$ -Producing Gamma/Delta T Cells in the Fetal Brain Following Intrauterine Inflammation: Possible Mechanism of Fetal Neuronal Injury.

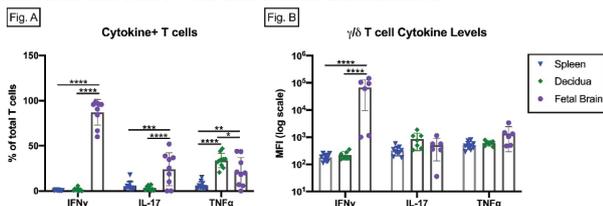
Emma L Lewis†, Natalia Tulina, Michal A Elovitz\*. *University of Pennsylvania, Philadelphia, PA, United States.*

**Introduction:** Intrauterine inflammation is associated with adverse neurobehavioral outcomes from schizophrenia to autism spectrum disorder. However, the mechanisms remain largely unknown. To address this gap in knowledge, this study sought to assess how inflammation transverse across multiple anatomic compartments in the maternal-fetal dyad and what specific immune cell types in the fetal brain may mediate long-term neuronal injury.

**Methods:** We utilized a well-established mouse model of intrauterine inflammation causing fetal brain injury. Timed-pregnant CD-1 mice received intrauterine injections of 50 $\mu\text{g}$  LPS or saline at E15. At 48 and 72 hours post-injections, dams were sacrificed and maternal spleen, uterus, placenta, fetal liver and fetal brain were analyzed for broad immune cell composition by flow cytometry (N=8-9 dams/treatment/time point). In follow-up experiments at 72 hours post-LPS injection, CD3+ cells were isolated from the maternal spleen, decidua, and fetal brain, stimulated *in vitro*, and stained for intracellular cytokine production (N=10).

**Results:** At 48 hours post-injection, neutrophils are elevated in the decidua ( $4 \text{ v. } 21 \times 10^3$ ,  $p < 0.0001$ ), placenta ( $9 \text{ v. } 13 \times 10^3$ ,  $p = 0.01$ ), and amniotic fluid ( $0.7 \text{ v. } 4 \times 10^3$ ,  $p = 0.01$ ) of LPS-treated dams. At 72 hours post-injection, but not 48 hours, neutrophils ( $19 \text{ v. } 43 \times 10^3$ ,  $p = 0.02$ ) and macrophages ( $22 \text{ v. } 33 \times 10^3$ ,  $p = 0.006$ ) are elevated in the fetal livers of LPS-treated dams. In LPS-treated dams,  $\gamma/\delta$  T cells are increased in fetal brains at 48 hours ( $230 \text{ v. } 750$ ,  $p = 0.003$ ) and 72 hours post-injection ( $110 \text{ v. } 470$ ,  $p = 0.02$ ). By 72 hours there also are increases in activated microglia ( $0.8 \text{ v. } 2 \times 10^3$ ,  $p = 0.0005$ ), granulocytes ( $0.3 \text{ v. } 2 \times 10^3$ ,  $p = 0.04$ ) and monocytes ( $1.6 \text{ v. } 3.3 \times 10^3$ ,  $p = 0.004$ ) in the fetal brain.  $\gamma/\delta$  T cells were more activated and differentiated to produce IFN $\gamma$ , in the fetal brain compared to maternal tissues, both in terms of the frequency of T cells making IFN $\gamma$  (Fig. A,  $p < 0.0001$ ) and the amount of IFN $\gamma$  produced (Fig. B,  $p < 0.0001$ ).

**Conclusion:** The presence of IFN $\gamma$  producing  $\gamma/\delta$  T cells, followed by an increase in activated microglia in the fetal brains of mice exposed to intrauterine inflammation, is a potential mechanism of fetal neuronal injury. Microglia exposure to IFN $\gamma$  is known to activate a neurotoxic microglial state, and prenatal exposure can create lasting alterations to microglial epigenetic states, which explain the long-term neurobehavioral outcomes associated with intrauterine inflammation.



### T-125

#### Ovarian Cancer Heterogeneity and Their Association with Differential Secretion of Extracellular Vesicles in Response to Hypoxia.

Nihar Godbole†,<sup>1</sup> Sharma Shayna†,<sup>1</sup> Priyakshi Kalita-de Croft†,<sup>1</sup> Carlos Salomon\*,<sup>1,2</sup> *The University of Queensland, Brisbane, Australia;* <sup>3</sup>*University of Concepcion, Concepcion, Chile.*

**Introduction:** Ovarian cancer is a broad term, encompassing a heterogeneous population of tumours, with both ovarian, and related origins. Recently, we have described that extracellular vesicles (EVs) are involved in ovarian cancer progression through regulation of the epithelial to mesenchymal transition, metastasis, and response to chemotherapy. Interestingly, the effect of EVs are enhanced under low oxygen tension, suggesting that hypoxia regulates the bioactivity of EVs. However, the term EVs comprises a heterogeneous family of vesicles, originating from different subcellular compartments. These EVs are of different sizes and have varying functions. Here, we evaluate the secretion of several EV populations, from a heterogenic panel of ovarian cancer cell lines, in response to low oxygen tension.

**Methods:** A panel of ovarian cancer cell lines were used in this study: low-grade serous (HEY), high-grade serous (SKOV-3, OV90, and CAOV-3), and clear cell (TOV-112D) and endometrioid (OVTOKO). Cells were cultured at 37°C (5% CO<sub>2</sub>-balanced N<sub>2</sub> to obtain 1% or 8% O<sub>2</sub>). Cell-conditioned media (CCM) was collected and different populations of EVs were isolated, and characterized using nanoparticle tracking analysis (NanoSight NS500). Different populations of EVs were classified according to size as i) exomere <35nm, ii) small exosomes (EXO) 35-80nm, iii) large EXO 80-200nm, and iv) large EVs >200nm. Two-way ANOVA was used to determine the effect of oxygen tension, and type of EVs, on the concentration of vesicles in the CCM, and Sidak's test was used for multiple comparisons analysis.

**Results:** No effects of hypoxia on cell viability were identified. Hypoxia significantly altered the release of different types of EVs from CAOV-3, SKOV-3, TOV-112D, OVTOKO, and OV90 cells (n=9, ANOVA,  $p < 0.01$ ), and no effects on HEY cells were identified. Hypoxia increased the release of small EXO (>20-fold) from the high-grade serous SKOV-3. Interestingly, higher levels of large EXO in CCM from SKOV-3, CAOV-3, TOV-112D, and OVTOKO cultured at 1% oxygen compared with 8% oxygen were observed. A significant (n=9,  $p < 0.05$ ) effect of low oxygen tension on the release of large EVs from the clear cells & endometrioid cell lines (TOV-112D, and OVTOKO) in comparison with 8% oxygen was observed.

**Conclusion:** We identified a specific pattern of secretion of different populations of EVs in response to low oxygen tension. We suggest that in response to hypoxia, different populations of EVs are secreted from ovarian cancer cells lines, and this is associated with morphological changes and their molecular heterogeneity.

### T-126

#### Applicability of Pre-Operative Patient Reported Duke Activity Scale Index (DASI) in Prediction of Postoperative Complications in Gynaecological Oncology.

Lusine Sevinyan†,<sup>1</sup> Anil Tailor,<sup>1</sup> Pradeep Prabhu,<sup>1</sup> Peter Williams,<sup>2</sup> Thumulu Kavitha Madhuri,<sup>1,3</sup> *Royal Surrey Hospital NHS Foundation Trust, Guildford, United Kingdom;* <sup>2</sup>*University of Surrey, Guildford, United Kingdom;* <sup>3</sup>*University of Brighton, Brighton, United Kingdom.*

**Introduction:** Increase in the incidence of gynaecological cancers has resulted in increased operative procedures, specifically in patients with multiple comorbidities including obesity and frailty. This is often associated with prolonged admission and higher rates of postoperative mortality and morbidity and presents a challenge with an unmet need for an accurate, personalised risk prediction. DASI is a 12 item scale in the form of self-reported questionnaire based around commonly performed activities of daily living. Currently, DASI is used to evaluate patients with cardiovascular diseases, however there is growing interest in utilising it in preoperative setting in different specialities. This study investigates the accuracy of DASI in preoperative prediction of postoperative outcomes in gynaecology.

**Methods:** A retrospective cohort study of 330 patients who had undergone an operative treatment at a tertiary oncology centre. Data collection undertaken through dedicated gynaecology database and missing data collected through patients' records. All patients had completed the DASI questionnaire prior to their consultation. Actual postoperative 30 day complications and the length of stay also recorded. DASI was then compared with the occurrence of postoperative complications.

**Results:** 181 patients had a *Da Vinci* robot-assisted procedure, 37 - laparoscopic and 112 - open surgery. 76/330 were classified as having any type of complications within 30 days of the operation. Our results have shown that the higher DASI score the less likely patients were to have postoperative complications. This result was statistically significant with odds ratio of 0.974 and confidence interval between 0.958 and 0.991. We were also able to demonstrate that for every 10 points further up the DASI score a patient was 0.768 times less likely to have a postoperative complication. Hence general morbidity prediction of DASI score has been found to statistically significantly predict postoperative complications (AUC=0.700).

**Conclusion:** Our study has shown that DASI self-reported score is a useful predictive tool of perioperative estimation of postoperative complications in the gynaecology setting. Further analysis with a larger sample size and a multicentre prospective study is currently underway to validate the findings.

Tumour site	
Uterus	163
Ovary	108
Tube	23
Cervix	26
Other	10

## T-127

### Placental Endocrine Function and Insulin-Like Growth Factor-2 (*Igf2*) Are Important Determinants of Maternal Metabolic State and Fetal Growth in Obese Mouse Pregnancies.

Samantha C Lean, Esteban Salazar Petres, Edina Gulacsi, Amanda N Sferuzzi-Perri\*. *University of Cambridge, Cambridge, United Kingdom.*

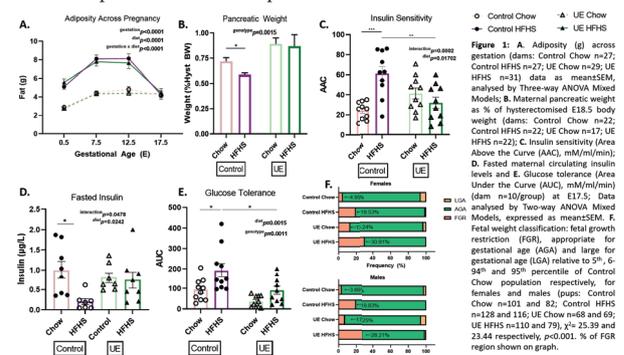
**Introduction:** In obesity, pregnancy-induced metabolic changes are often exacerbated contributing to fetal growth perturbations. Evidence suggests that maternal metabolism is modulated by placental endocrine function. Insulin-like growth factor-2 (*Igf2*) is an imprinted gene that regulates placental endocrine function (Aykroyd et al., 2020 *J Endo*) and *Igf2*'s placental expression is altered by maternal obesity (Sferuzzi-Perri et al., 2017 *J Physiol*). This study explored the interactions between obesity and placental endocrine function in determining materno-fetal outcomes.

**Methods:** Female *Igf2*Flox and *Tpba*-Cre mice were fed chow or high-fat, high-sugar (HFHS) diets for ~12wks. During which, they were mated with wildtype males to ensure fertility and then time-mated to *Tpba*Cre and *Igf2*Flox males to generate lean and obese dams control and placental endocrine zone-specific *Igf2* under-expression (*Iz-Igf2*UE; UE) litters. Maternal adiposity was assessed longitudinally by TdNMR. Insulin or glucose tolerance tests were performed at embryonic day (E)17.5. Mice at E18.5 were dissected to obtain pregnancy outcome data. Data were analysed by 2 or 3-way ANOVA and mixed model or  $\chi^2$  as required, significance at  $p < 0.05$ .

**Results:** Pre-pregnancy, HFHS fed mice had 19.3% adiposity vs 10.7% for chow fed. HFHS fed mice had a net loss of adiposity across gestation (Fig1A), ending pregnancy with comparable adiposity levels to chow fed; no differences were seen between genotypes. HFHS diet reduced pancreatic weight in control mice but increased by 25% in UE mice regardless of diet (Fig1B). Insulin sensitivity increased (Fig1C) and circulating fasted insulin decreased (Fig1D) in HFHS control but not UE dams versus their chow counterparts. HFHS UE dams had reduced insulin sensitivity compared to HSHF control dams. HFHS diet reduced glucose tolerance in control dams only (Fig1E). However, UE dams had greater glucose tolerance than controls overall. Fetal weight was reduced

by HFHS for both sexes, with more pups falling below 5<sup>th</sup> centile of the control chow group; an effect that was more pronounced in the UEs (Fig1F). HSHF reduced placental weight by 10% in females only. No effect of UE on placental weight was seen.

**Conclusion:** Placental endocrine function and *Igf2* are important determinants of maternal adiposity, insulin sensitivity, glucose handling and fetal growth in obese pregnancies. The mechanisms underlying this relationship still need to be explored.



## T-128

### Maternal Aging Impacts Vascular Adaptations to Pregnancy.

Mazhar Pasha, Raven Kirschenman, Amy Wooldridge, Floor Spaans, Sandra Davidge, Christy-Lynn Cooke. *University of Alberta, Edmonton, AB, Canada.*

**Introduction:** Advanced maternal age ( $\geq 35$  years) increases the risk of pregnancy complications, which may be due to vascular maladaptations to pregnancy. Aging is associated with vascular stiffness and endothelial dysfunction, potentially via oxidative stress, reduced nitric oxide (NO) and increased endothelin-1 (ET-1) levels. However, whether these vascular changes affect vascular adaptations during aged pregnancies and contribute to endothelial dysfunction remains unknown. We hypothesize that maternal aging impairs vascular adaptations, due to altered NO and ET-1-dependent mechanisms.

**Methods:** Pregnant young (4 months) and aged (9.5 months of age; ~35 year in humans) rats (n=6-10/group) were studied on gestational day 20 (term=22 days) and compared to age-matched non-pregnant rats. Blood pressure was measured with the CODA tail-cuff system and rats were euthanized for *ex vivo* vascular studies. Mesenteric artery endothelium-dependent relaxation to methylcholine (MCh) was assessed in the presence/absence of pan nitric oxide [NO] synthase inhibitor (L-NAME), or NADPH oxidase inhibitor (Apocynin). Big-endothelin-1 (bET-1) vasoconstriction responses in presence/absence of endothelin converting enzyme (ECE) inhibitor (CGS) were evaluated. Data were analyzed by two-way ANOVA with Sidak's post-test,  $p < 0.05$  was considered significant.

**Results:** Mean arterial pressure (MAP) was only elevated in aged non-pregnant rats (121.1±2.89 mmHg;  $p = 0.0001$ ), while similar between aged pregnant (101.3±2.60 mmHg), young non-pregnant (100.2±3.51 mmHg), and young pregnant rats (95.2±2.59 mmHg). Mch-induced vasodilation responses were not different between groups. However, pre-treatment with L-NAME decreased maximum vasodilation (Emax) in both young (control vs L-NAME: 99.22±0.41% vs 87.86±4.92%;  $p = 0.018$ ) and aged pregnant rats (98.9±0.47% vs 83.30±5.30%;  $p = 0.001$ ) but not in non-pregnant rats. Apocynin increased MCh sensitivity ( $pEC_{50}$ ) only in aged non-pregnant rats (control vs apocynin: 7.22±0.04 vs 7.54±0.08;  $p = 0.012$ ). Pre-treatment with CGS decreased big-ET-1 responses (area under the curve) in aged animals only (non-pregnant: 1.24±0.18 vs 0.55±0.09;  $p = 0.003$ ; pregnant: 1.14±0.15 vs 0.53±0.10;  $p = 0.003$ ).

**Conclusion:** Elevated MAP and NADPH oxidase-mediated modulation of vascular function in non-pregnant aged rats may suggest a constrictive systemic vasculature that was not evident in pregnant aged vasculature. ECE-mediated big-ET conversion was increased in aging regardless of pregnancy state; whereas NO contribution to vasodilation was increased

by pregnancy, independent of age. We speculate that in healthy aged vasculature, pregnancy may confer vascular protection. However, maternal aging is often associated with co-morbidities, thus future studies are needed.

### T-129

#### Circulating Placental Alkaline Phosphatase Correlates with Systemic Changes in Cardiovascular Function.

Maria Cristina Bravo, Carole McBride, Kathleen Brummel-Ziedins, Ira Bernstein. *University of Vermont, Colchester, VT, United States.*

**Introduction:** Recent research has begun to investigate changes in microvesicle (MV) concentration and function during pregnancy. We hypothesized that syncytiotrophoblast derived microvesicle (SDMV) concentration would be linked to cardiovascular changes during pregnancy.

**Methods:** This is a retrospective sub-analysis of a prospective study that had three study visits (follicular phase (V1), late first trimester at 12-14 wks (V2), mid third trimester at 29-34 wks (V3) in nulliparous women achieving a stable pregnancy (N=15). We examined plasma levels of placental alkaline phosphatase (PLAP) activity as a surrogate for circulating SDMVs at all visits and examined their relationship to changes in cardiovascular function. Cardiac output was measured using echocardiographic techniques. Continuous arterial blood pressures were measured non-invasively and mean arterial pressure (MAP) determined. Uterine blood flow was measured by uterine artery color Doppler ultrasound examinations at V1 and V2. All measurements and samples were collected prior to clinical diagnosis of any hypertensive disorder (late onset preeclampsia (PE) subsequently diagnosed in n=4 women). Correlation analyses were made for single visits and in comparison to the change of a measurement across two visits as indicated.

**Results:** PLAP activity levels at V3 ranged from 13.3-82.9  $\mu\text{g}/\text{mL}$  ( $44.2 \pm 18.3 \mu\text{g}/\text{mL}$ ); levels of PLAP at V1 and V2 ranged from 0-5.3  $\mu\text{g}/\text{mL}$  and 0-4.7  $\mu\text{g}/\text{mL}$ , respectively. V3 levels of PLAP and MAP had a correlation of  $r = 0.479$  ( $p = 0.07$ ), but PLAP showed no correlation with cardiac output at V3 ( $r = 0.013$ ) or with V2 measurements of uterine blood flow ( $r = 0.107$ ). Levels of PLAP at V3 were not different between normotensive pregnancies ( $44.3 \pm 21.3 \mu\text{g}/\text{mL}$ ) and pregnancies that were subsequently diagnosed with PE ( $44.0 \pm 7.4 \mu\text{g}/\text{mL}$ ). The changes in MAP and cardiac output between V2 and V3 were then examined relative to V3 levels of PLAP. A correlation was observed between PLAP and 1) the change in MAP ( $r = 0.602$   $p = 0.0502$  in normotensive pregnancies,  $r=0.569$   $p = 0.03$  all pregnancies ) and 2) the change in cardiac output ( $r = 0.597$   $p = 0.053$  in normotensive pregnancies,  $r=0.308$   $p = 0.26$  all pregnancies).

**Conclusion:** These findings indicate that changes in systemic maternal cardiovascular function, but not early uterine perfusion, correlate with changes in SDMV abundance as measured using PLAP activity as a surrogate. The correlation between systemic PLAP levels and changes in MAP in all pregnancies indicates that the subsequent development of PE in nulliparous women did not present as independent influences on systemic PLAP levels earlier in the third trimester.

### T-130

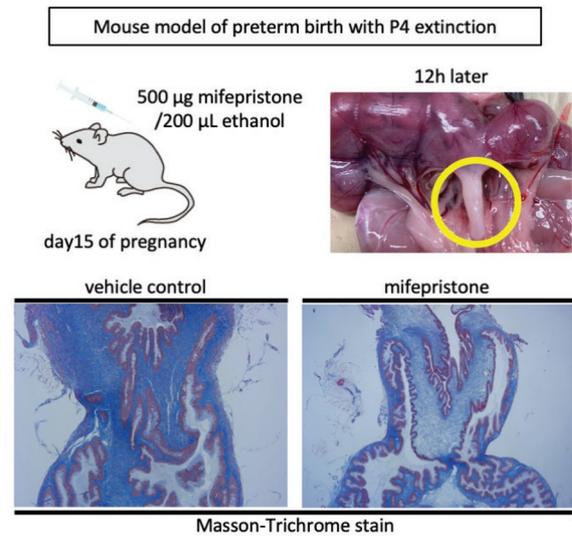
#### Progesterone Withdrawal Induces Eosinophilic Inflammation in the Process of Mouse Cervical Ripening.

Yosuke Sugita, Yoshimitsu Kuwabara\*, Shigeru Matsuda, Yumiko Oishi, Toshiyuki Takeshita. *Nippon Medical School, Tokyo, Japan.*

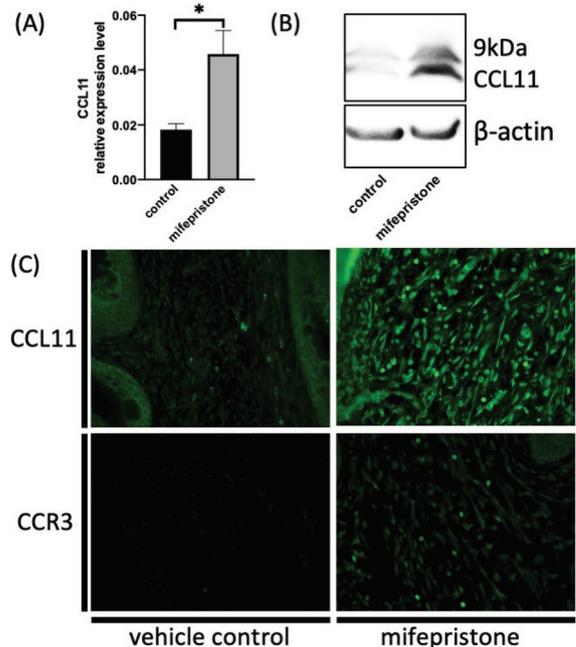
**Introduction:** Cervical ripening is a sterile process that induces an inflammatory response characterized by immune cell infiltration into the cervical stroma, and has been speculated to be triggered by functional progesterone (P4) withdrawal. In this study, we newly identified and analyzed the inflammatory markers associated with the cervical histological changes in a mouse model of preterm birth triggered by the anti-P4 drug.

**Methods:** C57BL/6J mice on day 15 of pregnancy were injected subcutaneously with a progesterone receptor antagonist (500  $\mu\text{g}$  mifepristone) or a solvent containing 200  $\mu\text{L}$  ethanol (n=5 vs. n=5), and each uterine cervix was collected 12 h later. RNA was extracted from the tissue and a polymerase chain reaction (PCR) array targeting

inflammatory cytokines and their receptors was performed. Molecules upregulated by mifepristone administration were subjected to comparative analysis by real-time PCR, western blotting (WB) of extracted proteins, and immunohistochemistry (IH) of tissue sections.



**Results:** Mice treated with mifepristone showed cervical ripening changes characterized by swelling of the cervical stroma (Figure 1). PCR array and real-time PCR identified the chemokine CCL11 (eotaxin-1), a major chemotactic factor of eosinophils, as a molecule whose expression was markedly increased by mifepristone treatment (Student's t-test  $P < 0.05$ ) (Figure 2 A). In WB, a 9 kDa band corresponding to CCL11 was clearly seen after mifepristone treatment (Figure 2 B). Based on IH results, CCL11 expression was enhanced throughout the cervical epithelium and stroma. Furthermore, the eosinophil cell surface marker CCR3, a specific receptor for CCL11, was widely detected, suggesting that eosinophil infiltration of the cervical tissue had occurred (Figure 2 C).



**Conclusion:** Eosinophilic inflammation caused by upregulation of CCL11 is suggested to be involved in the molecular mechanisms of cervical ripening triggered by P4 withdrawal.

**T-131****Maternal Obesity during Pregnancy Induces Oxidative Stress and Mitochondria Functional Alterations in Sheep Maternal Liver.**

Luis F Grilo†,<sup>1</sup> João D Martins†,<sup>1</sup> Mariana S Diniz†,<sup>1</sup> Carolina Tocantins†,<sup>1</sup> Chiara H Cavallaro†,<sup>1</sup> Inês Baldeiras,<sup>1,2</sup> Teresa Cunha-Oliveira,<sup>1</sup> Stephen Ford\*,<sup>3</sup> Peter W Nathanielsz\*,<sup>3</sup> Paulo J Oliveira\*,<sup>1</sup> Susana P Pereira†.<sup>4,1</sup> <sup>1</sup>University of Coimbra, Coimbra, Portugal; <sup>2</sup>Coimbra University Hospital, Coimbra, Portugal; <sup>3</sup>University of Wyoming, Laramie, WY, United States; <sup>4</sup>University of Porto, Porto, Portugal.

**Introduction:** Obesity and liver disease are increasing. Metabolic alterations and mitochondrial dysfunction are hallmarks of those conditions. Pregnancy represents a critical challenge to metabolism due to greater energy and nutrient requirements. In a compromised obese liver, the additional metabolic challenge imposed by pregnancy might exacerbate hepatic dysfunction's progression. We investigated effects of maternal obesity (MO) during gestation on maternal hepatic mitochondrial function.

**Methods:** Nonpregnant ewes were fed 150% (MO, n=8) or 100% (Control, C, n=10) recommended global nutrient intake 60 days before conception and throughout gestation. At 90% gestation, we euthanized ewes under general anesthesia and collected maternal livers. We measured mitochondrial protein expression and enzymatic activities (Western blot and spectrophotometric assays), glutathione (HPLC) and NAD<sup>+</sup>/NADH (luminescent assay). Data were compared using unpaired t-test: p<0.05 considered significant

**Results:** In MO we observed decreased protein kinase A activity (40%), a hepatic metabolism master regulator. MO induced changes in mitochondrial oxidative phosphorylation: complex II (C-II) activity and its subunits SDHA and SDHB, and C-IV subunit mtCO1 protein expression decreased while C-I and C-IV activities increased. Consistently, NAD<sup>+</sup>/NADH was higher in MO due to reduced NADH (19.9%). Mitochondrial mass indicators, mtDNA copy number, citrate synthase activity, and TOM20 protein were similar. MO increased Fis1 (34.3%) hepatic expression and reduced OPA-1 (51%), proteins involved in mitochondrial fission and fusion. Concomitantly, MO induced 81% increase in lipid peroxidation and 55% decrease in GSSG/GSH and altered antioxidant enzymatic activities: +35% SOD, -30% catalase and +32.3% mitochondrial catalase. Macroautophagy (LC3-II/LC3-I) was also increased (380%)

**Conclusion:** While the emphasis on programming by MO has been on fetal changes, few data are available in maternal organs. We showed that MO results in altered maternal hepatic mitochondrial function and redox state, likely predisposing to hepatic dysfunction. Monitoring maternal liver function during MO pregnancy provides new insights to understand and prevent problems in the mother and offspring.

FEDER/COMPETE/FCT-Portugal: PTDC/DTP-DES/1082/2014 (POCI-01-0145-FEDER-016657) and UIDB/04539/2020; SFRH/BPD/116061/2016, 2020.05539.BD; and NIH: R01HD070096-01A1

**T-132****Uterine Artery Adaptations to Pregnancy Are Impaired by Advanced Maternal Age.**

Amy L Wooldridge†, Mazhar Pasha†, Raven Kirschenman, Floor Spaans, Sandra T Davidge, Christy-Lynn M Cooke\*. *University of Alberta, Edmonton, AB, Canada.*

**Introduction:** Nowadays, an increasing number of pregnancies occur at advanced maternal age (>35 years), which is associated with pregnancy complications. This may be due to impaired adaptations to uteroplacental blood flow during pregnancy. In a rat model of maternal aging, we previously showed that constriction responses to increasing intraluminal pressures (i.e. myogenic tone) were greater in uterine arteries from aged compared to young pregnant dams. We hypothesized that age-related differences in vascular function develop as the result of poor adaptations during pregnancy.

**Methods:** Pregnant young (~4 months) and aged (~9 months; equivalent to ~35 years in humans) rats were studied on gestational day 20 (term=22 days) and compared to age-matched non-pregnant rats. Pregnancy outcomes from dams (n=16-23 dams/group) were recorded. Myogenic

tone (%; n=3-9/group) and mechanical properties (measures of elasticity and deformation across increasing pressure; n=10-24/group) were assessed in isolated main uterine arteries using pressure myography. Myography data were analyzed as area under the curve [AUC]. Data were analyzed by two-way ANOVA with Sidak post-hoc comparisons, or by unpaired t-test; mean ± SEM.

**Results:** Pregnancies in aged dams resulted in more reabsorptions (young: 0.7±0.2 vs aged: 3.3±0.7; p=0.001), fewer fetuses (young: 15±1 vs aged: 9±1; p<0.0001), greater placental weight (young: 0.48±0.01 g vs aged: 0.63±0.05 g; p<0.0001) and lower fetal weight (young: 3.8±0.1 g vs aged: 3.1±0.1 g; p<0.0001) than young dams. Myogenic tone was reduced in young pregnant compared to nonpregnant rats (AUC pregnant 11±3: vs non-pregnant: 44±6; p=0.027). However, this pregnancy adaptation did not occur in aged dams (AUC pregnant 37±2 vs non-pregnant: 30±3; NS). As a result, myogenic tone was lower in arteries from young dams than in arteries from aged dams (AUC young: 11±3 vs aged: 37±2; p=0.017). In arteries from non-pregnant rats, age did not affect myogenic tone. Circumferential stress and strain increased with pregnancy (p<0.0001 for both). The circumferential stress was not affected by age, however, the circumferential strain was lower in arteries from aged pregnant dams than arteries from young pregnant dams (AUC young: 139±6 vs aged: 120±4; p=0.029).

**Conclusion:** Maternal aging compromised pregnancy outcomes and was associated with impaired uterine artery adaptations to pregnancy. Of the arteries from pregnant rats, those from aged dams were less compliant, and more constrictive with increasing pressure, compared to those from young dams. These impairments due to aging were not shown in the non-pregnant state, suggesting that they developed during pregnancy, possibly due to pregnancy being a 'secondary hit'. These poor pregnancy adaptations may contribute to the increased risk of pregnancy complications with advanced maternal age.

**T-133****L-Citrulline Supplementation during Pregnancy Improves Maternal Vascular Dysfunction in a Preeclampsia-Like Mouse Model.**

Mary Gemmel†,<sup>1</sup> Elizabeth Sutton,<sup>2</sup> Marcia Gallaheer,<sup>1</sup> Robert W. Powers\*.<sup>1</sup> <sup>1</sup>University of Pittsburgh, Pittsburgh, PA, United States; <sup>2</sup>Woman's Hospital, Baton Rouge, LA, United States.

**Introduction:** Preeclampsia is a pregnancy-specific syndrome characterized by new onset hypertension and proteinuria. This disorder affects 3-5% of pregnancies worldwide, contributing to maternal morbidity, mortality and risk of future cardiovascular disease. Despite its prevalence, there is no cure for preeclampsia other than inducing delivery. Therefore, identifying therapeutic strategies to alleviate preeclampsia and associated vascular dysfunction is critical for maternal and fetal health. The nutraceutical L-citrulline promotes nitric oxide (NO) bioavailability and contributes to improved vascular function. The aim of this study was to investigate the effect of L-citrulline supplementation on vascular health during pregnancy in a preeclampsia-like mouse model.

**Methods:** Healthy wildtype C57BL/6 female mice were bred to male complement component C1q knockout (C1q<sup>-/-</sup>) mice. The C57BL/6xC1q<sup>-/-</sup> breeding scheme is documented to result in a preeclampsia-like pregnancy (PE) evidenced by pregnancy-specific hypertension, altered kidney and placental morphology, and vascular dysfunction. A subset of PE dams received L-citrulline (PE+CIT, 0.25%) in drinking water from gestation day (GD) 0.5-17.5. At the end of pregnancy, blood pressure and vascular function were assessed.

**Results:** Female preeclampsia-like mice supplemented with citrulline (PE+CIT) exhibited reduced systolic blood pressure (p<0.01), diastolic blood pressure (p=0.04), and mean arterial pressure (p=0.02) when compared to untreated PE mice. Wire myography data indicate that PE+CIT dams have improved vascular function compared to PE mice evidenced by reduced sensitivity to phenylephrine (p<0.03). Further, PE+CIT mice displayed improved endothelial-dependent (p<0.01) and -independent relaxation (p<0.01) compared to untreated PE mice, supporting improved vascular function in PE mice supplemented with L-citrulline.

**Conclusion:** L-citrulline supplementation during pregnancy improves blood pressure and vascular function in a mouse model of preeclampsia. The current work points to a potential novel therapeutic which may improve vascular health during pregnancy and may contribute to improved maternal health throughout the lifespan. This project was supported by the American Heart Association Go Red for Women 16SFRN27810001.

### T-134

#### Alterations in Inorganic Phosphate and Calcium Maternal Excretion Associated with Gestational Age and Parity.

Ana Correia-Branco<sup>†,1</sup>, Monica Rincon,<sup>2</sup> Leonardo Pereira,<sup>2</sup> Mary C Wallingford,<sup>1</sup> *Tufts Medical Center, Boston, MA, United States;* <sup>2</sup>*Oregon Health Science Center, Oregon, OR, United States.*

**Introduction:** Inorganic phosphate (P<sub>i</sub>) and calcium (Ca) are essential minerals that enable skeletal ossification, support cellular structure and organellar function, and serve key biochemical roles in energetics and molecular signaling. Disrupted P<sub>i</sub> and Ca homeostasis are associated with phosphate wasting, mineral and bone disorders, and vascular calcification. Homeostasis is also significantly altered during pregnancy, as bone resorption and deposition creates are uncoupled. P<sub>i</sub> and Ca homeostasis are regulated in part by fibroblast growth factor-23 (FGF-23), an endocrine hormone that is produced in osteocytes. FGF23 signaling downregulates P<sub>i</sub> transporters and has been described to decrease with parity. We hypothesize that parity regulates maternal P<sub>i</sub> and Ca homeostasis by reducing P<sub>i</sub> pools and accretion, and increasing vascular calcification.

**Methods:** In order to address these unknowns, we examined biological fluid characteristics of P<sub>i</sub> and Ca during pregnancy in accordance with parity. Amniotic fluid and maternal urinary P<sub>i</sub> levels were assessed with the P<sub>i</sub> Assay Kit (Sigma-Aldrich, MAK308) and Ca levels were determined with Calcium Reagent Set (Teco Diagnostics, C503-480). QuantiPro BCA Assay Kit (Sigma-Aldrich, QPBCA) was used to normalize to protein content. Correlations with gestational age at delivery (GAD), parity, preterm birth, preeclampsia, diabetes mellitus, and ectopic calcification were evaluated.

**Results:** During third trimester of pregnancy, we observed an uncoupling of maternal urinary P<sub>i</sub> and Ca levels. P<sub>i</sub> excretion increased with GAD (1.92 mmol/L at 28.7 weeks and 9.47 mmol/L at 33.0 weeks), whereas Ca excretion decreased with GAD (3.03 mg/L at 28.7 weeks and 0.13 mg/L at 33.0 weeks). Amniotic fluid P<sub>i</sub> levels decreased with gestation, ranging from 1.28 to 0.15 mmol/L, while low second trimester levels associated with preterm birth. Maternal phosphaturia was reduced with parity (14.92 ± 2.57 vs. 4.72 ± 1.59 mmol/L; p = 0.0045), whereas calciuria excretion increased (0.21 ± 0.09 vs. 1.20 ± 0.40 mg/L; p = 0.031052).

**Conclusion:** Together, this data supports that prior pregnancy (multigravida) associates with an uncoupling of P<sub>i</sub> and Ca excretion compared to primigravida (first pregnancies). No correlations were observed between urinary P<sub>i</sub> or Ca levels and preeclampsia or diabetes. In conclusion, P<sub>i</sub> and Ca levels provide clinical information regarding the temporal fluctuation of maternal-fetal phosphate homeostasis, in relation to parity, supporting that gestational P<sub>i</sub> homeostasis should be examined more closely and in larger populations.

### T-135

#### Predictors of Vaginal Delivery in Patients with Cardiac Disease.

Nicole Rose Gavin<sup>†,1</sup>, Jerome Federspiel<sup>†,2</sup>, Theresa Boyer<sup>†,1</sup>, Kristin Darwin<sup>†,1</sup>, Alexia Debrosse<sup>†,1</sup>, Anum Minhas<sup>†,1</sup>, Arthur Vaught\*. <sup>1</sup>*The Johns Hopkins Hospital, Baltimore, MD, United States;* <sup>2</sup>*Duke University School of Medicine, Baltimore, NC, United States.*

**Introduction:** Women with a history of cardiac disease are increasingly becoming pregnant. Despite this increase, knowledge gaps persist for appropriate timing and route of delivery. The aim of this study was to investigate if there are attributes associated with vaginal delivery in women with cardiac disease.

**Methods:** We performed a retrospective cohort study using data from two hospitals in an academic health system from 2016 through 2020. A population to screen for cardiac disease was identified by international classification of disease diagnosis codes from the electronic medical records (EMR), and data were assembled using both automated

extracts and chart review. The primary outcome was cesarean delivery (CD), with patients with obstetric contraindication to vaginal delivery excluded. Unadjusted associations between mode of delivery and clinical characteristics were assessed using t-tests and chi-squared tests as appropriate, with multiply-imputed logistic regression employed for adjusted associations.

**Results:** A total of 231 patients were included, including 78 (35%) with congenital heart disease (CHD), 36 (16.4%) with a cardiomyopathy, 58 (26.2%) with history of cardiac surgery, 69 (31.1%) with an arrhythmia, and 25 (12.2%) with aortic disease. CD was utilized for 86 (37%) percent of deliveries. The most common reasons for CD included history of prior CD, and non-reassuring fetal tracing (Table 1). In unadjusted comparisons of patients undergoing cesarean and vaginal delivery, increasing age, preeclampsia with severe features, and history of prior cesarean section were associated with increased risk of cesarean section. In adjusted analyses, preeclampsia with severe features and history of prior cesarean section remained statistically significant (Table 2).

**Conclusion:** In this study, risk of cesarean delivery was not increased, and was near the national average of 32%. In this cohort, no specific category of cardiac disease was associated with an increased risk of CD.

**Table 1: Indications for cesarean delivery**

Arrest of Descent	7 (10.3)
Elective	6 (8.8)
Failed Vacuum	1 (1.5)
Failure to Descend	1 (1.5)
Fetal Intolerance of Labor	9 (13.2)
Fetal tracing non-reassuring	8 (11.8)
Maternal Condition Precluding Vaginal Delivery	6 (8.8)
Multiple Gestation	2 (2.9)
Other (Specify)	5 (7.4)
Placenta Abruptio	1 (1.5)
Preeclampsia/PIH remote from Delivery	2 (2.9)
Prolonged Active Phase	3 (4.4)
Prolonged Latent Phase	1 (1.5)
Repeat Elective	16 (23.5)

**Missing information in 18 observations**

Data are Mean (Standard Deviation) or %

**Table 2: Unadjusted associations between patient characteristics and mode of delivery**

	Overall (N=231)	Cesarean section		P
		No (N=145)	Yes (N=86)	
Age (years)	29.9 (5.8)	29.1 (5.5)	31.1 (6.1)	0.01
Race				0.34
Asian	9 (3.9)	4 (2.8)	5 (5.8)	
Black or African American	76 (32.9)	44 (30.3)	32 (37.2)	
Declined to Answer	3 (1.3)	1 (0.7)	2 (2.3)	
Other	27 (11.7)	19 (13.1)	8 (9.3)	
White or Caucasian	116 (50.2)	77 (53.1)	39 (45.3)	
Ethnicity				0.83
Hispanic or Latino	21 (9.1)	15 (10.3)	6 (7.0)	
Not Hispanic or Latino	205 (88.7)	127 (87.6)	78 (90.7)	
Patient Refused	3 (1.3)	2 (1.4)	1 (1.2)	
Unknown	2 (0.9)	1 (0.7)	1 (1.2)	
Body mass index	28.5 (8.8)	28.0 (7.8)	29.4 (10.2)	0.24
History of smoking	19 (8.4)	16 (11.4)	3 (3.5)	0.04
History of alcohol use	4 (1.8)	2 (1.4)	2 (2.4)	0.61
Selected Comorbid Conditions				
Asthma	60 (26.0)	39 (26.9)	21 (24.4)	0.68
Cardiac valve disease	25 (10.8)	14 (9.7)	11 (12.8)	0.46
Congenital heart disease	35 (15.2)	23 (15.9)	12 (14.0)	0.70
Gestational diabetes	27 (11.7)	13 (9.0)	14 (16.3)	0.09
Pregestational diabetes	12 (5.2)	6 (4.1)	6 (7.0)	0.35
Drug abuse	14 (6.1)	8 (5.5)	6 (7.0)	0.65
Chronic hypertension	21 (9.1)	11 (7.6)	10 (11.6)	0.30
Gestational hypertension	19 (8.2)	8 (5.5)	11 (12.8)	0.05
Preeclampsia without severe features	7 (3.0)	4 (2.8)	3 (3.5)	0.75
Preeclampsia with severe features or eclampsia	22 (9.5)	6 (4.1)	16 (18.6)	<0.001
Multiple gestations	9 (3.9)	3 (2.1)	6 (7.0)	0.06
Obesity in pregnancy	66 (28.6)	37 (25.5)	29 (33.7)	0.18
Prior cesarean section	34 (14.7)	4 (2.8)	30 (34.9)	<0.001
Pulmonary hypertension	10 (4.3)	5 (3.4)	5 (5.8)	0.39
Any prior cesarean section	42 (18.2)	4 (2.8)	38 (44.2)	<0.001
Cardiac diagnosis				
Congenital heart disease	78 (35.1)	54 (39.1)	24 (28.6)	0.11
Cardiomyopathy	36 (16.4)	22 (16.1)	14 (17.1)	0.84
Cardiac surgery	58 (26.4)	41 (29.9)	17 (20.5)	0.12
Arrhythmia	69 (31.2)	42 (30.2)	27 (32.9)	0.67
Aortopathy	24 (11.8)	14 (10.9)	10 (13.3)	0.60
Gestational age at delivery (days)	265.3 (19.2)	267.0 (19.3)	262.5 (18.9)	0.09

Data are Mean (Standard Deviation) or %

P-values by t-test for continuous variables and chi2 test for binary / categorical variables

**T-136****Effect of Long-Term Storage and Pre-Pregnancy BMI on Lipid Parameters in Stored Maternal Plasma Samples.**

Theresa Boyer, Nada Elsayed, Kimberly Jones-Beatty, Irina Burd\*.

*Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Maternal lipid levels may serve as a marker for maternal inflammation and a predictor of adverse pregnancy outcomes. We sought to determine the effect of pre-pregnancy BMI on maternal lipid profile, and to assess the effect of long-term storage on the integrity of maternal lipoprotein level measurements.

**Methods:** Pregnant women were enrolled in a prospective, longitudinal cohort study. Women were categorized as normal (BMI  $\leq$  24.9), overweight (25.0  $\leq$  BMI  $\leq$  29.9), or obese (BMI  $\geq$  30.0) based on pre-pregnancy BMI. Plasma samples were analyzed for levels of LDL, HDL, and total cholesterol (TC). Samples were stored at -80°C. Standard statistical methods were employed and storage effect on lipoproteins was assessed using linear regression.

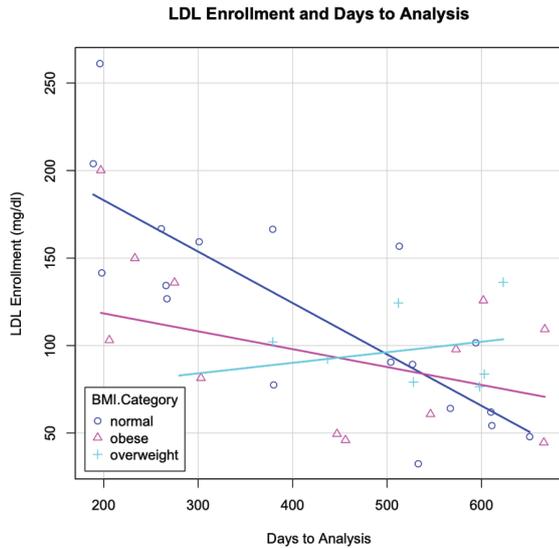
**Results:** EDTA-extracted plasma samples from normal (n=18), overweight (n=8), and obese women (n=13) were collected at study enrollment and at delivery. Among all women, LDL, HDL, and total cholesterol increased during pregnancy (p < 0.001, p = 0.028, p < 0.001, respectively). There was no significant difference in LDL, HDL, or TC at the time of enrollment or delivery between groups. Enrollment and delivery samples were stored at -80°C for an average of 433 and 263 days, respectively. A significant decay effect of storage time was noted. LDL degradation occurred at a significantly faster rate in enrollment samples from normal weight women ( $\beta$  = -0.294, p < 0.001) compared to both overweight ( $\beta$  = 0.060, p=0.012) and obese ( $\beta$  = -0.102, p=0.029) women.

**Conclusion:** While we did not find a significant difference in maternal lipid profiles among BMI categories, we established that long-term storage

of plasma from women with different BMIs may interfere with lipid level measurements in a non-linear fashion. Plasma lipid analysis should be conducted as soon as possible following collection to avoid potential degradation. Furthermore, consideration should be made for different lipid degradation rates or mechanisms in women with different BMIs.

Table 1. Sample Characteristics (shown as mean +/- std or n (%))

	Normal (n=18)	Overweight (n=8)	Obese (n=13)	P-value
Maternal Characteristics				
Age (years)	33.28 (3.82)	32.88 (6.88)	30.77 (6.10)	0.4577
Race				0.8133
African American/ Black	4 (22.2)	2 (25.0)	4 (30.8)	
Asian	2 (11.1)	0 (0)	0 (0)	
White/Caucasian	11 (61.1)	6 (75.0)	7 (53.8)	
Other	1 (5.6)	0 (0)	2 (15.4)	
Pre-pregnancy BMI (kg/m <sup>2</sup> )	21.71 (1.65)	27.29 (1.28)	42.23 (10.18)	< 0.001
Gestation at Enrollment (weeks)	15.76 (3.52)	13.63 (2.53)	17.18 (5.10)	0.09347
Gestation at Delivery (weeks)	39.01 (1.29)	38.71 (1.60)	37.89 (2.17)	0.2919
Time to Analysis Enrollment Sample (days)	419.28 (164.57)	495.00 (121.35)	414.92 (182.49)	0.3711
Time to Analysis Delivery Sample (days)	248.67 (166.39)	312.50 (126.84)	252.69 (184.88)	0.5411
Cholesterol Content				
LDL Enrollment (mg/dl)	118.70 (60.62)	95.90 (23.35)	96.44 (47.78)	0.3881
HDL Enrollment (mg/dl)	84.60 (50.48)	65.72 (35.58)	74.50 (47.55)	0.5699
Total Cholesterol Enrollment (mg/dl)	203.30 (106.31)	161.62 (42.21)	165.21 (82.70)	0.3722
LDL Delivery (mg/dl)	171.72 (97.32)	194.28 (78.48)	156.32 (78.17)	0.5888
HDL Delivery (mg/dl)	98.20 (63.04)	86.82 (45.98)	95.82 (70.14)	0.8739
Total Cholesterol Delivery (mg/dl)	270.44 (139.44)	281.10 (104.43)	242.21 (117.68)	0.7289



**T-137**

**Myocardial Bridge in Pregnancy: Beyond a ‘Normal Anatomic Variant’.**

Noor Joudi†, Imee Dato, Stephanie Leonard, Christine Lee, Ingela Schnittger, Abha Khandelwal, Katherine Bianco\*. *Stanford University Hospital and Clinics, Stanford, CA, United States.*

**Introduction:** A myocardial bridge (MB) is an anatomic variant where a segment of a major coronary artery courses through the myocardium and is susceptible to flow impairment and malignant arrhythmias. Physiologic changes of pregnancy cause cardiac stress and elevated heart rates. Our hypothesis was that during pregnancy MB patients are at increased risk compared with a healthy population and may be more comparable to those with other known cardiac conditions.

**Methods:** We conducted a matched cohort study of 10 cases of MB in pregnancy between 2014 to 2020 at a single quaternary care institution. The cases were compared to two control groups matched by parity and gestational age at delivery (weeks). The two control groups were (1) healthy pregnant patients and (2) pregnant patients with known cardiac disease cared for by a multidisciplinary team; both with no underlying chronic medical or obstetric conditions. We collected data via medical chart review and did statistical analysis via Fisher exact and chi-squared tests for comparison of MB cases and cardiac controls.

**Results:** MB cases were similar to the cardiac control group regarding NYHA classification, CARPREG II risk score, prior cardiac surgery, and ejection fraction (Table 1). MB cases were more likely than healthy controls to experience angina (60% vs 0%) or peripartum cardiac symptoms (80% vs 0%) (Table 2). Peripartum angina (60% vs 3.85%;  $p=0.0007$ ) and overall cardiac symptoms (80% vs. 38%;  $p=0.0256$ ) were experienced significantly more frequently among MB cases as compared to cardiac controls. MB cases were more likely to use beta blocker medication during pregnancy. There was no relationship between maternal MB and adverse neonatal outcomes.

**Conclusion:** Medical recommendations for pregnant women with MB vary greatly in clinical practice. This single-institution study suggests that MB patients experience cardiac symptoms more frequently than pregnancies complicated by other cardiac conditions and healthy controls. We propose that larger studies be conducted to further assess increased risk in pregnancy with presence of myocardial bridging.

Characteristics	Cases N= 10	Cardiac Controls N= 26	Healthy Controls N= 21	
Age at Delivery	Mean	32.5	33.3	30.4
	SD	4.46	3.31	5.38
Parity	Mean	0.5	0.385	0.286
	SD	0.671	0.496	0.463
Gestational age at delivery	Mean	39.4	38.9	39.9
	SD	1.23	1.06	0.825
Maternal Race/Ethnicity	Caucasian	40.0%	30.8%	76.2%
	Hispanic	40.0%	3.85%	0.00%
	Asian	20.0%	57.7%	23.8%
	Other	0.00%	3.85%	0.00%
Family History	Yes	80.0%	38.5%	47.6%
	No	20.0%	57.7%	52.4%
Maternal Insurance	Public	40.0%	3.85%	52.4%
	Private	60.0%	96.2%	47.6%
Pre-Pregnancy BMI	Mean	24.9	23.2	22
	SD	3.98	3.69	1.9
Chronic medical conditions	Autoimmune	10.0%	0.00%	0.00%
	Cardiac	20.0%	0.00%	0.00%
	Endocrine	20.0%	0.00%	0.00%
	Gastrointestinal	50.0%	0.00%	0.00%
	Infectious	0.00%	0.00%	0.00%
	Neurologic	10.0%	0.00%	0.00%
	Psychiatric	20.0%	0.00%	0.00%
	Renal	10.0%	0.00%	0.00%
	Respiratory	20.0%	0.00%	0.00%
	Chronic medication use in pregnancy	Aspirin	30.0%	0.00%
Beta Blocker		40.0%	19.2%	0.00%
Calcium Channel blocker		10.0%	3.85%	0.00%
Digoxin		0.00%	3.85%	0.00%
Immunosuppressant		10.0%	0.00%	0.00%
Levothyroxine		20.0%	0.00%	0.00%
Prior cardiac surgery		Open	20.0%	26.9%
	Catheterization	20.0%	23.1%	0.00%
	None	60.0%	50.0%	100%
EF	Mean	59.2%	62.0%	n/a
	SD	7.15	0.065	n/a
NYHA Classification	Mean	1.1	1	1
	SD	0.3	0	0
CARPREG II Score	Mean	1.82	1.58	0
	SD	1.402	1.75	0

Characteristics	Cases N= 10	Cardiac Controls N= 26	Healthy Controls N= 21	P-value	
Cardiac Symptoms	Angina	60.0%	3.85%	0.00%	<b>0.0007</b>
	Arrhythmia	50.0%	19.2%	0.00%	0.0997
	Palpitations	20.0%	11.5%	0.00%	0.6034
	Shortness of Breath	30.0%	7.69%	0.00%	0.1186
	Symptoms, Total	80.0%	38.0%	0.00%	<b>0.0256</b>
	New cardiovascular medication required in labor	10.0%	3.85%	0.00%	0.4841
Telemetry	Intrapartum	30.0%	34.6%	0.00%	1.0
	Postpartum	20.0%	30.8%	0.00%	0.6895
Labor Analgesia	Epidural	60.0%	65.4%	33.3%	
	CSE	20.0%	23.1%	61.9%	
	Spinal	0.00%	7.69%	0.00%	
	IV Fentanyl	0.00%	3.85%	0.00%	
MOD	None	20.0%	0.00%	4.76%	
	SVD	60.0%	61.5%	85.7%	
	OVD	10.0%	19.2%	4.76%	
Disposition	CS	30.0%	19.2%	9.52%	
	Maternity Unit	80.0%	73.1%	100%	
	Telemetry Unit	20.0%	23.1%	0.00%	
Fetal Sex	ICU	0.00%	3.85%	0.00%	
	Male	40.0%	53.9%	52.4%	
Birthweight	Female	60.0%	46.2%	47.6%	
	Mean	3608g	3155	3363	
1 min APGAR	SD	390.1g	548	404	
	Mean	8.1	8	7.57	
5 min APGAR	SD	0.3	1.06	1.21	
	Mean	9	8.81	8.9	
Cord pH	SD	0	0.694	0.301	
	Mean	7.18	7.23	7.14	
Cord BE	SD	0.051	0.0734	0.0414	
	Mean	-4.27	-5.5	-7.24	
Length of neonatal hospitalization	SD	4.1	2.3	1.94	
	Mean	2.2	5.77	1.86	
	SD	0.872	8.6	0.478	

**T-138****Nursing Modifies the Immune Profile in Postpartum Mice.**

Pauline DiGianivittorio†, Marlena Tyldesley†, Kirtika Prakash†, Elizabeth A Bonney\*. *University of Vermont, Larner College of Medicine, Burlington, VT, United States.*

**Introduction:** In women, breast vs. bottle feeding may modify cardiovascular and autoimmune disease. Few studies examine the phenotype of immune cells relative to nursing. Studies in rodent models have linked altered immunity and lack of nursing to impaired postpartum (PP) vascular remodeling. We compared immune cells in non-nursing (NN) and nursing (N) PP mice.

**Methods:** Adult (~4 months) C57BL/6 females were mated to same-strain males and allowed to litter. At 48 hours after delivery, pups were either removed or allowed to stay and nurse. Mothers (n=4/group) were euthanized 2 weeks PP, and mesenteric and uterine nodes, spleen and thymus were removed for examination. Additionally, tissues from late gestation mice (LG, 17 days of 19 total, n=3) were studied. Tissues were examined by flow cytometry using antibodies (BD Biosciences) to the T cell receptor beta chain, T cell delta chain, CD4, CD8, a B cell marker (B220), and to the NK cell marker (NK1.1) which was also used, along with anti T cell beta chain, to delineate NK T cells. Studies also used antibodies to the activation/memory marker CD44 and to the receptor for the homeostatic cytokine, IL-7. After gating for single lymphocytes, the % of each cell population was determined using FlowJo (Ashland OR). Population numbers (millions) in the spleen and thymus were also calculated. Differences were compared by ANOVA with GraphPad Prism (San Diego CA). Significance was set at  $P < 0.05$ . Data presented is mean±SEM.

**Results:** In the thymus, NN led to a lower % of TCR+ cells (LG,  $7.2 \pm 0.5$ ; N,  $5.7 \pm 0.8$ ; NN,  $4.5 \pm 0.2$ ,  $p=0.035$ ) and tended to decrease that of NKT+ cells ( $p=0.054$ ). In the spleen, NN resulted in a lower % of TCR+ cells (LG,  $27 \pm 2$ ; N,  $31 \pm 1$ ; NN,  $24 \pm 1$ ;  $p=0.007$ ) and a higher % (LG,  $42 \pm 4$ ; N,  $44 \pm 1$ ; NN,  $56 \pm 5$ ,  $p=0.048$ ) and number (LG,  $66 \pm 8$ ; N,  $63 \pm 6$ ; NN,  $88 \pm 3$ ;  $p=0.027$ ) of B220+ cells. In mesenteric lymph nodes, NN resulted in lower % of CD4+ TCR+ cells (LG  $40 \pm 0.1$ ; N,  $26 \pm 1$ ; NN,  $10 \pm 5$ ;  $p=0.002$ ) and the tendency to lower %CD8+ TCR+ ( $p=0.053$ ) but had no effect on %B220+ cells ( $p=0.18$ ). In uterine nodes, NN led to lower % NKT cells (LG,  $0.3 \pm 0.1$ ; N,  $0.8 \pm 0.3$ ; NN,  $0.4 \pm 0.1$ ;  $p=0.01$ ) and tended to decrease %CD4 T cells ( $p=0.051$ ), with no effect on CD8 T cells ( $p=0.7$ ) or B220+ cells ( $p=0.5$ ). NN had no significant effect on expression of CD44 or the IL-7 receptor. Further, NN did not change gamma-delta T cells.

**Conclusion:** Overall, NN prematurely contracts the PP T cell pool, and this is not likely due to impaired response to activating or homeostatic signals. In the context of data that immune deficient mice have defects in PP vascular remodeling, this contraction may in part explain the recent finding that removal of pups results in defective PP remodeling and altered response to vasoactive agents. *Supported by NIH R01 HL141747; P30 GM118228; UVM-COM Flow Cytometry and Cell Sorting Facility.*

**T-139****Differential Shedding of Endothelial Cell Proteins during the Peripartum Period.**

Maria Cristina Bravo, Ira Bernstein, Kelley McLean, Thomas Orfeo, Kathleen Brummel-Ziedins. *University of Vermont, Colchester, VT, United States.*

**Introduction:** The glycocalyx is a network of proteoglycans and glycoproteins projecting from the endothelial cell (EC) surface that plays a key role in maintaining endothelial integrity. Proteolytic release of components of the glycocalyx (*i.e.* shedding) has been shown to occur in response to a number of physiologic challenges. Syndecan-1 is a component of the endothelial glycocalyx and is substituted with heparan sulfate chains which promote its interactions with key anticoagulants such as tissue factor pathway inhibitor (TFPI) and antithrombin. Thrombomodulin (TM), another EC transmembrane protein, and TFPI are key components of the primary dynamic anticoagulant mechanism limiting thrombus growth and are also subject to proteolytic release. In this longitudinal study of the peripartum period, we evaluate the circulating plasma levels of these proteins in healthy women.

**Methods:** Healthy women (N=10) were prospectively enrolled in a longitudinal study at the University of Vermont Medical Center in a protocol approved by the Institutional Review Board. Two blood collections were obtained, Visit 1 was up to 1 week before a vaginal delivery (n = 5) or scheduled Cesarean-section (n = 5) and Visit 2 was within 18-36 hours after delivery. Blood was collected into citrate and platelet poor plasma aliquots were prepared. Syndecan-1, soluble TM (sTM), Total TFPI and Free TFPI were measured on all samples by ELISA. Comparisons were made across Visits using a paired Student's t-test. Data are shown as mean ± SD.

**Results:** Pre- and post-delivery levels of syndecan-1, sTM, Free TFPI and Total TFPI are reported in the Table. A comparison of the four analytes at each Visit segregated by route of delivery did not reveal any differences (data not shown). Syndecan-1 levels fell to  $21 \pm 5\%$  of the pre-delivery level. sTM, Free TFPI, and Total TFPI fell on average to 87% of pre-delivery levels ( $86 \pm 11\%$ ,  $87 \pm 11\%$ , and  $89 \pm 7\%$ , respectively).

**Conclusion:** After delivery women show significant decreases in plasma levels of four endothelial cell markers. The greater impact on syndecan-1 levels suggest differential regulation of the proteolytic enzymes targeting syndecan-1 than sTM. A potential inference from these data is that after delivery the endothelial cell surface expresses or retains higher levels of these potent anticoagulants thus restoring the non-thrombogenic character of the endothelial surface.

**T-140****Long Term Patient Follow-Up of Cardiac Disease in Pregnancy: Multidisciplinary Teams Tether At-Risk Patients to the System.**

Sarah E Miller†, Danielle Panelli, Elizabeth Sherwin, Christine Lee, Hayley Miller†, Alisha Tolani†, Alana O'Mara†, Abha Khandelwal, Ylaly Katherine Bianco\*. *Stanford University, Stanford, CA, United States.*

**Introduction:** Cardiovascular disease is the leading cause of maternal mortality, with a significant number of deaths occurring up to one year postpartum. While postpartum loss to follow-up is an issue for many patients, little is known about the clinical course of pregnant patients with cardiac disease during this period. We sought to evaluate postpartum follow-up and complications among a cohort of cardiac patients cared for by a multidisciplinary team including obstetricians, anesthesiologists and cardiologists.

**Methods:** Surveys were sent to English-speaking patients with cardiac disease who were followed as a part of a multidisciplinary cardiac care program and who delivered at a single institution from 2012 to 2019. The survey included questions about clinical follow-up, maternal complications, and infant development. To estimate bias in survey results, we compared maternal demographics and neonatal outcomes between survey responders and non-responders using Fisher's Exact or Wilcoxon rank-sum tests.

**Results:** Of 126 patients, 51 (40.5%) completed the survey. Median follow-up time from delivery to response was 2.4 (IQR 1.8-3.7) years. Survey respondents were less likely than non-respondents to be of non-White race (37.3% versus 58.7%,  $p=0.03$ ). Thirteen of 51 (25.5%) reported a postpartum diagnosis of anxiety or depression, though the median quality of life score was 5/5 (IQR 4-5). Importantly, the vast majority reported following up with their cardiologist (41 of 51, 80.3%) and/or primary care provider (41 of 51, 80.3%) after delivery.

**Conclusion:** The cohort of patients with cardiac conditions who responded to the survey were well connected with their healthcare providers as evidenced by high rates of follow-up postpartum. These patients also reported a high quality of life despite significant rates of depression. Taken together, these results may represent a unique benefit for patients with cardiac disease treated under the umbrella of a multidisciplinary care team.

Comparison of maternal demographics between survey respondents and non-respondents			
	Respondents N=51 n (%)	Non-respondents N=75 n (%)	p-value
Age at delivery ≥ 35	17 (33.3)	26 (34.7)	1.00
Hispanic ethnicity	11 (21.6)	17 (22.7)	1.00
Non-White race	19 (37.3)	44 (58.7)	0.03
Public insurance	11 (21.6)	19 (25.3)	0.67
Congenital cardiac disorder	24 (47.0)	27 (36.0)	0.26
Cardiologist established prior to pregnancy	39 (76.5)	58 (77.3)	0.57
Nulliparous	28 (54.9)	40 (53.3)	1.0

Survey responses	
	N=51n (%)
Years since delivery	2.4 (1.8-3.7)
Quality of life, rated 1-5	5 (4-5)
Activity level	
Sedentary or mildly active	19 (37.3)
Moderately or very active	31 (60.8)
Cardiac-related procedure or complication since delivery	8 (15.7)
Visit to cardiologist since delivery	41 (80.4)
Visit to primary care provider since delivery	41 (80.4)
Contraception plan	
Used LARC	15 (29.4)
Female sterilization	4 (7.8)
Pregnancy since delivery	13 (25.5)
Planning to get pregnant again	
Yes	6 (11.8)
Uncertain	14 (27.5)
No	31 (60.8)
Diagnosed with anxiety or depression since delivery	13 (25.5)
Infant diagnosed with cardiac disease	5 (9.8)
Child development disorders	4 (7.8)

**T-141**

**Relationship between Fetal Position and Obstetric Laceration Location and Severity.**

Gillian Horwitz†, Megan Trostle†, Iffath Hoskins\*, Ashley S. Roman\*. NYU Langone Health, New York, NY, United States.

**Introduction:** While previous studies have linked occiput posterior (OP) position to severe perineal lacerations, the relationship between fetal position and all other obstetric lacerations has not been studied. The objective of this study was to characterize the association of fetal position with obstetric laceration location and severity. We hypothesized that women with OP and occiput transverse (OT) fetuses would have higher rates of sulcal and severe perineal lacerations.

**Methods:** This was a single-center, retrospective, cohort study of all vaginal deliveries of vertex, singleton gestations ≥34 weeks in women with no prior vaginal deliveries from 7/2013 to 10/2018. We compared the rates of anterior vulvar, 1<sup>st</sup>-degree, 2<sup>nd</sup>-degree, severe perineal, sulcal, and cervical lacerations in women with fetuses in occiput anterior (OA), OT, and OP positions. Fischer’s exact test, Chi-square test, Kruskal Wallis H test, and multivariate regression were performed with p<0.05 considered statistically significant.

**Results:** Among 6,875 deliveries meeting inclusion criteria, fetal position was OA in 6,600 (96.0%), OP in 222 (3.2%), and OT in 53 (0.8%). We found OP to be associated with increased risk of 3<sup>rd</sup>-degree laceration (p=0.002) and 4<sup>th</sup>-degree laceration (p=0.014). Multivariate analysis was performed to adjust for demographic and labor differences between the groups, and fetal position remained significantly associated with severe perineal laceration. In the multivariate analysis, other significant factors associated with severe perineal laceration included race, BMI ≥35, second stage duration, and birth weight. OP was also associated with increased risk of sulcal laceration (p=0.011), but fetal position was not significant in the multivariate regression. There was no association between fetal position and anterior vulvar (p=0.154), cervical (p=0.593), 2<sup>nd</sup>-degree (p=0.397), or perineal (p=0.765) lacerations.

**Conclusion:** The most notable association between fetal position and obstetric laceration was the increased risk of severe perineal laceration in OP position.

	OA;N=6600;n (%)	OP;N=222;n (%)	OT;N=53;n (%)	OR (95% CI)	p-value
Sulcal laceration	362 (5.5)	21 (9.5)	3 (5.7)	N/A	<b>0.042</b>
	X	X		0.56(0.35 - 0.88)	<b>0.011</b>
Severe perineal (3 <sup>rd</sup> or 4 <sup>th</sup> degree) laceration	213 (3.2)	18 (8.1)	5 (9.4)	N/A	<b>&lt;0.001</b>
	X	X		0.38(0.23 - 0.62)	<b>&lt;0.001</b>

	aOR (95% CI)	p-value
Gestational age	0.93 (0.79 - 1.09)	0.373
2 <sup>nd</sup> stage duration	0.78 (0.69 - 0.89)	<b>&lt;0.001</b>
Birth weight	0.9985 (0.9981 - 0.999)	<b>&lt;0.001</b>
Maternal age	0.97 (0.94 - 1.01)	0.134
Race	1.27 (1.08 - 1.50)	<b>0.004</b>
BMI ≥ 35	5.3 (1.66 - 32.38)	<b>0.02</b>
Fetal position	0.59 (0.39 - 0.93)	<b>0.014</b>

**T-142**

**Innate Lymphoid Cell Subsets Are Uniquely Distributed within the Maternal-Fetal Interface.**

Stephen A McCartney,<sup>1</sup> Nicholas Maurice,<sup>2</sup> Marie Frutos,<sup>2</sup> Florian Mair,<sup>2</sup> Shree S Raj,<sup>1</sup> Martin Prlic\*.<sup>2</sup> <sup>1</sup>University of Washington, Seattle, WA, United States; <sup>2</sup>Fred Hutchinson Cancer Research Center, Seattle, WA, United States.

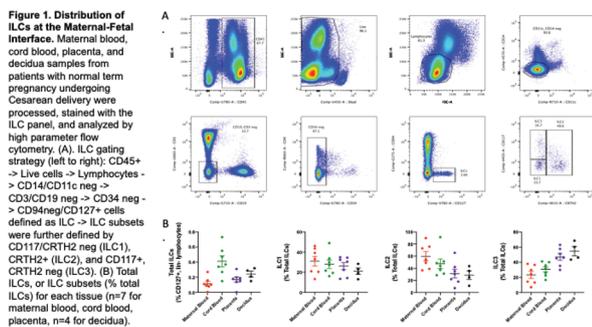
**Introduction:** Innate lymphoid cells (ILCs) are an immune cell type which lack expression of antigen receptors but rather sense environmental signals such as soluble cytokines, prostaglandins and metabolites. In other tissues, ILCs act as inflammatory amplifiers or suppressors depending on the stimuli, and they have been hypothesized to perform a similar role in pregnancy, which requires immune adaptation throughout gestation. Recent studies consistently demonstrate that ILCs are present at the maternal-fetal interface (MFI) during pregnancy, however, the distribution of ILC subsets throughout the MFI is currently unclear. We evaluated total ILCs and ILC subset distribution throughout the MFI in term pregnancy.

**Methods:** Maternal blood, cord blood, placenta (n=7 each), and uterine decidua (n=4) specimens were obtained from women with uncomplicated pregnancies at the time of scheduled Cesarean delivery at term (37-40 weeks). Mononuclear cells were isolated from blood samples, and placenta and decidua samples were digested with collagenase to isolate immune

cells. High-parameter flow cytometry was performed using a panel of 20 cell surface markers to identify total ILC (CD127+, lineage negative), ILC1 (CD117/CRTH2 neg), ILC2 (CD117neg/CRTH2+), and ILC3 (CD117+/CRTH2neg) subsets (Figure 1A). Kruskal Wallis testing was performed to determine significant differences in ILCs between tissues.

**Results:** Among the tissues at the MFI, we found that cord blood contained a significantly greater concentration of ILCs (0.42% of total lymphocytes) compared to decidua (0.24%), placenta (0.17%), and maternal blood (0.12%),  $p=0.013$  (Figure 1B). There was a significant difference between ILC2 ( $p=0.038$ ) and ILC3 ( $p=0.004$ ) but not ILC1 subsets between the tissues of the MFI. Maternal blood contained the highest percentage of ILC2 and the lowest percentage of ILC3, while decidua contained the highest percentage of ILC3 and the lowest percentage of ILC2.

**Conclusion:** There are significant differences in the distribution of ILCs and ILC subsets between the tissues of the MFI. These differences may reflect functional requirements as ILC2 are thought to have primarily anti-inflammatory properties, while ILC3 can have both pro- or anti-inflammatory functions. Further studies are necessary to demonstrate the activation status and stimuli of ILCs at the MFI, which are currently unknown.



## T-143

### Migraine and Adverse Pregnancy Outcomes: The nuMoM2b Study.

Eliza C Miller,<sup>1</sup> Sarah E. Vollbracht,<sup>1</sup> Cynthia Gyamfi-Bannerman,<sup>1</sup> Whitney Booker,<sup>1</sup> Leslie Moroz,<sup>1</sup> Marianna S. Yigrakh,<sup>1</sup> Lisa D. Levine,<sup>2</sup> David M. Haas,<sup>3</sup> William A. Grobman,<sup>4</sup> Mary D'Alton,<sup>1</sup> Ronald Wapner,<sup>1</sup> Natalie A. Bello\*.<sup>1</sup> <sup>1</sup>Columbia University, New York, NY, United States; <sup>2</sup>University of Pennsylvania, Philadelphia, PA, United States; <sup>3</sup>Indiana University, Indianapolis, IN, United States; <sup>4</sup>Northwestern University, Chicago, IL, United States.

**Introduction:** Migraine affects more than 1 in 4 women of childbearing age and is associated with endothelial dysfunction and inflammation. Migraine history has been associated with adverse pregnancy outcomes (APOs) including hypertensive disorders of pregnancy, preterm delivery, and low birth weight, but few prospective studies have investigated this association, and most studies were done in non-US populations.

**Methods:** The Nulliparous Pregnancy Outcomes Study Monitoring Mothers-to-be (nuMoM2b) study enrolled 10,038 nulliparous US women with singleton gestation in early pregnancy and followed them through delivery. Medical histories, including history of migraine, were collected from participants by self-report at each study visit. We defined APO as 1 or more of the following: gestational hypertension, preeclampsia/eclampsia, preterm delivery, gestational diabetes, small for gestational age (SGA), or stillbirth. All APOs and self-reported medical comorbidities including migraine were adjudicated by study investigators. We calculated odds ratios (OR) and 95% confidence intervals (95%CI) for the association of migraine with APOs, adjusting for self-reported race/ethnicity, recent smoking, and APO-associated comorbidities including chronic hypertension, obesity, chronic kidney disease, pre-gestational diabetes and autoimmune disorders. We tested for interactions between migraine and obesity, and migraine and chronic hypertension.

**Results:** Of the 10,038 women in the sample, 1,181 (11.8%) had a migraine diagnosis. A higher proportion of women who self-identified as White reported migraine. Adjusting for covariates, migraineurs had higher

odds of any APO (adjusted OR 1.3, 95%CI 1.2-1.5). For individual APOs, migraineurs had higher odds of gestational hypertension, preeclampsia, and preterm birth, but not gestational diabetes, SGA, or stillbirth (**Table**). There were no significant interactions between migraine and obesity or chronic hypertension.

Bold type indicates confidence interval does not cross 1.0.				
Table: Association of migraine with APOs in nulliparous women				
	APO among unexposed (N=8857)	APO among exposed (N=1181)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
	n (%)	n (%)		
Any APO	3075 (34.7)	495 (41.9)	<b>1.4 (1.2-1.5)</b>	<b>1.3 (1.2-1.5)</b>
Gestational diabetes	347 (3.9)	49 (4.2)	1.1 (0.8-1.4)	1.0 (0.8-1.4)
Hypertensive disorder of pregnancy	1869 (21.1)	320 (27.1)	<b>1.4 (1.2-1.6)</b>	<b>1.3 (1.1-1.5)</b>
Gestational hypertension	1149 (13.0)	206 (17.4)	<b>1.4 (1.2-1.7)</b>	<b>1.4 (1.2-1.6)</b>
Preeclampsia/eclampsia	720 (8.1)	114 (9.7)	1.2 (1.0-1.5)	1.1 (0.9-1.4)
Preterm birth (all)	672 (7.6)	114 (9.7)	<b>1.3 (1.1-1.6)</b>	<b>1.3 (1.0-1.6)</b>
Iatrogenic	269 (3.0)	41 (3.5)	1.1 (0.8-1.6)	1.1 (0.8-1.6)
Spontaneous	403 (4.6)	73 (6.2)	<b>1.1 (1.1-1.8)</b>	<b>1.4 (1.0-1.8)</b>
Small for gestational age	901 (10.2)	117 (9.9)	1.0 (0.8-1.2)	0.9 (0.8-1.1)
Stillbirth	51 (0.6)	6 (0.5)	0.9 (0.4-2.1)	0.6 (0.2-1.7)

*Adjusted for obesity (BMI≥30), race/ethnicity, recent smoking, chronic hypertension, chronic kidney disease, pregestational diabetes, and autoimmune disorders.*

**Conclusion:** In a large, prospective cohort of nulliparous US women, migraine was independently associated with increased odds of APOs, particularly hypertensive disorders of pregnancy and preterm birth. Migraine may be an under-recognized risk factor for APOs.

## T-144

### Periconceptional Maternal Renin-Angiotensin-Aldosterone System Activation and the Association with Maternal Telomere Length: The Rotterdam Periconception Cohort.

Damiat Aoulad Fares†, Rosalieke E Wiegelt†, Alex J Eggink\*, Joyce B.J. Van Meurs\*, Jan A.H. Danser\*, Eric A.P. Steegers\*, Régine P.M. Steegers-Theunissen\*. Erasmus Medical Center, Rotterdam, Netherlands.

**Introduction:** The maternal renin-angiotensin-aldosterone system (RAAS) has a great impact on the cardiovascular and hemodynamic adaptations to pregnancy. RAAS activation induces oxidative stress and inflammation. Accelerated attrition of telomeres has been described as a consequence of oxidative DNA damage. Therefore, telomere length (TL) has been suggested a long term biomarker of chronic oxidative stress and inflammation. Because cardiovascular-related adverse pregnancy outcomes originate in the periconceptional period, efforts for early prediction and prevention should be pursued. From this background, we hypothesize that increased chronic activation of RAAS enhances oxidative stress exposure resulting in shortening of maternal TL. Here we aim to investigate this hypothesis in women in the periconceptional period.

**Methods:** In the Virtual Placenta study, embedded in the Rotterdam Periconception Cohort, we selected 145 singleton pregnancies. The maternal RAAS determinants, i.e., renin, prorenin and aldosterone, are measured in blood plasma at 9 weeks gestational age (GA). Venous

blood samples, drawn approximately at 20 weeks GA, are used for Genomic DNA extraction and TL is measured by qPCR. After logarithmic transformation of the RAAS determinants, multivariable linear regression is applied to assess crude and adjusted associations with TL, i.e., Model 1: adjusted for maternal age, Model 2: Model 1 and adjusted for mode of conception, pre-pregnancy body-mass index, mean arterial blood pressure and smoking.

**Results:** A significantly negative association was found between renin and TL (crude  $\beta$  -0.094 [95% CI: -0.17, -0.01],  $p=0.02$ , Model 1  $\beta$  -0.092 [95% CI: -0.17, -0.01],  $p=0.025$  and Model 2  $\beta$  -0.086 [95% CI: -0.17, -0.00],  $p=0.047$ ). Prorenin tended to show a negative association with TL (crude  $\beta$  -0.07 [95% CI: -0.14, -0.003],  $p=0.06$ , Model 1  $\beta$  -0.065 [95% CI: -0.14, -0.01],  $p=0.079$  and Model 2  $\beta$  -0.058 [95% CI: -0.13, -0.02],  $p=0.14$ ), albeit not significantly. No associations were found between aldosterone or aldosterone/renin ratio and maternal TL and also not after adjustment. After stratification by mode of conception, no association between RAAS determinants and maternal TL was found in pregnancies conceived spontaneously or after in vitro fertilization treatment.

**Conclusion:** Negative associations independent from maternal age were found between renin and maternal TL. This finding supports the hypothesis that increased renin detrimentally impacts maternal TL due to chronic exposure to excessive oxidative stress and inflammation. No role for aldosterone, as an oxidative stressor, was found. Additional studies are needed to investigate whether TL is also a marker of cardiovascular hemodynamic adaptation to pregnancy.

#### T-145

##### Corpus Luteum Contribution and the Maternal Renin-Angiotensin-Aldosterone System as Underlying Mechanism to (Utero) Placental Vascular Development Throughout Pregnancy: The Rotterdam Periconception Cohort.

Rosalieke E Wiegelt, A.H. Jan Danser\*, Maud J.H. Karsten†, Igna F Reijnders†, L van Rossem\*, Sten P Willemsen\*, Annemarie G.M.G.J. Mulders\*, Eric A.P. Steegers\*, Régine P.M. Steegers-Theunissen\*. *Erasmus Medical Center, Rotterdam, Netherlands.*

**Introduction:** Pregnancies without a corpus luteum (CL) display impaired maternal cardiovascular adaptation and show increased risk of preeclampsia. The renin-angiotensin-aldosterone system (RAAS) is involved in early cardiovascular pregnancy adaptation, and the CL contributes to RAAS activity by secreting prorenin. An adequate adaptation is crucial for placental development, which starts during the first trimester. We aimed to investigate whether the CL number, at spontaneous or assisted conception, and maternal RAAS component levels influence (utero)placental vascular development throughout pregnancy.

**Methods:** In the Virtual Placenta study, embedded in the Rotterdam Periconception cohort, 201 women with 0 ( $n=8$ ), 1 ( $n=143$ ), or  $>1$  ( $n=51$ ) CL were selected. Renin, prorenin and aldosterone levels were determined at 11 weeks gestational age (GA). Placenta Volume (PV) and utero-Placental Vascular Volume (uPVV) were measured from transvaginal 3D ultrasound scans at 7, 9 and 11 weeks GA. Pulsatility (PI) and resistance indices (RI) of the uterine arteries (UtA) were assessed by pulsed wave Doppler ultrasounds at 7, 9, 11, 13, 22 and 32 weeks GA. At birth placental weight was obtained using standardized procedures. Associations between CL number and RAAS components (prorenin and the aldosterone/renin ratio, indicative for angiotensin- and aldosterone-mediated effects, respectively) with PV, uPVV and UtA indices were studied using linear mixed models and adjusted for patient and fertility treatment characteristics.

**Results:** Absence of a CL showed lower UtA PI and RI throughout pregnancy than 1 CL and  $>1$  CL pregnancies ( $p=0.026$  and  $p=0.02$ , respectively). Placental development parameters were comparable between CL groups. Prorenin associated positively with trajectories of UtA PI and RI ( $\beta = 0.10$ , 95% CI 0.01;0.20,  $p=0.036$  and  $\beta = 0.06$ , 95% CI 0.01;0.12,  $p=0.04$ , respectively), and negatively with uPVV ( $\beta = -0.23$ , 95% CI -0.44;-0.02,  $p=0.036$ ) and placental weight ( $\beta = -93.8$ , 95% CI -160.3; -27.4,  $p=0.006$ ). The aldosterone/renin ratio associated positively with PV ( $\beta = 0.12$ , 95% CI 0.01;0.24,  $p=0.036$ ). We found no significant association regarding the aldosterone/renin ratio and placental weight.

**Conclusion:** The absence of a CL, resulting in low prorenin levels, reduces UtA pulsatility and resistance, while high prorenin levels reduce placental vascular volume and weight. These data likely reflect the constrictor effects of angiotensin II, leading to diminished placental flow and (vascular) development. Interestingly, aldosterone, when corrected for renin, shows the opposite. This illustrates its indispensability with regard to volume regulation.

#### T-146

##### CDKN1C Is a Conserved Regulator of Trophoblast Cell Development.

Regan L Scott†, Khursheed Iqbal, Kaela M Varberg, Marija Kuna, Keisuke Kozai, Michael J Soares\*. <sup>1,2</sup> *University of Kansas Medical Center, Kansas City, KS, United States; <sup>2</sup>Children's Mercy Research Institute, Kansas City, MO, United States.*

**Introduction:** The interface formed between the placenta and the uterus during pregnancy is an area of complex interplay between trophoblast cells and maternal cells. The communication between these cells facilitates adaptations necessary to accommodate the needs of the growing fetus. Early in pregnancy, trophoblast stem (TS) cells differentiate and invade into the uterus to remodel uterine spiral arteries. Insufficient development of the invasive trophoblast cell lineage can lead to pregnancy complications such as miscarriage, preeclampsia, intrauterine growth restriction, and preterm birth. However, little is known about the mechanisms that regulate trophoblast cell differentiation to the invasive trophoblast cell lineage. Rats, like humans, possess a hemochorial placenta with deep trophoblast invasion and uterine spiral artery remodeling.

**Methods:** To identify conserved regulators of invasive trophoblast cells, we performed single-cell RNA sequencing (scRNA-seq) of the rat uterine-placental interface and examined gene expression in human TS cells. Cyclin-dependent kinase inhibitor 1C (CDKN1C) expression was monitored in rat and human placentation sites. Loss-of-function strategies were utilized in human TS cells and rats to investigate roles for CDKN1C in trophoblast cell development and placentation.

**Results:** scRNA-seq interrogation of the rat uterine-placental interface identified several cell clusters, including a trophoblast cluster defined by the expression of cytokeratins and members of the expanded prolactin gene family. CDKN1C transcripts were specific to invasive trophoblast cells, a property shared with human extravillous trophoblast cells of the human placentation site. CDKN1C, a paternally imprinted gene, is a regulator of cell cycle progression, and its dysregulation has been implicated in abnormal placentation. Expression of CDKN1C was mapped in rat and human placentas and in human TS cells. In all cases, CDKN1C expression was a feature of invasive trophoblast cells. Short-hairpin RNAs and Crispr/Cas9 mediated genome-editing were used to investigate roles for CDKN1C in human TS cells and in the rat, respectively. In both experimental loss-of-function models, CDKN1C expression was disrupted. Phenotypes of control and CDKN1C knockdown human TS cells, and wild type and CDKN1C null rat placentas were examined.

**Conclusion:** CDKN1C is a conserved regulator of trophoblast cell differentiation and healthy placental development. CDKN1C deficiency is linked to placental disease. [Supported by NIH grants HD020676, HD099638; Sosland Foundation]

#### T-147

##### Global DNA Methylation Differences in Chorionic Villi from Euploid Miscarriages.

Winfred Mak\*, Jawon Song.<sup>2</sup> <sup>1</sup>*Dell Medical School, UT Austin, Austin, TX, United States; <sup>2</sup>TACC, Austin, TX, United States.*

**Introduction:** Pregnancy loss is the most common complication of pregnancy. About 60% of first trimester miscarriages are due to genetic abnormalities such as aneuploidy. In a genetically normal pregnancy, epigenetics could have a role in pregnancy loss. The mammalian placental epigenome has two distinct features: 1) Genomic imprinting; 2) Global DNA hypomethylation. Imprinted genes are expressed from one parental allele only and are epigenetically regulated. Species-specific differences between mouse and human placenta occur, for example, some genes are imprinted in human placenta and not in mouse; large contiguous genomic regions ( $>100$ kb) of hypomethylation called *partial methylated domains*

(PMD) are found in human placenta and not in mouse. The PMDs are interspersed between *highly methylated domains (HMD)* and are stable across the three trimesters of pregnancy. The genes in the PMDs are tissue-specific and repressed. Genes in PMDs have hypomethylated gene bodies and hypermethylated promoter regions. The function of PMD/HMD regions in human placenta is unknown. Several studies show that miscarriages are associated with abnormal methylation in a subset of imprinted genes. However, it is unknown if changes in PMDs occur in chorionic villi in euploid miscarriages. Our study shows preliminary evidence that there are differences in the DNA methylation levels in PMDs in chorionic villi of euploid miscarriages compared to control villi. **Methods:** The Illumina Human Methylation450K bead chip array was performed on eight first trimester euploid chorionic villi from miscarriages and eight first trimester chorionic villi obtained from elective terminations. Array data was processed and normalized using *minfi* R package. Distributions of methylation ratios of CpG that are within PMD were compared between euploid miscarriages and terminations using Mann-Whitney test.

**Results:** The differentially methylated regions (DMR) (beta value > 20%) in euploid miscarriages were not more frequently overlapping with PMD in contrast to prior studies which found that most DMR were in PMDs in normal human placenta. Furthermore, we found that imprinted genes (7.5%) were more likely to have significantly differentially methylated CpGs than non-imprinted genes (1.4-1.9%) in euploid miscarriages consistent with prior studies. Interestingly, we found an overall significant increase of 2.5% in the DNA methylation of CpGs in PMDs in euploid miscarriages compared to elective terminations ( $p < 0.001$ ). These methylation differences are primarily found in the protein-coding regions and not at the transcription start sites of the PMD genes.

**Conclusion:** Our study shows preliminary evidence that changes in DNA methylation occur in the PMD regions of placenta from first trimester human pregnancy loss. Our findings contribute to our knowledge regarding the placental epigenome of human pregnancy loss.

## T-148

### Mapping Enhancer - Transcription Factor Interactions in Human Placenta Development and Trophoblast Differentiation.

David Owen, Xuan Huang, Anusha Nagari, Tulip Nandu, W. Lee Kraus\*. *UT Southwestern Medical Center, Dallas, TX, United States.*

**Introduction:** The architecture of placenta villi changes over gestation from immature forms with a low syncytiotrophoblast/volume ratio in early pregnancy to highly branched mature villi with much greater syncytiotrophoblast surface area at term. These changes facilitate increased nutrient and gas exchange to support fetal growth. The transcription factors responsible for this process, and their genomic targets, particularly within enhancer elements, are not well understood. The objective of this study is to identify changes in active enhancer elements and their associated transcription factors in immature and mature human placenta tissue, and to correlate these findings with a human trophoblast stem cell model of syncytiotrophoblast differentiation. The transcription factors identified could serve as targets to modulate placenta development, while the genomic loci where they act could facilitate genetic risk assessment for placenta pathologies such as delayed villous maturation, associated with stillbirth.

**Methods:** We identified ~ 20,000 enhancer sites in human placenta tissue across gestation by enhancer RNA transcription (PRO-seq) and characteristic histone marks (ChIP-seq). We then implemented the Total Functional Score of Enhancer Elements (TF-SEE) methodology, which integrates enhancer activity, transcription factor binding site motif analysis, and transcription factor expression levels (RNA-seq) to predict the most active transcription factors at enhancers in a given set of samples. We performed ChIP experiments to validate these relationships in a human trophoblast stem cell model of syncytiotrophoblast differentiation.

**Results:** TF-SEE identified about 100 transcription factors with predicted activity at enhancers in human placenta tissue. Of these, 14 were significantly enriched in immature placenta tissue (<22 weeks gestation), while 12 were enriched at term. The majority of the transcription factors enriched by TF-SEE in immature placenta tissue also showed increased

expression by RNA-seq in trophoblast stem cells as compared to the syncytiotrophoblast state. This suggests a possible role for these TFs in maintenance of the stem cell state. Of note, some transcription factors showed a difference in TF-SEE activity score despite being expressed at similar levels, highlighting the importance of accessible binding sites for activity.

**Conclusion:** TF-SEE identifies transcription factor - enhancer interactions associated with placenta development and syncytiotrophoblast differentiation. Mapping these interactions can form the basis for future detailed mechanistic studies of their role in placenta development and pathology.

## T-149

### Placental Pathology Associated with Liveborn and Stillbirth Infants in Women with and without Antiphospholipid Antibodies.

Jhenette Lauder<sup>1,2</sup>, Jessica Page<sup>1,2</sup>, Amanda Allshouse<sup>1</sup>, Uma Reddy<sup>3</sup>, Robert Goldenberg<sup>4</sup>, Halit Pinar<sup>5</sup>, Silver Robert<sup>1</sup>, Ware Branch<sup>1,2</sup>. <sup>1</sup>University of Utah Health, SLC, UT, United States; <sup>2</sup>Intermountain Health Center, SLC, UT, United States; <sup>3</sup>Yale University, New Haven, CT, United States; <sup>4</sup>Columbia University, NYC, NY, United States; <sup>5</sup>Brown University, Providence, RI, United States.

**Introduction:** Antiphospholipid antibodies (aPL) are associated with adverse perinatal outcomes, which has been attributed to placental inflammation and thrombosis. However, these findings are inconsistent and non-specific. Thus, we sought to compare placental pathology among women with and without aPL with livebirths (LB) and stillbirths (SB).

**Methods:** This is a secondary analysis of the Stillbirth Collaborative Research Network (SCRN), a case-control study that enrolled LB and SB. Standardized evaluation of placentas was performed by perinatal pathologists with centralized training. We included all participants who had a non-anomalous, singleton pregnancy with placental pathology and aPL results. Testing included IgG and IgM anticardiolipin (aCL) and anti-beta 2 glycoprotein 1 (aβ2GPI) antibodies. A positive test was defined as greater than 20 units for any of the four antibodies. Primary outcomes included placental lesions adopted from Amsterdam Criteria and included five categories previously associated with SB: maternal vascular lesions (MVL), fetal vascular lesions (FVL), maternal inflammation, fetal inflammation, and immune/idiopathic lesions. Placental lesions were compared between aPL groups and stratified by LB and SB. Statistical analysis incorporated analytical weights using Wald chi-square and ANOVA where appropriate, reflecting the SCRN sampling design. Placental-analysis-specific analytical weights were used for analysis of placental lesions.

**Results:** 1,380 (970 LB; 410 SB) were included for analysis. There were no clinically significant differences in maternal demographic and clinical characteristics between aPL groups among LB and SB. Placental abnormalities were extremely common in both LB and SB (Table 1). For most lesions, there were no differences in their incidence in placentas with and without aPL. Among SB, aPL positive patients were more likely to have any vascular lesion. However, the difference among groups was small and influenced by weighting (98.2 vs 92%).

**Conclusion:** Among LB and SB, most placental abnormalities are not associated with aPL.

Table 1. Placental histopathological lesions

Characteristic	Stillbirth			Livebirth		
	aPL	Non-aPL	p	aPL	Non-aPL	p
Unweighted N	41 (%)	369 (%)		64 (%)	906 (%)	
Weighted N	41 (%)	367 (%)		46 (%)	744 (%)	
Maternal Vascular lesions	31 (74)	252 (69)	0.437	20 (43)	360 (49)	0.424
Fetal Vascular lesions	34 (83)	274 (75)	0.172	26 (57)	406 (55)	0.76
Any Vascular lesions	40 (98)	338 (92)	0.011	34 (73)	565 (76)	0.605
Maternal Inflammatory	18 (43)	119 (33)	0.227	8 (17)	117 (16)	0.835
Fetal Inflammatory	4 (10)	59 (16)	0.285	3 (7)	68 (9)	0.543
Any Inflammatory lesions	19 (46)	127 (35)	0.204	9 (20)	136 (19)	0.8
Immune/Idiopathic	6 (16)	35 (10)	0.317	2 (3)	36 (5)	0.527

**T-150****Novel Mechanisms of Disrupted Placental Development: Lipid Mediators and Inflammatory Signaling.**

Yuliya Fakhr†,<sup>1,2</sup> Kirsten Webster†,<sup>1,2</sup> Denise G Hemmings\*,<sup>1,2,3</sup> <sup>1</sup>Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Women and Children's Health Research Institute, Edmonton, AB, Canada; <sup>3</sup>Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, AB, Canada.

**Introduction:** The pathophysiology of preeclampsia, an inflammatory pregnancy disorder, is rooted in poor trophoblast fusion that forms the multinucleated syncytium, the site of maternal-fetal exchange. In preeclampsia, elevated pro-inflammatory tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) hinders trophoblast fusion into the syncytium. However, the mechanism of this response remains unclear. In endothelial cells, TNF- $\alpha$  exerts its disruptive effects by modulating the levels of sphingosine 1-phosphate (S1P), a signaling lipid, and its receptors (S1PRs). Similarly to TNF- $\alpha$ , S1P hinders trophoblast fusion. We hypothesize that TNF- $\alpha$  disrupts syncytium fusion by modulating S1P regulatory enzymes and S1PRs in the human placenta, similar to effects in endothelial cells.

**Methods:** Chorionic villous explants from term placentas were treated after syncytial sloughing (day 4) with or without 1ng/mL TNF- $\alpha$ . Syncytium re-formation and cell death were examined up to 48hrs later. The role of S1P was assessed using 1 $\mu$ M PF-543, which inhibits S1P main synthesizing enzyme, sphingosine kinase 1 (SK1). Cell fusion (n=4) was assessed by E-cadherin staining, demarcating cell membranes that are lost during syncytialization. Cell death and syncytium function were measured with lactate dehydrogenase (LDH) and chorionic gonadotropin (CG) assays (n=8). S1P regulatory enzymes and S1PR1-3 mRNA expressions were measured by qRT-PCR (n=5-7). Results were analyzed by 2-way ANOVA for combined treatments and mixed effects model for time courses.

**Results:** TNF- $\alpha$  treatment decreased formation of multinucleated units by 37.2 $\pm$ 0.09% (p=0.008). Inhibiting S1P formation with PF-543 in the absence of TNF- $\alpha$  also decreased fusion by 30.9 $\pm$ 0.09% (p=0.04). Combined treatment led to a 41.3 $\pm$ 0.09 % decrease (p=0.04) with a TNF- $\alpha$  and PF-543 interaction (p=0.06), indicating that SK1 inhibition is part of TNF- $\alpha$  signalling. LDH and CG were unaltered in response to TNF- $\alpha$ . S1PR2, SK1, and S1P lyase (irreversibly degrades S1P) levels were unchanged throughout syncytialization with or without TNF- $\alpha$ . S1P phosphatase-1 (reversibly degrades S1P) increased after 48hrs of re-syncytialization with and without TNF- $\alpha$  (7.0 $\pm$ 1.72 fold, p=0.04 and 5.8 $\pm$ 1.37-fold, p=0.02, respectively). Under normal culture conditions, only S1PR1 (17.5 $\pm$ 3.82 fold, p=0.01) mRNA expression was upregulated after 48hrs. TNF- $\alpha$  treatment blocked this normal S1PR1 surge and increased S1PR3 expression (5.4 $\pm$ 1.47 fold, p=0.03).

**Conclusion:** SK1-induced S1P synthesis is crucial for trophoblast fusion. TNF- $\alpha$  disrupts fusion by decreasing SK1 activity. Fusion hindered by TNF- $\alpha$  is associated with a decrease in S1PR1 and increase in S1PR3 levels and potentially signalling. **Grant:** CIHR

**T-151****AMPK Signaling Stimulates Mitophagy in Human Trophoblast Cell Line via Pathways Mediated by PINK1/PARKIN and FUNDC1.**

Bin Wu,<sup>1</sup> Seyedeh Alaie†,<sup>2</sup> Yun Chen,<sup>3</sup> Guoyang Luo,<sup>2</sup> Haijun Gao\*.<sup>2</sup>

<sup>1</sup>Central Hospital Affiliated to Shandong First Medical University, Jinan, China; <sup>2</sup>Howard University, Washington, DC, United States;

<sup>3</sup>Rocket Pharmaceuticals, Inc., Cranbury, NJ, United States.

**Introduction:** Our previous study and others indicated that mitophagy, a subtype of autophagy targeting dysfunctional mitochondria, was impaired in GDM placentas, which was coincident with reduced AMPK signaling. However, to date, mitophagy pathways have not been characterized in human trophoblast cells. To fill this gap in knowledge, we hypothesized that AMPK signaling stimulates mitophagy in human trophoblast cells via classical mitophagy pathways.

**Methods:** In Study One, to determine whether AICAR (AMPK activator) can stimulate AMPK signaling in human trophoblast cells, BeWo cell line was treated with AICAR (0.5mM) for 12 or 24 hours, and the phosphorylated AMPK at Thr<sup>172</sup> (p-AMPK; AMPK activity indicator)

and ACC at Ser<sup>79</sup> (p-ACC; phosphorylated by AMPK) were analyzed by Western blotting. In Study Two, to determine whether and how AICAR induces mitophagy, BeWo cells were treated with AICAR (0.5mM), chloroquine (CLQ; 40 $\mu$ M; blocking the degradation of mitochondria) and their combination for 24 hours. The abundance of LC3II proteins (mitochondria receptor in mitophagy) in both whole cell lysates and mitochondrial fractions, and PINK1, PARKIN, BNIP3 and BNIP3L (mediators of mitophagy) in mitochondrial fractions was analyzed by Western blotting. The abundance of proteins was normalized to ACTB or total proteins in blots after MemCode staining. The effects of AICAR, CLQ and/or their combination were analyzed by one or two way ANOVA (n=3).

**Results:** The main findings include: 1) The p-AMPK levels were increased (P<0.05) by 1.59- and 1.54-fold by AICAR after 12 and 24 hours' treatment, respectively, compared to the control group, while total AMPK protein levels were unchanged; coincidentally, changes in p-ACC levels followed the same pattern to p-AMPK; 2) The abundance of LC3II in whole cell lysates was increased (P<0.001) by 3.10-fold by AICAR and further elevated (P<0.001) by 9.69-fold by the combination of AICAR and CLQ; similarly, the abundance of LC3II in mitochondrial fractions was increased (P<0.05) by 1.92-fold by AICAR and further elevated (P<0.05) by 1.95-fold by the combination of AICAR and CLQ; 3) In mitochondrial fractions, the abundance of PINK1, PARKIN, and FUNDC1 was increased (P<0.05) by 1.21-, 1.24-, 1.58-fold, respectively, by AICAR, while the abundance of BNIP3 and BNIP3L was reduced (P<0.05) by 1.66- and 1.29-fold by AICAR, respectively.

**Conclusion:** Taken together, we report for the first time that AICAR induced AMPK signaling can stimulate mitophagy in human trophoblast cells via pathways mediated by PINK1/PARKIN and FUNDC1, but not by BNIP3/BNIP3L, suggesting that mitophagy in the human placenta may be finely tuned by involvement of multiple pathways.

**T-152****Placental Pathology Associated with Liveborn and Stillbirth Infants in Women with and without an Inherited Thrombophilia.**

Jhenette Lauder†,<sup>1,2</sup> Jessica Page\*,<sup>1,2</sup> Amanda Allshouse\*,<sup>1</sup> Robert Goldenberg\*,<sup>3</sup> Carol Hogue\*,<sup>4</sup> Halit Pinar\*,<sup>5</sup> Ware Branch\*,<sup>1,2</sup> Robert Silver\*.<sup>1</sup> <sup>1</sup>University of Utah Health, SLC, UT, United States; <sup>2</sup>Intermountain Health Center, SLC, UT, United States; <sup>3</sup>Columbia University, NYC, NY, United States; <sup>4</sup>Emory University, Atlanta, GA, United States; <sup>5</sup>Brown University, Providence, RI, United States.

**Introduction:** Inherited Thrombophilia's (IT) have been linked to adverse perinatal outcomes via placental thrombosis and infarction. However, data are mixed, and the association is controversial. Thus, we sought to characterize placental pathology among women with and without IT, who experienced either livebirths (LB) or stillbirths (SB).

**Methods:** This is a secondary analysis of data from the Stillbirth Collaborative Research Network (SCRN), a case-control study that enrolled LB and SB at the time of delivery. Standardized evaluation of placentas was performed by perinatal pathologists with centralized training. We included all enrollees who had a non-anomalous, singleton pregnancy with placental pathology and completion of IT testing (Factor V Leiden (FV), Prothrombin G20210A (PT), Plasminogen Activator Inhibitor-1 (PAI-1), and MTHFR A1298C and C677T). Primary outcomes included placental lesions adopted from Amsterdam Criteria and included five categories previously associated with SB: maternal vascular lesions (MVL), fetal vascular lesions (FVL), maternal inflammation, fetal inflammation, and immune/idiopathic lesions. Placental lesions were compared between IT groups and stratified by LB and SB. Statistical analysis incorporated analytical weights using Wald chi-square and ANOVA where appropriate, reflecting the SCRN sampling design. Placental-analysis-specific analytical weights were used for analysis of placental lesions.

**Results:** Of the 2,595 women enrolled in SCRN, 1,085 were included for analysis. Placental abnormalities were extremely common in both LB and SB (Table 1). For most lesions, there were no differences in their incidence in placentas with and without maternal IT. When all thrombophilia's are considered, the IT group had more immune lesions in LB, but fewer FVL in SB. For FV and PT alone, there were more FVL in LB but fewer MVL

in SB. These differences were small and given the frequency of these abnormalities in normal pregnancies and women without IT, the findings are unlikely to be discriminatory or consequential.

**Conclusion:** Among LB and SB, most placental abnormalities are not associated with maternal IT.

Table 1. Placental histopathological lesions

Characteristic	Stillbirth			Livebirth		
	Inherited Thrombophilia	Normal	p	Inherited Thrombophilia	Normal	p
<b>Any Thrombophilia</b>						
Unweighted N	306 (%)	64 (%)		696 (%)	163 (%)	
Weighted N	300 (%)	65 (%)		576 (%)	122 (%)	
Maternal Vascular lesions	212 (70)	45 (68)	0.745	290 (51)	53 (43)	0.084
Fetal Vascular lesions	223 (74)	58 (87)	0.007	312 (55)	68 (55)	0.995
Any Vascular lesions	282 (93)	63 (95)	0.478	437 (77)	90 (73)	0.359
Maternal Inflammatory	94 (31)	28 (43)	0.087	86 (15)	20 (16)	0.722
Fetal Inflammatory	42 (14)	10 (16)	0.696	54 (10)	11 (9)	0.854
Any Inflammatory lesions	100 (33)	29 (45)	0.094	104 (18)	23 (19)	0.946
Immune/diopathic	30 (10)	7 (11)	0.872	30 (5)	2 (2)	0.008
<b>Factor V or Prothrombin</b>						
Unweighted N	29	341		36	823	
Weighted N	29	339		28	667	
Maternal Vascular lesions	13 (44)	244 (72)	0.008	15 (53)	328 (49)	0.729
Fetal Vascular lesions	24 (81)	257 (76)	0.553	22 (80)	358 (54)	0.002
Any Vascular lesions	28 (97)	317 (93)	0.395	23 (84)	504 (76)	0.258
Maternal Inflammatory	7 (24)	115 (34)	0.27	4 (15)	102 (15)	0.987
Fetal Inflammatory	3 (11)	49 (15)	0.559	3 (11)	62 (9)	0.836
Any Inflammatory lesions	7 (25)	122 (36)	0.211	5 (19)	121 (18)	0.939
Immune/diopathic	2 (8)	35 (11)	0.56	3 (9)	30 (5)	0.465

### T-153

#### A Mechanistic Framework for Cytotrophoblast to Extravillous Trophoblast Differentiation.

Sonia C. DaSilva-Arnold, Stacy Zamudio, Abdulla Al-Khan, Nicholas P. Illsley\*. *Hackensack University Medical Center, Hackensack, NJ, United States.*

**Introduction:** Despite the critical importance of invasive extravillous trophoblast (EVT) to events such as uterine spiral artery remodeling, a mechanistic framework controlling EVT differentiation from cytotrophoblast (CTB) has not been developed. We have suggested previously that this process is mediated by the epithelial-mesenchymal transition (EMT), which transforms anchorage-dependent, epithelial cells such as CTB into invasive, mesenchymal-like EVT. As gestation advances, we have shown that the EVT lose their invasiveness, but appear to maintain mesenchymal characteristics. We hypothesized that differentiation and the later reversion to non-invasive status, is reflected in unique, 3rd trimester, trophoblast-specific gene expression profiles.

**Methods:** In this study we compared global patterns of gene expression between third trimester CTB and EVT using RNA-sequencing. CTB and EVT (n=8,7) were prepared from normal placentae by immunomagnetic affinity separation, using anti-integrin  $\beta 4$  and anti-HLA-G antibodies. Cellular RNA was ribo-depleted (Ribo-Zero kit, Illumina) and cDNA libraries were constructed (NEBNext Ultra II RNA kit and Multiplex oligos) for RNA sequencing, which was performed on an Illumina NextSeq 500.

**Results:** Out of ~23K genes assessed, 4206 genes were differentially expressed (FDR < 0.05; >2-fold increase/decrease). Cellular phenotypic differences were highlighted by up-regulation of mesenchymal markers (*HLA-G*, *ITGA5*) and down-regulation of epithelial markers (*EGFR*, *ITGA6*) in the EVT. Compared to a library of 486 genes associated with EMT in other tissues, 215 genes were observed in common, including many seen in multiple EMT types (e.g. *FNI*, *ERBB2*, *KRT19*, *VIM*, *CDH1*, *BMP7*). There were a number of other notable trophoblast-specific changes, including down-regulation of genes involved in syncytialization (*ERVFRD-1*, *ERVW-1*), alterations in extracellular signaling (*CSH1*, *CSH2*, *CGA*) and in genes related to placental steroid synthesis (*ESRRG*, *CYP11A1*, *CYP19A1*).

**Conclusion:** Despite the fact that they are no longer invasive, third trimester EVT display clear signs of having undergone the EMT process when compared to CTB. With these results, EMT is clearly defined as the mechanistic framework for CTB differentiation. The novel, placental EMT profile derived from this data encompasses placenta-specific elements including genes involved in placental hormonal signaling, syncytialization and steroid synthesis. These data now provide the essential baseline necessary for assessment of EMT-related gene expression changes in

pregnancy pathologies. This is crucial for those pathologies associated with aberrant invasion, such as preeclampsia and placenta accreta, disorders often not apparent until the late second or early third trimester.

### T-154

#### Transcriptomic Analysis of Human Placenta Reveals a Distinct Gene Expression Pattern Associated with Dysregulated Apoptosis and Autophagy Leading to Preterm Birth.

Khondoker Mehedi Akram\*, Neha S Kulkarni†, Dilichukwu O Anumba\*. *University of Sheffield, Sheffield, United Kingdom.*

**Introduction:** Preterm birth (PTB) is the leading cause of death for neonates and children under five. The cause, and the pathogenesis of PTB remain widely unknown. Inflammation with dysregulated apoptosis (programmed cell death) and autophagy (cell recycling process) may be involved in the pathogenesis. The pathological events occur within the placenta, particularly in the villous compartment.

Our aim was to explore gene expression in the preterm placenta to understand the underlying pathomechanism of PTB.

**Methods:** Total RNA was extracted from villous tissue of 12 term and 12 preterm human placentas. Next Generation Sequencing of RNA (RNAseq) was conducted for differential gene expression analysis and targeted qRT-PCR was done for further mechanistic exploration.

**Results:** RNAseq analysis identified 803 upregulated and 273 downregulated genes (FDR 0.05). Pathway analysis revealed an involvement of upregulated genes in immunity-related pathways and downregulated genes in cell cycling-related pathways along with apoptosis and autophagy. qRT-PCR on the same samples detected a 50% downregulation of master autophagy gene BECN1 in preterm placenta (p = 0.002). Pro-apoptotic gene Bax and anti-apoptotic gene Bcl-2 were downregulated in preterm placenta by 30% (p = 0.006) and 70% (p = 0.005) respectively. The balance between Bax/Bcl-2 is crucial for normal tissue function. We found a strong correlation between Bax/Bcl-2 expression in term placenta (r = 0.70, p = 0.02), which was perturbed in preterm placenta (r = 0.31, p = 0.32). Ratio calculation revealed a significant relative loss of Bcl-2 compared to Bax in preterm placenta (term = 1.05 vs preterm = 0.81, p = 0.002). Bcl-2 is a negative regulator of BECN1. The Ct value of BECN1/Bcl-2 ratio was significantly reduced in preterm placenta (p = 0.006), whereas, BECN1/Bax ratio was unaltered.

**Conclusion:** Together, we postulate that relative loss of Bcl-2 may augment trophoblast apoptosis and potentiate autophagy within villous tissue which may lead to early labour and preterm birth.

### T-155

#### Myostatin Increases Human Trophoblast Cell Invasion by Upregulating N-Cadherin via SMAD2/3-SMAD4 Signaling.

Faten Fa Ahmed†, Christian Klausen\*, Hua Zhu\*, Peter Leung\*. *UBC, Vancouver, BC, Canada.*

**Introduction:** Placental insufficiency disorders, including preeclampsia, intrauterine growth restriction and preterm labor, are major obstetric complications that can have devastating effects on both the mother and the fetus. These syndromes share a common phenomenon of poor placental trophoblast cell invasion into the uterine tissue. To date there are no effective treatments for these illnesses. Myostatin is a transforming growth factor (TGF)- $\beta$  superfamily member well-known for its important role in muscle growth control. Recently myostatin was shown to be secreted in the placenta where its role in trophoblast cell proliferation and migration was highlighted. However, nothing is known about its role in trophoblast cell invasion. My research hypothesizes that myostatin increases trophoblast cell invasion by upregulating N-cadherin.

**Methods:** HTR8/SVneo immortalized extravillous cytotrophoblast cells and primary cultures of human cytotrophoblast cells were used as study models. Matrigel-coated transwell invasion assay was used to study the effects of human recombinant myostatin on trophoblast cell invasion. RT-qPCR and Western blot were used to measure myostatin effects on N-cadherin mRNA and protein levels, respectively. TGF- $\beta$  type I receptor inhibitor (SB431542) as well as small interfering RNA targeting SMAD2/3/4 were used to block myostatin receptor and downstream signaling, respectively. Each experiment was repeated with at least 3

different passages (HTR8/SVneo) or cultures from at least 3 different patients. Data were analyzed either by unpaired Student T test or one-way ANOVA followed by Tukey's test for multiple group comparisons.

**Results:** Myostatin significantly increased primary and HTR8/SVneo trophoblast cell invasion. Moreover, myostatin up-regulated N-cadherin mRNA and protein levels in a time dependent manner, with maximal effects occurring at 24-48 hours, in primary and HTR8/SVneo trophoblast cells. The effects of myostatin on N-cadherin expression were blocked by inhibition of TGF- $\beta$  type I receptor. Moreover, combined knockdown of SMAD2/3 or common SMAD4 abolished the up-regulation of N-cadherin by myostatin.

**Conclusion:** Myostatin may increase human trophoblast cell invasion by up-regulating N-cadherin via SMAD2/3-SMAD4 signaling.

## T-156

### Hypoxia Activates NOTCH1 Signaling to Promote HTR-8/SVneo Trophoblast Cell Migration but Not Invasion.

Barry E Perlman<sup>†</sup>. Rutgers-NJMS, Newark, NJ, United States.

**Introduction:** Proper placentation requires the complex regulation of extravillous trophoblast (EVT) migration and invasion into the maternal decidua in physiologic hypoxic conditions. Previous studies using the first trimester trophoblast (TB) cell line, HTR-8/SVneo (HTR-8), support a role for Notch and hypoxia as individual mediators of TB motility, but crosstalk has yet to be described. We hypothesized that hypoxia activates NOTCH1 to promote TB migration and invasion in the physiologic microenvironment of early pregnancy.

**Methods:** HTR-8 cells were exposed to normoxia (21% O<sub>2</sub>) or hypoxia (2.5% O<sub>2</sub>), which mimics early placentation, for 6hrs and 24hrs. mRNA sequencing (mRNA-seq) was performed to identify differentially expressed genes and upregulated cellular pathways. Changes in gene and protein expression were determined by qPCR and western blot, respectively. Transwell assays were performed to assess HTR-8 cell migration and invasion after exposure to DAPT in hypoxic vs normoxic conditions for 24hrs. For transwell migration assays (n=6 per condition), the number of migrated cells through culture inserts were counted. For invasion assays (n=6 per condition), the number of cells that invaded into Matrigel and collagen I matrices were counted. Means were compared using unpaired t-tests and statistical significance was defined as  $p < 0.05$ .

**Results:** Exposure of HTR-8 cells to 2.5% O<sub>2</sub> significantly altered expression of 8353 genes, with 4169 down regulated and 4184 up regulated (FDR < 0.05 by mRNA-seq). Gene ontology analyses revealed that Notch signaling and cellular movement pathways are enriched in HTR-8 cells following exposure to hypoxia. Expression of *NOTCH1* was significantly increased by mRNA-seq and qPCR (5-fold,  $p = 0.03$ ). Exposure of HTR-8 to 2.5% O<sub>2</sub> for 24hrs increased expression of the active form of NOTCH1 protein and significantly increased expression of Notch effector, *HES1* (2-fold by qPCR,  $p = 0.003$ ). Exposure of HTR-8 cells to DAPT in hypoxic conditions for 24hrs decreased expression of cleaved NOTCH1 protein (0.3-fold,  $p = 0.03$ ) and the *HES1* gene (0.6-fold,  $p = 0.02$ ). Introduction of DAPT to HTR-8 cells under hypoxic conditions for 24hrs significantly reduced HTR-8 migration (0.7-fold,  $p = 0.0001$ ), but did not impact HTR-8 invasion through collagen I or Matrigel.

**Conclusion:** Inhibition of  $\gamma$ -secretase with DAPT in a hypoxic microenvironment, reduced HTR-8 cell migration, suggesting that hypoxia-induced HTR-8 migration, but not invasion, is a NOTCH1 dependent process. These data along with previously reported data suggest that TB cells require NOTCH1 activation via hypoxia to promote cellular migration, supporting a role for crosstalk between the hypoxia and Notch pathways in EVT migration during placentation.

## T-157

### Do DNA Methylation Changes Contribute to the Phenotypic Differences between Human Cyto- and Extravillous Trophoblast?

Sonia C. DaSilva-Arnold,<sup>1</sup> Martha Salas,<sup>2</sup> Stacy Zamudio,<sup>1</sup> Abdulla Al-Khan,<sup>1</sup> Benjamin Tycko,<sup>2</sup> Nicholas P. Illsley\*.<sup>1</sup> <sup>1</sup>Hackensack University Medical Center, Hackensack, NJ, United States; <sup>2</sup>Hackensack Meridian Health Center for Discovery and Innovation, Nutley, NJ, United States.

**Introduction:** Epigenetic regulation via DNA methylation is recognized as a means of regulating gene expression at the level of transcription, usually associated with suppression of gene expression but which can also lead to activation. Human placental cytotrophoblast (CTB) to extravillous trophoblast (EVT) differentiation is accompanied by a broad array of gene expression changes as epithelial CTB are transformed into invasive, mesenchymal-like EVT. As gestation advances, we have shown that the EVT lose their invasiveness, an apparently irreversible event. Previous research has shown significant changes in methylation during differentiation and thus we wondered whether methylation might be associated with the non-invasive EVT phenotype. We hypothesized that reversion of EVT to a non-invasive status, might involve both global and gene-specific changes in DNA methylation. In this study we compared methylation changes between paired, third trimester CTB and EVT.

**Methods:** CTB and EVT were prepared from normal placentae by immunomagnetic affinity separation, using anti-integrin  $\beta 4$  and anti-HLA-G antibodies. Genomic DNA was extracted, and CpG methylation patterns were assessed genome-wide using Illumina EPIC (800K) Methylation Beadchips (n=3,3).

**Results:** The EPIC data showed a widespread loss of DNA methylation in EVT (affecting ~12K genes) compared to the paired CTB. By contrast, a much smaller group of ~100 genes showed a gain of methylation of more than 20%, at more than one CpG site in the EVT. Of these genes, half also showed differential mRNA expression. When tabulated against known EMT-associated genes, a small group of common genes was identified. Several of these genes were substantially down regulated (>10-fold) including *ALDH1A3*, *BMP7*, *FGFR2*, *MSX2*, *SEMA3F* and *SLC27A2*. Interestingly, several genes showed up-regulation associated with methylation; *FSCN1*, *RUNX1* and *COL5A1*. Some of the top biological processes associated with these genes, as assessed by gene ontology (apart from epithelial-mesenchymal transition), include embryonic morphogenesis and mesenchymal cell differentiation.

**Conclusion:** It is widely accepted that the placenta, like certain cancers, displays a degree of hypomethylation relative to other somatic tissues. Examining specific placental cell types, we find that there is extensive hypomethylation in EVT compared to CTB, further defining the epigenetic status of this tissue. There are however specific genes that show gains in methylation, several of which are associated with the EMT mechanism. The altered gene expression associated with these gains of methylation may serve to regulate the non-invasive EVT phenotype.

## T-158

### Palmitic Acid Impedes Extravillous Trophoblast Activity by Increasing MRP1 Expression and Function.

Yunali V Ashar<sup>†</sup>, Qiu-Xu Teng<sup>†</sup>, John ND Wurlpel, Zhe-Sheng Chen, Sandra E Reznik\*. St. John's University, Queens, NY, United States.

**Introduction:** The thrifty phenotype hypothesis proposes that an unfavorable *in utero* environment leads to increased susceptibility to disease and decreased life expectancy. We and others have previously shown that maternal consumption of a diet high in saturated fatty acids such as palmitic acid (PA) compromises the *in utero* milieu and results in fetal programming for cardiometabolic disease. Normal function of placental extravillous trophoblasts (EVT), which are responsible for uteroplacental vascular remodeling in the first half of gestation, is critical for adequate delivery of oxygen and nutrients to the developing fetus and normal fetal programming. EVT proliferation and invasion of spiral arteries both depend upon adequate levels of folate, which is removed from these cells by multidrug resistance-associated protein 1 (MRP1), an ATP-binding cassette transporter, encoded by the *ABCC1* gene. We tested the novel hypothesis that PA increases MRP1-mediated folate removal from EVT and thereby interferes with EVT's role in early placental vascular remodeling.

**Methods:** HTR8/SVneo cells, immortalized first trimester human EVT cells, were grown in the absence or presence of 0.5 mM PA for 72 h. The effect of PA on expression of *ABCC1*, the gene encoding MRP1, was determined by qPCR and MRP1 protein expression was probed by Western blotting. Folate efflux was determined by transport assay, using

radiolabeled folate. Migration and invasion were determined by separate *in vitro* assays monitoring movement and penetration, respectively. MRP1 localization was evaluated by immunofluorescence.

**Results:** PA increased both *ABCC1* gene expression and MRP1 protein expression in HTR8/SVneo cells ( $P < .05$ ). PA also increased the rate of folate efflux from the cells into the media and decreased migration and invasion functions of the cultured cells ( $P < .05$ ). Finally, while it did increase the level of expression of MRP1, PA had no effect on its localization in the extravillous trophoblast plasma membrane.

**Conclusion:** We show here, for the first time, a mechanistic rationale for the known adverse effect of PA, the most common saturated fat in the Western diet, on maternal vascular perfusion of the placenta. Previously, we have shown that PA increases oxidative stress in HTR8/SVneo cells. Others have shown that oxidative stress increases MRP1 expression. The novel finding in this study that PA increases MRP1-mediated folate efflux provides a missing link explaining how PA leads to compromise of the *in utero* environment. Elucidation of the precise molecular pathways connecting maternal high fat diet consumption to fetal programming for cardiometabolic disease provides insights that can lead to future therapeutic approaches to safeguard the developmental origins of human health.

### T-159

#### Decorin-Induced MicroRNAs in Trophoblast Functions: Roles in Preeclampsia.

Chidambra D Halari†, Maria Sbirnac, Jasmine Sidhu, Pinki Nandi, Peeyush K Lala\*. *University of Western Ontario, London, ON, Canada.*

**Introduction:** Extravillous trophoblasts (EVT) of the human placenta invade the uterus and its arteries to derive  $O_2$  and nutrients for the fetus. A hypo-invasive placenta is implicated in a serious maternal disease preeclampsia (PE). Our lab discovered that decorin (DCN), a leucine-rich proteoglycan produced by uterine decidual cells restrains multiple trophoblast functions: self-renewal & differentiation of trophoblast stem cells, migration, invasion & endovascular differentiation of EVT cells. Moreover, decidual over-production of DCN was associated PE, and a rise in DCN levels in maternal blood during the second trimester was a predictive biomarker for PE. The anti-proliferative, anti-migratory and anti-invasive functions of DCN resulted from binding to multiple tyrosine kinase receptors on the EVT. We wish to identify additional epigenetic mechanisms. Objectives: MicroRNAs are 15-20 nucleotide long small RNAs which block gene transcription by binding to mRNAs. Many miRNAs were reported to be dysregulated in PE. Since DCN overproduction is associated with PE, we wish to identify miRNAs that may compromise DCN mediated EVT functions known to be altered in PE. **Methods:** Since EVT cells do not express DCN, we conducted a miRNA micro-array using ectopically DCN-over-expressing EVT (HTR-8/SVneo) and control EVT cells. Of multiple DCN-regulated miRNAs, we selected some which were reported to be upregulated in PE and validated them by qPCR using exogenous DCN (250nM)-treated EVT cells. Selected miRNAs were then manipulated in EVT cells by transfection with either miRNA inhibitor (for knockdown) or mimic (for over-expression), and validated with qPCR. Then we measured proliferation (24hr EdU uptake), migration (24hr across  $8\mu\text{M}$  pore membranes inserted in transwells) and endovascular differentiation (tube formation on matrigel in the presence of VEGF) with the miRNA-manipulated and control (mock-transfected) EVT cells.

**Results:** We validated two miRNAs hsa-miR-512-3p and hsa-miR-let-7c-5p to be significantly upregulated by DCN. We decided to knockdown hsa-miR-let-7c-5p and overexpress hsa-miR-512-3p in EVT cells for functional assays. Suppression of miR-let-7c-5p did not affect proliferation, but increased migration and endovascular differentiation of EVT cells. Conversely, overexpression of miR-512-3p inhibited EVT cell proliferation and migration and totally blocked endovascular differentiation. Finally qRT-PCR analysis showed significantly increased expression of both miRNAs in PE-associated placentas relative to those in healthy normotensive pregnancies (control) (n=5 each). N=3, \* $p < 0.05$ .

**Conclusion:** DCN can epigenetically influence trophoblast functions by dysregulating miRNAs in PE. We shall validate a small number of gene targets of these miRNAs and their expression levels PE.

### T-160

#### Trophoblast-Derived Soluble Fms-Like Tyrosine Kinase-1 Production Is Modulated by pICln in Preeclampsia.

Yuko Matsubara,<sup>1</sup> Keiichi Matsubara\*,<sup>2</sup> Yuka Uchikura,<sup>1</sup> Katsuko Takagi,<sup>1</sup> Takashi Sugiyama.<sup>1</sup> *Ehime Univ. SOM, Toon, Ehime, Japan;* <sup>2</sup>*Ehime University Graduate SOM, Toon, Ehime, Japan.*

**Introduction:** pICln is one of chloride channel-forming proteins which mediates chloride intercellular transportation. It is also known that it is involved in the vascular endothelial production of soluble vascular endothelial growth factor (VEGF) receptor (fms-like tyrosine kinase 1: sFlt-1) which can inhibit neovascularization. It is reported that sFlt-1 is a main cause of the pathogenesis of preeclampsia (PE). Therefore, pICln could be also involved in the pathogenesis of PE through the modulation of sFlt-1 production. In this study, we investigated the effect of pICln on the production of sFlt-1 in trophoblasts.

**Methods:** Expression of pICln of placental tissues derived from normal pregnant women and PE patients were evaluated using immunohistochemistry. mRNA of pICln and sFlt-1 was extracted from placenta and analyzed using real-time RT-PCR. sFlt-1 production was evaluated by pICln DNA and siRNA transfection in TBT (rat syncytiotrophoblast) and HTR-8 (human extravillous trophoblast) cells.

**Results:** pICln and Flt-1 was strongly expressed in PE placenta. mRNA of pICln was significantly decreased in PE placenta; however, sFlt-1 mRNA was significantly increased in TBT. pICln DNA transfection did not change sFlt-1 production in TBT; however, the production was reduced in HTR-8. On the other hand, pICln siRNA transfection significantly increased sFlt-1 production in TBT; however, the production was not changed in HTR-8.

**Conclusion:** pICln-mediated sFlt-1 production is different between in syncytiotrophoblast and extravillous trophoblast. It is thought that pICln plays an important role in the pathogenesis of PE through sFlt-1 production in trophoblast.

### T-161

#### Sustained Hypoxemia Activates Nutrient Shuttles between the Placenta and Fetus.

Amanda Jones,<sup>1</sup> Ashebo Betelhem,<sup>1</sup> Ramon Lorca,<sup>1</sup> Colleen Julian,<sup>1</sup> Lorna Moore,<sup>1</sup> Brown Laura,<sup>1</sup> Paul Rozance,<sup>1</sup> Sean Limesand,<sup>2</sup> Stephanie Wesolowski\*.<sup>1</sup> *University of Colorado, Aurora, CO, United States;* <sup>2</sup>*University of Arizona, Tucson, AZ, United States.*

**Introduction:** Gestational hypoxemia can reduce birth weight, yet how hypoxemia affects placental metabolism and nutrient supply to the fetus is unclear. Hypoxemia has been proposed to increase placental glucose utilization and lactate production, thus decreasing glucose and increasing lactate supply to the fetus. However, other reports indicate no change in placental glucose or amino acid transport capacity. This study aimed to test the hypothesis that sustained hypoxemia will activate placental-fetal nutrient shuttles that allow the fetus to maintain its metabolic rate.

**Methods:** We produced hypoxemia in pregnant sheep (HOX, n = 11) via maternal intratracheal nitrogen gas insufflation for 10 days, between 0.8 to 0.9 gestation, compared to ewes receiving air (CON, n = 7). Studies were performed to measure blood flow and net oxygen and nutrient uptake rates across the uterine, placental, and umbilical circulation. Gene expression, protein expression, and enzyme activities were measured in placental cotyledon tissue. Data were analyzed by t-test ( $P < 0.05$ ).

**Results:** Uterine blood flow and uterine and placental oxygen and glucose uptake rates were similar in CON and HOX groups. Placental lactate production was increased, and glutamate uptake was decreased in the HOX group. Fetal glucose, lactate, and oxygen uptake rates were not different, yet fetal pyruvate output was increased in HOX fetuses and lower glucose oxidation rates were associated with severity of hypoxemia. HOX fetuses also had decreased alanine (Ala) uptake and trends for decreased glutamate (Glu) and serine (Ser) output. Concentrations of oxygen, Ser, Glu, and aspartate were decreased, and lactate, pyruvate, and Ala concentrations were increased in HOX fetuses. There was no

difference in fetal glucose concentrations or weight. In HOX placenta tissue, expression of glycolytic genes (*PFK1*, *PKM2*) was decreased, and lactate production (LDH protein) was increased.

**Conclusion:** Our results support increased fetal pyruvate output during hypoxemia and a shuttle between the fetus and placenta whereby pyruvate from the fetus, rather from increased placental glucose uptake and glycolysis, is used by the placenta for lactate production. HOX fetuses had increased Ala concentrations yet decreased Ala uptake without a decrease in placental Ala output, suggesting increased Ala synthesis by the fetus. Decreased fetal release of Ser and Glu and increased pyruvate output, as result of limited glucose oxidation, may reflect liver metabolism as it normally releases pyruvate and these amino acids to the placenta, in exchange for lactate, glycine, and glutamine. Together, these data support activation of placental-fetal nutrient shuttles that alter substrate milieu and enable maintained growth and oxidative metabolism in the HOX fetus.

## T-162

### Interaction of Inorganic Phosphate and Unfolded Protein Response (UPR) in Placenta.

Ana C Correia-Branco†,<sup>1</sup> Olga C Kashpur†,<sup>1</sup> Ciara C Benson,<sup>2</sup> Nirmala Jayaraman,<sup>1</sup> Sasha A Singh,<sup>3</sup> Mark C Blaser,<sup>3</sup> Hideyuki Higashi,<sup>4</sup> Shiori Kuraoka,<sup>4</sup> Elena Aikawa,<sup>3</sup> Eugene W Hinderer III,<sup>5</sup> Mary C Wallingford\*,<sup>1</sup> <sup>1</sup>Tufts Medical Center, Boston, MA, United States; <sup>2</sup>Global Alliance to Prevent Prematurity and Stillbirth, Seattle, WA, United States; <sup>3</sup>Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States; <sup>4</sup>Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States; <sup>5</sup>Clinical and Translational Science Institute-CTSI, Boston, MA, United States.

**Introduction:** Maternal-fetal inorganic phosphate ( $P_i$ ) performs numerous critical functions during embryonic development, but chronic exposure to  $P_i$  is detrimental to vascular health. Our previous work has shown that the sodium-dependent placental  $P_i$  transporter Slc20a2 is a critical  $P_i$  transporter in mouse placenta, and loss of Slc20a2 results in ectopic placental calcification. Clinically, expression of Slc20a2 is decreased in early preeclampsia, while the homeostatic endoplasmic stress unfolded protein response (UPR<sup>ER</sup>) is activated. UPR<sup>ER</sup> activation increases X-box binding protein 1 (XBP1) splicing, and activates protein disulfide isomerase (PDI), a molecular chaperone.

**Methods:** In this study, we hypothesized that Slc20a2 loss contributes to cellular stress in the placenta, and evaluated the role of  $P_i$  in placental vascular health at the molecular level by analyzing placental proteomes from control and Slc20a2 knockout (KO) mice.

**Results:** Proteomics of WT and Slc20a2 KO placental tissue identified 3,050 unique proteins. Differences in protein abundance between WT and KO models and at two gestational time points (13.5 days and 17.5 days) were calculated using an independent t-test ( $p < 0.05$ ). Those with significant changes were analyzed on the STRING database which confirmed that 23 endoplasmic reticulum and Golgi-associated proteins were significantly altered (7 decreased, 9 increased, 7 discordant). In support of failed UPR<sup>ER</sup> activation, XBP1 mRNA splicing was decreased in E17.5 Slc20a2 KO placenta. To determine whether UPR<sup>ER</sup> is influenced by  $P_i$ , we examined XBP1 splicing in placental Jeg3 cells in response to high extracellular  $P_i$  (1-4 mM). Splicing was further attenuated with increasing extracellular fluid  $P_i$  levels. Ongoing work investigates UPR<sup>ER</sup> activation in mouse placenta with PDI immunofluorescence and immunoblotting.

**Conclusion:** We conclude that  $P_i$  and Slc20a2 both interact with placental UPR<sup>ER</sup>. We now propose that Slc20a2 protects the placenta from ectopic calcification by maintaining homeostatic  $P_i$  levels in balance with UPR<sup>ER</sup>. In our future work, we will test the hypothesis that Slc20a2 loss upregulates extracellular  $P_i$  and UPR<sup>ER</sup> in placenta to advance our understanding of the link between maternal  $P_i$  homeostasis and placental calcification, a condition leading to preterm birth.

## T-163

### Chromosomal Microarray (CMA) and Fetal Growth in Stillborn Fetuses.

Susan Dalton†\*,<sup>1</sup> Tsegallassie Workalemahu,<sup>1</sup> Amanda A. Allshouse,<sup>1</sup> Jessica M. Page,<sup>2</sup> Silver M. Robert\*,<sup>1</sup> <sup>1</sup>University of Utah, Salt Lake City, UT, United States; <sup>2</sup>Intermountain Healthcare, Salt Lake City, UT, United States.

**Introduction:** Abnormal fetal growth is associated with stillbirth (SB), with small for gestational age (SGA) and for large for gestational age (LGA) infants incurring a 3-6, and 2-3 fold increased risk, respectively. Although diabetes, hypertension, and placental insufficiency have been associated with growth aberrations, the role of genetic abnormalities is uncertain. Thus, our objective was to assess the relationship between chromosomal microarray (CMA) and fetal growth in stillbirths.

**Methods:** We performed a secondary analysis of the Stillbirth Collaborative Research Network (SCRN) including singleton stillbirths with CMA results. Copy number changes (CNC) were categorized into abnormal (including pathogenic and variants of unknown clinical significance) and normal. Our primary outcome was SGA, defined as less than the 10% birthweight for gestational age. We estimated a logistic regression model of SGA which incorporated analytical weights that reflected the SCRN sampling design. We report weighted frequency and percent for categorical variables, and weighted geometric means with 95% confidence intervals for continuous measures.

**Results:** Among 434 stillbirths with CMA data, 15% had CMA abnormalities. Table 1 demonstrates the relationship between abnormal CMA results and fetal growth. An abnormal CMA was more common in SGA than appropriate for gestational age (AGA) infants (29.5% vs. 16.5%,  $p=0.048$ ). This result remained significant after adjusting for smoking, diabetes, hypertension, obesity, and fetal sex (aOR 2.49, 95% CI 1.29-4.82). In the final model, only abnormal CMA remained significantly associated with SGA (OR 2.12, 95% CI 1.12-4.02). Table 2 presents abnormal CMA results in SGA stillbirths, along with previously published clinical associations.

**Conclusion:** Among stillbirths, genetic abnormalities are significantly associated with SGA. Further analysis of specific genes associated with SGA and stillbirth may provide insight into mechanisms of fetal growth and placental function.

**Table 1.** Fetal growth parameters in association with microarray results.

Characteristic	Value	Abnormal Microarray	Normal	p-value	
		Unweighted	N=67		N=367
		Weighted	N=66		N=63
Birthweight	LBW <2500g	57 (85.6)	266 (76.5)	0.07	
	≥2500g or more	10 (14.4)	81 (23.5)		
Birthweight (g)	Geometric mean (95% CI)	887 (713-1104)	911 (821-1010)	0.82	
Placental weight (g)	Geometric mean (95% CI)	180 (154-211)	197 (184-211)	0.30	
Birthweight percentile	<10th	18 (29.5)	54 (16.5)	0.07	
	10th-90th	40 (64.2)	261 (80.2)		
	>90th	4 (6.2)	11 (3.3)		
Small for gestational age (SGA)	<5 <sup>th</sup> percentile	10 (15.5)	31 (9.5)	0.25	
	<10 <sup>th</sup> percentile	18 (29.5)	54 (16.5)		0.048

Cell values for categorical variables reported as weighted frequency (%), with p-values from weighted wald chi-square. Continuous variables reported as a geometric mean with p-value from weighted t-test. Birthweight percentile and SGA referenced the Alexander growth curves.

**Table 2.** Chromosomal microarray results found in small for gestational age (<5%) stillbirths.

Array result	GA at stillbirth	N=	Clinical associations
Duplication 18p11.32q23	23-36	4	Intellectual disability, epilepsy, bilateral cryptorchidism, abnormal pinna
Duplication 16p13.11p12.3	34	1	Developmental delay, congenital heart defects, skeletal anomalies
Duplication 17p13.3	27	1	Fetal growth restriction, 5 <sup>th</sup> finger clinodactyly, intellectual disability
Deletion Yq11.221	25	1	Azoospermia and male infertility
Deletion 22q11.21q11.23	36	1	DiGeorge syndrome: congenital heart defects, cleft palate, developmental delay, immunodeficiency
Duplication 7p12.3	35	1	Short stature, growth restriction, facial asymmetry and 5th finger clinodactyly

## T-164

### Localization and Kinetics of the Transferrin-Dependent Iron Transport Machinery in the Mouse Placenta.

Chang Cao†, Mark D Fleming. *Boston Children's Hospital, Boston, MA, United States.*

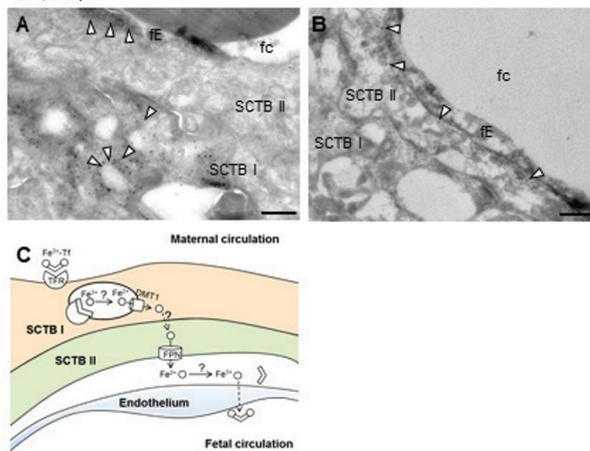
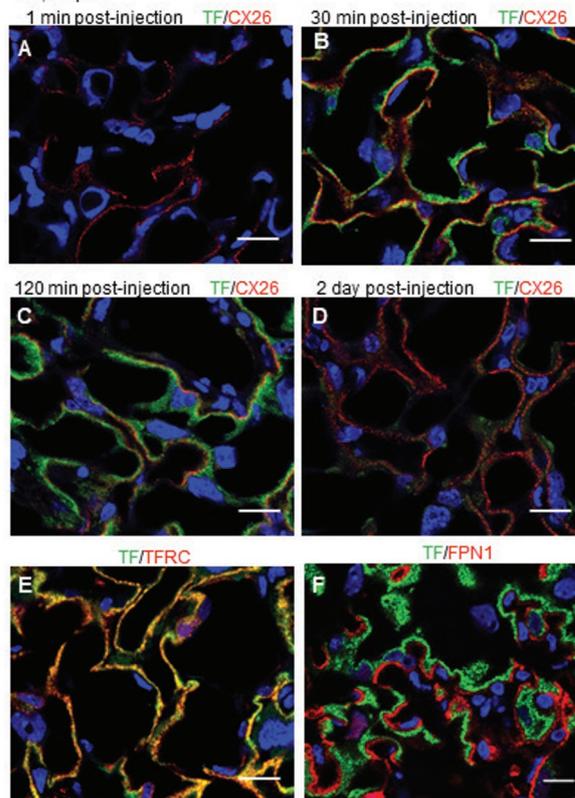
**Introduction:** All the iron needed for fetal growth is transferred from the maternal circulation by the placenta. Mice are commonly used to study placental iron transport. However, it is unclear where the major iron transporters including transferrin receptor (Tfrc) and ferroportin (Fpn) are located within the multiple placental cell layers involved in iron delivery to the fetus. Similarly, how maternally transferrin iron is trafficked across the syncytiotrophoblasts and fetal endothelium before reaching fetal circulation is unknown. Answers to these fundamental questions are critical for future research on the mechanism of placental iron transport.

**Methods:** Using high resolution immunogold electron microscopy, we determined the subcellular locations of the iron importer protein Tfrc and the iron exporter protein Fpn in the mouse placenta at embryonic day 15.5. Fluorescently labeled transferrin iron was injected in the mother and placental uptake of maternal transferrin was studied at different time points (1, 2, 5, 30, 120 minutes, and 2 days) post-injection by immunofluorescence.

**Results:** In the mature mouse placenta, Tfrc was localized to the intracellular vesicles in the syncytiotrophoblast layer 1. Fpn was localized to syncytiotrophoblast layer 2 and is absent from fetal endothelium (Figure 1). Maternally injected transferrin iron was taken up by the placenta within 120 minutes and colocalized with Tfrc in syncytiotrophoblast layer 1 (Figure 2).

**Conclusion:** We show for the first time the precise locations of Tfrc and Fpn in the mouse placenta. We provide evidence that transferrin-iron cycle is confined in syncytiotrophoblast layer 1 and that iron transfer in syncytiotrophoblast layer 2 and fetal endothelium may be independent of transferrin. Taken together, these data strongly suggest that iron transport mechanism is distinct in different placental cells involved in

iron delivery to the fetus. This study also demonstrates the usefulness of modern imaging techniques to study the cellular mechanism and kinetics of nutrient transport in the placenta.

**Figure 1.** Immunogold localization of Tfrc (A) and Fpn (B) in the mouse placenta. Schematic of updated placental iron transport mechanism (C). SCTB, syncytiotrophoblast; fc, fetal circulation; fE, fetal endothelium. Scale bar, 0.5 μm**Figure 2.** Placental uptake of maternally transferrin (green) at different time points post-injection (A-D). Connexin 26 (red, CX26) is a marker of the border between syncytiotrophoblast 1 and 2. Co-staining of Tfrc (red, E) and Fpn (red, F) in transferrin (green) injected placenta. Scale bar, 15 μm

## T-165

**Maternal Inositol Supplementation during Pregnancy Impacts Placental Metabolic Pathways in an Obese Murine Model.**

Lidia Di Cerbo<sup>†,1,2</sup> Ahmed R Hamed<sup>\*,2</sup> Daniela Menichini<sup>†,1</sup> Francesca Ferrari<sup>\*,1</sup> Baha M Sibai<sup>\*,2</sup> Fabio Facchinetti<sup>\*,1</sup> Sean Blackwell<sup>\*,2</sup> Monica Longo<sup>\*,2</sup> <sup>1</sup>University of Modena and Reggio Emilia, Modena, Italy; <sup>2</sup>McGovern Medical School at The University of Texas Health Science Center at Houston (UTHealth), Houston, TX, United States.

**Introduction:** We have shown that wild type (WT) mice fed a high fat (HF) diet develop an obese phenotype with increased gestational weight gain that was improved by inositol (INO) supplementation. Obesity in pregnancy affects fetal metabolic programming leading to an increased risk of metabolic disorders later in life. Placenta plays a role in this fetal abnormal development. Our hypothesis is that INO regulates placental metabolic pathways in a gender-specific manner.

**Methods:** Female WT at 8 weeks of age were placed on HF diet for 4 weeks to develop an obese phenotype, then bred with WT males. On gestational day (GD) 1, dams were randomly allocated to receive either INO supplementation (HF-INO) or water as control. Three groups were obtained: CTR, HF and HF-INO. At term (GD 18) dams were euthanized. Placental biopsy from fetal side were genotyped for gender. In male (M-PL) and female placenta (F-PL), glycogen (Gly) was measured by an ELISA assay, while placental proteins involved in glucose uptake (GLUT4 and IR- $\beta$ ), glycogen synthesis (Akt, pAkt<sup>T308</sup>) and ATP production (pPDH) were measured by Western blot. 1-way or 2-way ANOVA were used for statistical analysis.

**Results:** Gly storage was higher in M-PL and F-PL of HF group dams vs. CTR and was significantly reduced by INO supplementation in both genders (Fig. 1A). Active pAkt<sup>T308</sup> was higher in M-PL of HF group dams vs. CTR and was increased by INO supplementation in F-PL (Fig. 1B). No differences were seen in GLUT4, IR- $\beta$ , total Akt and pPDH expression between groups (Table 1).

**Conclusion:** The time and duration of maternal obesity play a role in the degree of placental metabolic abnormalities predisposing to altered fetal programming. Maternal INO supplementation seems to have a beneficial metabolic effect that is gender dependent.

## T-166

**Dynamic Activation and Regulation of the Placental Unfolded Protein Response in Pregnancy.**

Arren Simpson<sup>†,1</sup> Kyathanahalli Chandrashekar<sup>†,1</sup> Andrew D Kane,<sup>2</sup> Wen Tong,<sup>2</sup> Pancharatnam Jeyasuria,<sup>1</sup> Dino A Giussani,<sup>2</sup> Jennifer C Condon<sup>\*,1</sup> <sup>1</sup>Wayne State University, Detroit, MI, United States; <sup>2</sup>University of Cambridge, Cambridge, United Kingdom.

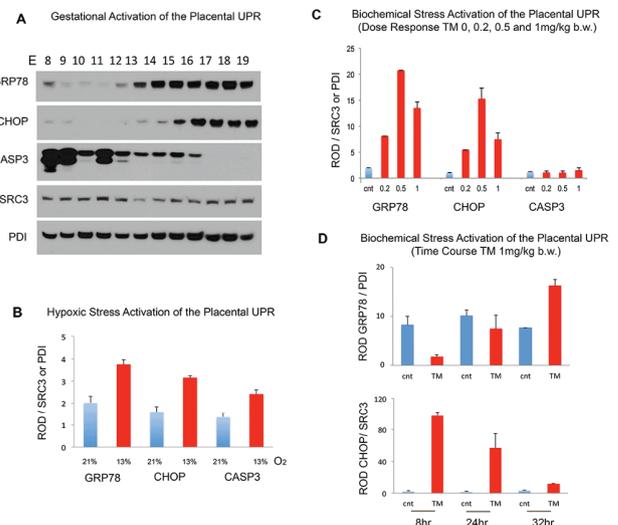
**Introduction:** Increased placental endoplasmic reticulum (ER) stress and activation of its related unfolded protein response (UPR) have been implicated in preeclampsia, intrauterine growth restriction and disrupted placental morphogenesis. However, to date, the function of placental ER stress during healthy pregnancy remains undetermined. We hypothesize that placental proteotoxic stresses generated by the normal physiological and biochemical changes that occur throughout healthy pregnancy, are facilitated by the adaptive action of the ER. In this study we compared the activation of the normal placental UPR across gestation and that challenged physiologically with hypoxic pregnancy and chemically with Tunicamycin.

**Methods:** Chemical Stress Model: Placental tissues were obtained from CD1 mice at gestation day (E) 8-19 and those undergoing an ER stress and UPR activation 32h post i.p. administration at E15 with Tunicamycin (TM; 0, 0.2, 0.5 and 1.0 mg/kg b.w.; n=3 per group). Placental and birth weights were recorded. Maternal Hypoxia Model: On day 6 of pregnancy, Wistar rat dams at 10-12 weeks of age were randomly divided into control (21%) and hypoxic (13%) pregnancy from E6 to 20 (n=10 per group). On day 20, animals were euthanized and weighed, and the placentas from male fetuses were processed for molecular studies. The expression of ER stress and UPR markers BiP, CHOP and apoptotic markers were analyzed (WB, IHC and TUNEL).

**Results:** BiP and CHOP were actively upregulated from E13 and increased over 20 fold towards term. In contrast, CASP3 declined to undetectable

levels as term approached (Fig. 1A). Hypoxic pregnancy led to a further 2 fold upregulation of the UPR without modification to birth or placental weight (Fig.1B). TM-induced ER stress actively modified the UPR in a gestational, spatial, dose and time-dependent manner (Fig.1C, D).

**Conclusion:** We show the dynamic capacity of the placental UPR to mediate cellular adaptation to advancing gestation in healthy pregnancy and in pregnancy complicated by physiological and chemical stress. We suggest that the placenta utilizes the ER and UPR to avoid apoptotic endpoints in response endogenous and exogenous stimuli, allowing for successful placentation and normal embryo development and viability. Supported by The British Heart Foundation (D.A.G.) and AMAG, Ferring and March of Dimes (J.C.C.)



**Figure 1.**

A. Western blot analysis of mouse placenta from E8 to term (E19) demonstrates increased activation of the normal adaptive placental UPR across gestation, as indicated by elevated levels of placental GRP78 and CHOP towards term. In contrast active caspase-3 (CASP3) declines to barely detectable levels at term.

B. Early onset maternal hypoxic stress in the rat model from E8 to E20 of pregnancy also revealed activation of the adaptive placental UPR. Placental GRP78, CHOP and CASP3 are upregulated 2-fold.

C. Tunicamycin administered i.p. to the pregnant mouse at E15 initiates biochemical activation of the adaptive placental UPR in a dose and D. in a time dependent manner. SRC-3 and PDI are utilized as nuclear and cytoplasmic protein loading controls.

## T-167

**Placental DAGLbeta Regulates 2-arachidonoylglycerol Levels and Is Involved in Pregnancy Induced Inflammation.**

Natascha Berger<sup>†,1</sup> Thomas Bärnthaler<sup>\*,2</sup> Jürgen Gindlhuber<sup>†,1</sup> Nermeen Girgis<sup>\*,2</sup> Tom van der Wel<sup>\*,3</sup> Birgit Hirschmugl<sup>\*,1</sup> Ruth Birner-Gruenberger<sup>\*,1</sup> Mario van der Stelt<sup>\*,3</sup> Robert Zimmermann<sup>\*,2</sup> Christian Wadsack<sup>\*,1</sup> <sup>1</sup>Medical University of Graz, Graz, Austria; <sup>2</sup>University of Graz, Graz, Austria; <sup>3</sup>Leiden University, Leiden, Netherlands.

**Introduction:** Diacylglycerollipase  $\alpha/\beta$  (DAGL $\alpha/\beta$ ) possess hydrolytic activity for arachidonic acid (AA)- esterified diacylglycerols to generate 2-arachidonoylglycerol (2-AG) which is one of the main endocannabinoids (EC). 2-AG is the precursor of AA and both lipid species serve as substrates for pro-inflammatory cellular pathways. Given the emerging evidence for an enhanced inflammatory status and altered lipid metabolism in compromised pregnancies like preeclampsia (PE), we aim to identify the specific function and regulation of DAGL $\alpha/\beta$  in healthy and PE placentas.

**Methods:** *In situ* hybridization complemented with immunohistochemical staining was used to determine the cellular localization and distribution of DAGL $\alpha/\beta$  in CTRL (n=4) and PE (n=6) placental tissue (2-way ANOVA). The expression was quantified by RT-qPCR of CTRL (n=12) and PE (n=10) placentas (unpaired two tailed T-test). Immunoblot analysis (n=5, unpaired two tailed T-test) and activity-based protein profiling (ABPP) served to identify protein expression and active sites of isoforms. Placentas were perfused with DAGL inhibitor (DH376 [1  $\mu$ M]) and changes in lipid profiles were determined by LC-MS.

**Results:** DAGL $\alpha$ / $\beta$  isoforms were *in situ* detectable with varying expression in different cell types of CTRL and PE term placentas. We identified DAGL $\beta$  as the predominant DAGL isoform ( $p < 0.001$ ) which is mainly located to trophoblasts (54%). DAGL $\beta$  transcripts and protein expression were significantly increased ( $p = 0.009$ ,  $p = 0.018$ , respectively) in PE. Activity of DAGL $\beta$  in placental tissue was detected and *ex vivo* perfusion underlined the physiological role of this enzyme by decreased 2-AG ( $p = 0.036$ ) tissue levels upon pharmacological inhibition.

**Conclusion:** Our results suggest DAGL $\beta$  dependent induction of 2-AG signaling events in the human term placenta. Pharmaceutical inhibition of DAGL $\beta$  activity directly impacts the lipid profile in the tissue. Exaggerated chronic inflammation during pregnancy as observed in PE, increases placental DAGL $\beta$  expression and activity. We conclude that placental DAGL $\beta$  synthesizes bioactive lipids cell type specifically upon metabolic demand and thereby triggers inflammatory processes in PE. We aim to identify the role of DAGL $\beta$  in placental physiology and disease, to test the enzyme as a potential target for pharmaceutical intervention.

### T-168

#### Circulating microRNA Signatures Associated to Gestation Events Along the Same Healthy Human Pregnancy.

Erika Chavira-Suárez,<sup>1,2</sup> Alma Lilia Hernández-Olvera†,<sup>1</sup> Mariana Flores-Torres†,<sup>2</sup> Karen Celaya-Cruz†,<sup>1</sup> Sofía Gitler†,<sup>1</sup> Juan Carlos de la Cerda-Ángeles\*,<sup>3</sup> Nidia Carolina Espinosa-Maldonado\*,<sup>1</sup> Carlos Fabián Flores-Jasso\*,<sup>2</sup> Humberto Gutiérrez\*,<sup>2</sup> Felipe Vadillo-Ortega\*.<sup>1,2,4</sup>  
<sup>1</sup>Universidad Nacional Autónoma de México, CDMX, Mexico; <sup>2</sup>Instituto Nacional de Medicina Genómica, CDMX, Mexico; <sup>3</sup>Secretaría de Salud de la Ciudad de México, CDMX, Mexico; <sup>4</sup>University of Michigan School of Public Health, Ann Arbor, MI, United States.

**Introduction:** Pregnancy-related circulating microRNAs (c-miRNAs) has been identified in different groups of healthy and pathologic pregnancies. However, no evidence exists of the potential use of c-miRNAs as biomarkers for different gestation processes. In this study, we aimed to characterize c-miRNA profiles at different periods of gestation, following the pregnancy of healthy women from CDMX's perinatal cohort with the purpose of identifying potential c-miRNA signatures associated to specific events of gestation, and performing computational genomic approaches.

**Methods:** 32 plasma samples collected during pregnancy (1T, 2T, and 3T trimesters) and after birth (AB) of eight clinically healthy pregnant women (PW) were used for small RNA (sRNA) profiling by next generation sequencing. sRNA profiles in plasma of ten healthy age-matched non-pregnant women (NPW) were employed as controls. c-miRNA expression levels were quantified carrying out the optimal discovery procedure and generalized likelihood ratio tests. Chromosome 14 microRNA cluster (C14MC) and C19MC were identified in our c-miRNA expression dataset, as well as the top 25% of miRNAs expressed in placenta, umbilical cord plasma, amniotic fluid were obtained from: GSE112343, GSE112343, GSE107524. Wilcoxon signed-rank tests were used to assess significant collective changes of these datasets, between each pregnancy stage, and NPW. Data handling and statistical analyses were carried out in R.

**Results:** 1,449 c-miRNAs were identified in the plasma of PW and NPW. A moderate subset of 46 c-miRNAs were differentially expressed in association to progressive stages of pregnancy, and AB were significantly down regulated (FDRs  $< 0.05$ ). Members of C14MC were down regulated ( $p < 4 \times 10^{-6}$ ) and C19CM were up regulated ( $p < 4 \times 10^{-4}$ ) throughout pregnancy. Comparing the profiling of our dataset with available GEO expression data, we found that c-miRNAs most prominently expressed in reproductive tissues are also collectively down regulated throughout pregnancy ( $p < 0.02$  in placenta and umbilical cord plasma,  $p < 0.009$  in amniotic fluid, and umbilical cord plasma,  $p < 0.05$  in breast milk) and many of those returned to a basal expression AB ( $p < 0.4$ ).

**Conclusion:** Temporal changes of c-miRNA signatures and subpopulations associated to distinct aspects of gestation, including correlates of reproductive tissues supports a wider potential of peripheral miRNAs as biomarkers of normal pregnancy.

### T-169

#### Using iHumanPlacenta Network and Longitudinal Metabolomics Data to Identify Metabolic Signatures Associated with Preterm Birth.

Priyanka Baloni,<sup>1</sup> Nagendra Monangi,<sup>2</sup> Alison Paquette,<sup>3</sup> Gina Huynh,<sup>1</sup> Heather Brockway,<sup>4</sup> Yoel Sadovsky,<sup>5</sup> Louis J Muglia,<sup>6</sup> Jones Jones\*,<sup>4</sup> Nathan D Price\*.<sup>1</sup> <sup>1</sup>Institute for Systems Biology, Seattle, WA, United States; <sup>2</sup>Cincinnati Children's Hospital Medical Center, Cincinnati, OH, United States; <sup>3</sup>Seattle Children's Research Institute, Seattle, WA, United States; <sup>4</sup>University of Florida College of Medicine, Gainesville, FL, United States; <sup>5</sup>Magee-Womens Research Institute, Pittsburgh, PA, United States; <sup>6</sup>Burroughs Wellcome Fund, Research Triangle Park, NC, United States.

**Introduction:** The placenta is the interface between mother and fetus and plays a crucial role throughout pregnancy by performing critical physiological and endocrinological functions. Alterations in the exchange of metabolites between the placenta and fetus may impact fetal development, perinatal outcomes, and long-term health.

**Methods:** We analyzed transcriptome data from 112 term non-pathological placentas, collected at Magee Womens Research Institute, using paired-end RNA sequencing. We leveraged this data to generate the first genome-scale metabolic network of human placenta, iHumanPlacenta, using mCADRE algorithm and Recon3D as the template model. We used an aggregated publicly available placental villous microarray dataset that consisted of 55 preterm and 78 term samples. We also prospectively collected urine samples twice weekly from 100 pregnant women with a previous history of preterm birth beginning at 24 weeks of gestation through the end of pregnancy. We have extracted the untargeted metabolome of urine from 58 pregnant women across multiple timepoints and identified metabolites that are significantly associated with preterm delivery, as determined by linear regression with gestational age.

**Results:** The placenta specific metabolic network, iHumanPlacenta has 7104 reactions, 1896 metabolic genes and 3643 metabolites. We integrated gene expression dataset with the metabolic network and identified reactions associated with RRM2B and FADS2 having significant flux differences between term and preterm samples. Our dense sampling of 2256 urine samples identified 26 unique metabolites that were consistent with the effect size and significant across multiple time points. Among those that decreased included N-methylnicotinamide and creatine and succinate and lactate increased with gestational age. We also developed a workflow to integrate tissue-specific transcriptional regulatory network with iHumanPlacenta network to identify transcription factors that regulate the metabolic landscape in placenta. The clinical impact of this work lies in the identification of metabolic signatures for normal placental development and pregnancy complications.

**Conclusion:** The iHumanPlacenta metabolic reconstruction provides insights into normal placental development and identifying metabolic signatures associated with pregnancy complications.

### T-170

#### Early Gestation T2\*-Based BOLD Effect in Human Placenta.

Ruiming Chen†,<sup>1</sup> Ante Zhu,<sup>2,1</sup> Jitka Starekova,<sup>1</sup> Daniel Seiter†,<sup>1</sup> Kevin M Johnson,<sup>1</sup> Sean B Fain,<sup>1</sup> Scott B Reeder,<sup>1</sup> Dinesh M Shah,<sup>1</sup> Oliver Wieben,<sup>1</sup> Diego Hernando.<sup>1</sup> <sup>1</sup>University of Wisconsin - Madison, Madison, WI, United States; <sup>2</sup>GE Research, Niskayuna, NY, United States.

**Introduction:** Blood oxygen level-dependent (BOLD) based T2\* measurements are associated with placental oxygenation. Here, we investigated early gestation placental T2\* values for obese and non-obese pregnant women.

**Methods:** MRI scans were acquired on a wide-bore clinical 1.5T MRI scanner in supine position with an axial 3D multi-echo SPGR sequence. Water, fat and T2\* maps were generated via chemical shift encoded processing. Image quality of the water-only anatomical images and T2\* maps was scored on a 5-point Likert scale by a radiologist with 10 years of experience, with score  $< 3$  excluded from analysis.

**Results:** 72 non-obese (BMI 23.8 $\pm$ 2.5 kg/m<sup>2</sup>) and 25 obese (BMI 33.5 $\pm$ 2.7 kg/m<sup>2</sup>) pregnant women underwent MRI at up to two gestation time points (14 and 20 weeks). Images from 68 non-obese and 20 obese subjects scored  $\geq 3$ , considered to be of analyzable quality. Fig 1 shows that the

non-obese subjects have a trend of increased T2\* with increased gestation age; the obese subjects show the opposite trend. However, there are no statistically significant differences in T2\* values either between obesity groups or for exploratory comparisons between normal and complicated pregnancies.

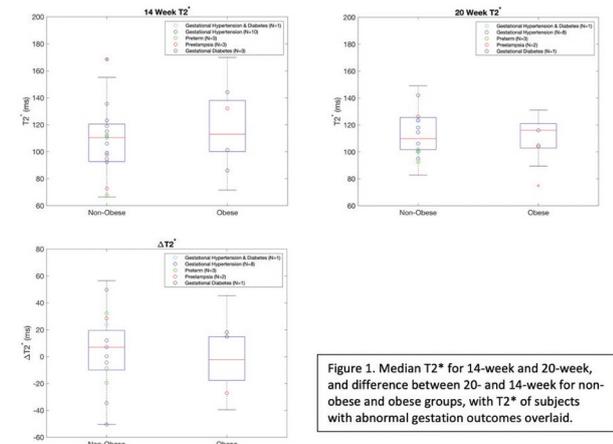


Figure 1. Median T2\* for 14-week and 20-week, and difference between 20- and 14-week for non-obese and obese groups, with T2\* of subjects with abnormal gestation outcomes overlaid.

Qualitatively, Fig 2 shows that the T2\* histogram for preeclampsia subjects is characterized by a shift to lower T2\* values and a less symmetric shape at 14 weeks. All placental volumes increase at 20 weeks, as reflected in the increased overall counts. Patients with diabetes and preeclampsia show lower volume than controls; patients that delivered preterm show a slightly steeper histogram shape that is shifted towards lower T2\* values.

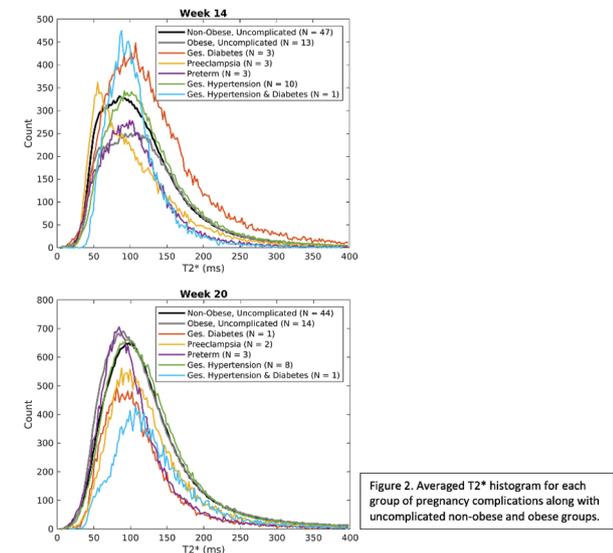


Figure 2. Averaged T2\* histogram for each group of pregnancy complications along with uncomplicated non-obese and obese groups.

**Conclusion:** Our analysis shows median T2\* values alone were not significantly different between subjects with and without adverse events or between non-obese and obese subjects, which could suggest low inter-subject variation in global oxygenation at early gestation. However, the differences in the shape of T2\* histograms between pregnancies of different complication groups could suggest potential differences in local T2\* heterogeneities.

T-171

**Maternal Doxycycline Treatment Causes Murine Fetal Cardiac Dysfunction Associated with Altered Placental Morphology and Endothelin-1 Expression.**

Yuliya Fakhr†,<sup>1,2</sup> Saba Saadat†,<sup>1,2</sup> Lisa K Hornberger,<sup>2,3</sup> Denise G Hemmings\*,<sup>1,2</sup> Luke Eckersley\*,<sup>2,3</sup> <sup>1</sup>Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada; <sup>2</sup>Women and Children's Health Research Institute (WCHRI), University of Alberta, Edmonton, AB, Canada; <sup>3</sup>Department of Pediatrics, University of Alberta, Edmonton, AB, Canada.

**Introduction:** Low levels of endothelin-1 (ET-1) lead to cardiac dysfunction but high levels of this potent constrictor increase afterload, also negatively impacting cardiac function. Doxycycline (Doxy), a commonly prescribed antibiotic, reduces active ET-1 levels by lowering matrix metalloproteinase (MMP) expression. Similarly, chronic exposure to Doxy in non-pregnant animals leads to cardiac dysfunction. During pregnancy, ET-1 plays a physiological role by promoting trophoblast survival and proliferation. Proper placental function is crucial for fetal heart development. However, the effect of Doxy on placental function, ET-1 levels and the association with fetal heart disease is unclear. We hypothesized that treating pregnant mice with Doxy would decrease placental ET-1 expression and alter placental function, leading to fetal cardiac dysfunction.

**Methods:** Pregnant mice received Doxy feed (200mg/kg, n=4 dams) or control feed (n=3 dams) from embryonic day 6.5 (E6.5) till E16.5. Placental ET-1 expression was assessed by Western blot, qRT-PCR, and ELISA. MMP2,9 mRNA expression was assessed by qRT-PCR. Placental morphology was analyzed in H&E stained placentas. Inflammation in maternal blood and placentas and placental cell damage were examined using C-Reactive Protein (CRP) and Lactate Dehydrogenase (LDH) assays. Fetal heart dimensions, flow, and function were assessed by M-Mode and Doppler ultrasound. Values were analyzed using two-tailed Student's t-test.

**Results:** Doxy-treated dams gained less weight during pregnancy and had fewer fetuses. Placental weights (p=0.0034) and placental/fetal weight ratios (p=0.06) were higher in Doxy pregnancies. As hypothesized, placental ET-1 mRNA expression was lower in Doxy pregnancies (p=0.0098), but without detected differences in active ET-1 protein levels. The placental labyrinth/junctional zone ratio was higher in Doxy pregnancies (p=0.01). No differences in placental or maternal blood LDH or CRP were detected. In Doxy pregnancies, the early to late ventricular inflow ratio for both ventricles in fetal hearts was reduced, indicating diastolic dysfunction.

**Conclusion:** Doxycycline causes fetal diastolic dysfunction, with associated changes in placental morphology and reduced ET-1 mRNA. The increased placental size and labyrinth zone in the Doxy group might indicate an adaptive response to suboptimal uterine conditions and reflect poor trophoblast function. Extrinsic maternal or fetal ET-1 protein sources and / or reduced ET-1 degradation may explain maintained MMPs and active ET-1 despite reduced ET-1 mRNA. **Funding:** WCHRI

T-172

**Zika Virus (Zikv) Infection at Early or Mid-Gestation Results in Persistent Uterine and Lymph Node Infection and Adverse Fetal CNS Pathology by Term Gestation in the Olive Baboon (*Papio anubis*).**

Sunam G Dockins†, Darlene N Reuter, Marta E Maxted, Krista R Singleton, Molly E Dubois, James F Papin, Dean A Myers\*. *OUHSC, Oklahoma City, OK, United States.*

**Introduction:** In women, ZIKV infection from early to mid-gestation (G) is associated with highest rate of Congenital Zika Syndrome (CZS). We reported that ZIKV infection at mid-G of a non-human primate (NHP), the Olive baboon, produced transient viremia, placental targeting and vertical transfer (~50%) resulting in loss of radial glial (RG) fibers, astrogliosis and oligodendrocyte damage in the fetal cortex 21 days post-infection (dpi; PMID: 30657788). The present study examined the outcome of ZIKV infection of baboons during early or mid-G on viremia, tissue distribution and fetal brain pathology at term. We hypothesized that ZIKV infection would result in significant neuropathology by term gestation in the baboon.

**Methods:** Timed-pregnant baboons were inoculated subcutaneously with ZIKV (PRVABC59;  $10^4$  ffu) during early (n=4;55-58 dG) or mid-G (n=3; 92-97 dG). Maternal blood, saliva/vaginal swabs were obtained at 0,4,7,14,21,42,80,115 dpi (early) and 0,7,14,21,42,73 dpi (mid). Dams were euthanized and tissues collected at 167-172 dG, term ~183 dG). Control fetal brains (n=3) were obtained at 167-172 dG. ZIKV RNA was quantified by qPCR. H&E staining was performed on fetal cortical and cerebellar sections.

**Results:** Viremia was observed in 6/7 dams between 4-7 dpi, resolving <14 dpi. Fetal death occurred at 5 dpi in 1/4 early G dams. ZIKV RNA was detected in uterus (3/4 early; 0/3 mid-G), lymph nodes (2/4 early; 3/3 mid-G) but not placenta (0/7) and in fetal tissues. Preliminary analysis showed gross structural fetal brain defects (abnormal gyri, sulci) and smaller frontal lobe. H&E staining of fetal frontal cortex showed disrupted formation of the ordered six-layered cortex, decreased pyramidal neurons in the cortical plate and significant cerebellar neuropathology in 3 fetuses (2 early and 1 mid-G).

**Conclusion:** Despite rapid resolution of viremia, ZIKV RNA was found in lymph nodes and uterus at term, indicative that lymphatics and critically the uterus may be privileged sites for ZIKV persistence and immune evasion in pregnancy. Systemic viremia in pregnant baboons is primarily transient similar to ZIKV infected pregnant women yet differing from that reported in macaques. We observed significant gross and histological neuropathology in fetal brains consistent with early to mid-G being the most vulnerable to CZS. (NIH: NS103772, OD010988).

ZIKV RNA copies/ml or mg tissues							
Gest	ID	Blood	Saliva	V. Swab	Uterus	Lymph Node	Birth
Early	1	1.9E4	4.0E3	<LD	7.5E4	2.5E3	V
	2	1.6E4	3.9E4	<LD	<LD	<LD	V
	3	2.1E4	3.3E3	4.5E5	4.4E6	<LD	V
	4	1.9E4	<LD	<LD	5.0E6	6.2E5	FD
Mid	5	3.4E3	<LD	<LD	<LD	8.6E4	V
	6	<LD	7.0E3	<LD	<LD	8.1E3	V
	7	1.4E5	1.0E5	<LD	<LD	2.7E4	V

### T-173

#### A Dual Role for Progesterone in Mediating Influenza A Virus H1N1-Associated Injury in the Lung, but Protecting the Placenta.

Miranda Li†, H Huang\*, A Li†, K Adams Waldorf\*. *University of Washington, Seattle, WA, United States.*

**Introduction:** The influenza A virus (IAV) 2009 H1N1 pandemic was associated with an increased risk of maternal mortality, preterm birth, and stillbirth. The underlying mechanism for severe maternal lung disease and stillbirth is incompletely understood, but IAV infection is known to activate innate immunity triggering the release of cytokines. Elucidating the impact of progesterone (P4), a key hormone elevated in pregnancy, on the innate immune and inflammatory response to IAV infection is a critical step in understanding the pathogenesis of adverse maternal-fetal outcomes.

**Methods:** IAV H1N1 CA/04/09 was used to infect cell lines Calu-3 (lung) and ACH-3P (extravillous trophoblast) with or without P4 (100 nM) at multiplicity of infections (MOI) 0, 0.5, and 3. Cells were harvested at 24 and 48 hours post infection (hpi) and analyzed for cytopathic effects (CPE), replicating virus (TCID50), cytotoxicity (LDH assay), and NLRP3 inflammasome activation (caspase-1 activity, fluorometric assay). Activation of antiviral innate immunity was quantified (RT-qPCR) by measuring biomarker gene expression of innate immune activation (*ifit1*, *ifnb*), inflammation (*il6*) and interferon signaling (*mxr*). All experiments were replicated four times independently and samples tested in triplicate. Statistical analyses used Wilcoxon rank sum.

**Results:** Both Calu-3 and ACH-3P were highly permissible to IAV infection at each timepoint as demonstrated by CPE and replicating virus. Pre-treatment of ACH-3P (trophoblast line) with P4 significantly suppressed cytotoxicity (55% vs. 69% untreated,  $p<0.05$ ) and expression of *ifnb* (7.4 vs. 10.4 untreated log2fold,  $p<0.05$ ), *il6* (4.4 vs 6.2 untreated

log2fold,  $p<0.05$ ), and *il1b* (2.1 vs. 5.8 untreated log2fold,  $p<0.05$ ). Conversely, progesterone treatment in the lung cell line (Calu-3) was associated with a significant increase in cytotoxicity (51.8% vs. 65.2% untreated,  $p<0.05$ ) and upregulation of *il6* expression (7.6 vs. 6.4 untreated log2fold,  $p<0.05$ ). Caspase-1 activity was significantly decreased in both cell lines after treatment with P4 ( $p<0.05$ , both).

**Conclusion:** Pre-treatment with P4 was associated with divergent innate immune and inflammatory responses in placental and lung cell lines marked by less viral cytotoxicity and innate immune activation in ACH-3P, but greater cytotoxicity and *il6* expression in Calu-3. In both ACH-3P and Calu-3, caspase-1 activity was significantly decreased indicating NLRP3 inflammasome activation was not involved in innate immune activation. These data provide evidence that progesterone has a dual role in mediating an antiviral response in the placenta, but may exacerbate influenza A virus-associated injury in the lung.

### T-174

#### Maternal Administration of siRNA-SAA Alleviates Preterm Birth through PD-1/PD-L1 Signaling in a Mouse Model of Sub-Chronic Maternal Inflammation.

Yang Liu†, Jin Liu†, Anguo Liu†, Irina Burd\*, Jun Lei\*. *Johns Hopkins University, Baltimore, MD, United States.*

**Introduction:** Preterm birth (PTB) is the leading cause of perinatal injury, and leads to many long-term sequelae. Maternal inflammation (MI) is a risk factor for PTB with little available intervention to prevent preterm birth. SAA has been studied as a sensitive inflammatory biomarker. PD-1/PD-L1 pathway has been shown to play a role in the regulation of adaptive immune system. Given that PDL1 expression is regulated by SAA, we hypothesized that SAA regulates PD-1/PD-L1 pathway associated immune cells during sub-chronic MI.

**Methods:** At embryonic (E) day 14, CD-1 dams (n=36) were randomly allocated into four groups: PBS+PBS, IL-1 $\beta$ +PBS, IL-1 $\beta$ +siRNA (SAA) and PBS+siRNA (SAA). Dams received intraperitoneal injection (IP) of 0.5 $\mu$ g IL-1 $\beta$  in 100  $\mu$ L PBS or 100  $\mu$ L of PBS only for four consecutive days. siRNA-SAA (1.5 mg/kg) or PBS was injected into caudal vein 1 hour post injection (hpi) after IL-1 $\beta$  injection. The PTB and viability were observed. Maternal blood, maternal liver and placenta were harvested at 24 hpi. Flow cytometry was performed to characterize the PD-1 and PD-L1 expressions in leukocytes of maternal blood. Western blot and qRT-PCR were performed to characterize the levels of *Saa*. Standard statistical analyses were employed.

**Results:** At 24hpi, PTB was significant decreased in IL-1 $\beta$ +siRNA compared to IL-1 $\beta$ +PBS (0% vs 33.3%). The fetal viability was significantly higher ( $P<0.05$ ) in IL-1 $\beta$ +siRNA compared to IL-1 $\beta$ +PBS (39.4% vs 25.5%). Following exogenous IL-1 $\beta$  treatment, the expressions of SAA2 in liver ( $P<0.01$ ) and placenta ( $P<0.001$ ) increased compared to PBS+PBS. Validation on the effect of siRNA of SAA demonstrated that SAA2 expression was inhibited in maternal liver and placenta. IL-1 $\beta$  treatment decreased the number of T cells ( $P<0.001$ ) in maternal blood and increased the PD-1 expression in T cells ( $P<0.05$ ) compared to PBS+PBS. siRNA-SAA treatment decreased the number of T cells ( $P<0.005$ ) while there was no change in PD-1 expression on T cells compared to IL-1 $\beta$ +PBS. Maternal administration of IL-1 $\beta$  had no effect on PD-L1 expression on leukocytes, while siRNA-SAA significantly increased PD-L1 expression compared to IL-1 $\beta$ +PBS ( $P<0.001$ ) and PBS+PBS groups ( $P<0.001$ ).

**Conclusion:** Our study demonstrated that PD-1/PD-L1 associated immune responses was regulated by SAA2 during sub-chronic MI. Maternal administration of siRNA-SAA alleviated PTB and stillbirth through PD-1/PD-L1 pathway. SAA2 appears to be a promising therapeutic target for sub-chronic maternal inflammation.

### T-175

#### Functional Properties of Cytotoxic T Cells in Placenta after Sub-Chronic Inflammation Exposure.

Jin Liu†, Yang Liu†, Anguo Liu†, Jun Lei\*, Irina Burd\*. *Johns Hopkins University, -Baltimore, MD, United States.*

**Introduction:** We have previously determined that placental CD8+T cells play a crucial role in neonatal outcomes following maternal inflammation. However, specific subsets of CD8+ T cell and their functional properties in placenta following inflammation exposure require further investigation.

**Methods:** At embryonic day (E)14, timed-pregnant CD1 dams (n=14) were randomly allocated into two groups: intraperitoneal injection (IP) of 100uL phosphate-buffered saline (PBS) (n=4) or 0.5ug/100uL PBS of mouse recombinant IL-1 $\beta$  (rIL-1 $\beta$ ) (n=10). The dams were injected for four consecutive days to simulate maternal sub-chronic inflammation (MI). After 24 hours (24hpi), placenta tissue was harvested and digested into cells. Flow cytometry was used to analyze immune cell infiltrates and their phenotype in placenta at 24hpi. Intracellular staining for flow cytometry was performed to analyze the cytokines produced by CD8+ T cells, including IL4, IL13, IFN- $\gamma$  and TNF- $\alpha$ .

**Results:** Effector CD8+ T cells and exhausted CD8+ T cells increased in placenta under maternal sub-chronic inflammatory condition (p<0.01). The production of IFN- $\gamma$  and TNF- $\alpha$  in placental CD8+ T cells increased (p<0.05) but there was no significance for IL4 and IL13.

**Conclusion:** The heterogeneity and functional properties of placental CD8+ T cells changed following maternal sub-chronic inflammation exposure. Following maternal sub-chronic inflammation, CD8+ T cells functioned via production of IFN- $\gamma$  and TNF- $\alpha$ , cytokines implicated in hypoxia.

## T-176

### Protective Effect of IL-1Ra on GBS-Induced Chorioamnionitis and Neurobehavioural Impairment of the Progeny.

Taghreed A. Ayash†. McGill University Health Center, Montréal, QC, Canada.

**Introduction:** Group B Streptococcus (GBS) is one of the most common bacteria isolated in human chorioamnionitis, which is a major risk factor for preterm birth, fetal brain injuries, and neurodevelopmental disorders such as cerebral palsy and autism spectrum disorder. Despite the risk of placental inflammation induced by GBS in human, still little known about it. Previous preclinical work from our lab, showed that placental inflammation triggered by GBS induces placental chemokine (CXCL-1), which triggers massive infiltration of the placenta by PMN - which is the hallmark of human chorioamnionitis. In addition, GBS-induced chorioamnionitis triggers an upregulation of interleukin-1B (IL-1B) in the placenta and maternal/fetal blood, which associated to a higher risk of having autism spectrum disease (ASD) and attention-deficit hyperactivity disorder (ADHD) in human. Based on this previous finding we hypothesized that counteracting live GBS-induced placental inflammation using IL-1 Receptor antagonist (IL-1Ra) will prevent chorioamnionitis and subsequent neurobehavioral impairments in the offspring.

**Methods:** We used preclinical Lewis rat model of live GBS-induced chorioamnionitis to study the protective effect of IL-1 Receptor antagonist (IL-1Ra). Dams were treated with 10 mg/kg/12 h, i.p of IL-1Ra. ELISA was used to titrate the protein expression level of proinflammatory cytokines including IL-1 $\beta$ , IL-6 and TNF- $\alpha$ . In addition, *in situ* immunohistochemical (IHC) experiments were used to measure the PMNs infiltration density in the placenta tissue.

**Results:** We showed that administration of 10 mg/kg of IL-1Ra treatment improved the weight gain of the GBS+IL-1Ra dams. Most of the pro-inflammatory cytokines including IL-6, TNF- $\alpha$  and IL-1B were decreased in the placenta, as well as in maternal and fetal blood circulation of GBS+IL-1Ra compared to GBS treated group. Moreover, IL-1Ra treatment, showed a protective effect on the placental infiltration of PMNs in GBS+IL-1Ra compared to GBS-exposed dams.

**Conclusion:** Together, these results suggest the protective effect of IL-1Ra treatment on the causal pathway associating GBS-induced IL-1B-driven chorioamnionitis and resulting neurobehavioural impairments. This work was supported by a grant from the Canadian Institutes of Health Research (CIHR)

## T-177

### Chronic Intervillositis Series with 2 Cases of Recurrence: Successful Rescue Treatment.

Kateri Lévesque, Dorothee Dal Soglio, Michele David, Evelyne Rey\*, Line Leduc\*. CHU Ste-Justine, Montréal, QC, Canada.

**Introduction:** Chronic intervillositis is a rare complication of pregnancy. Experimental preventive treatment has shown promises with a lower recurrence rate of 36% and an increase of babies born alive (36% to 67%). We present our experience with prevention/treatment therapies for chronic intervillositis.

**Methods:** The diagnosis is based on obstetrical history with compatible placental findings and treatment follows the recommendations of Mekinian & al (2015)<sup>1</sup>. Patients are seen in the first trimester and followed monthly.

**Results:** Since 2015, 6 women were diagnosed with this condition. They had 8 pregnancies afterward. They all had suffered at least 1 intrauterine death (median 1.5). All patients received low dose aspirin, dalteparin and low dose prednisone (median 10 mg/d). Half the patients also received hydroxychloroquine. Patients delivered, at a median term of 37 weeks, babies with a median weight above the 10<sup>th</sup> percentile. Five of eight pregnancies were complicated by gestational diabetes. Two women suffered from a recurrence of chronic intervillositis. Both started their preventive treatment before 8 weeks of pregnancy. The first one presented preterm preeclampsia but delivered at 37 weeks a girl of 1870 grams. The second one presented with an intrauterine growth restriction (IUGR) at 16 weeks. The fetal weight of the second woman was less than the 3<sup>rd</sup> percentile at 20 weeks with an increase in alkaline phosphatase (ALKP) levels to 302 U/L (N<126 for the 2nd trimester). The ALKP was used as a marker of placental inflammation. We considered a recurrence of placental chronic intervillositis as the most probable diagnosis and administered IVIG 1g/kg/wk. We observed a decrease in ALKP and improvement in fetal growth along pregnancy. At 30 weeks, as ALKP was increasing, the dose of IVIG was increased to 1.5g/kg/wk. At 32 weeks, the fetal middle cerebral artery doppler showed some vasodilatation and she had a c-section at 33 weeks after betamethasone administration. A healthy boy of 1775 g (10<sup>th</sup> percentile) was born. No intrauterine death occurred.

**Conclusion: Discussion** In our series, the recurrence rate was 25% and all babies were born alive at a median term of 37 weeks (32-40). We present, to our knowledge, the first report of rescue treatment for recurrent chronic intervillositis in the 2nd trimester. Presence of IUGR and increased levels of ALKP suggested recurrence. Monitoring ALKP levels allowed us to adjust therapy and the addition of IVIG helped avoid extreme prematurity and favored fetal growth.

**Conclusion** The preventive treatment reported currently in the literature is efficient to avoid recurrence. The use of IVIG, as a rescue treatment for a presumed recurrence of chronic intervillositis, need validation in similar cases.

1. Mekinian & al. Autoimmunity. 2015 Feb;48(1):40-5

## T-178

### Direct Induction of Trophoblast Stem Cells from Human Fibroblasts.

Moriyah Naama Shacham†, Valery Zayat,<sup>2</sup> Shulamit Sebban,<sup>1</sup> Ahmed Radwan,<sup>1</sup> Rachel Lasry,<sup>1</sup> Ofra Sabag,<sup>1</sup> Silvina Epsztejn-Litman,<sup>3,4</sup> Michal Novoselsky Persky,<sup>5</sup> Kirill Makedonski,<sup>1</sup> Dana Orzech,<sup>1</sup> Noy Dery,<sup>1</sup> Debra Goldman-Wohl,<sup>5</sup> Howard Cedar,<sup>1</sup> Simcha Yagel,<sup>5</sup> Rachel Eiges,<sup>3</sup> Yosef Buganim\*.<sup>1</sup> <sup>1</sup>The Institute for Medical Research Israel-Canada, The Hebrew University-Hadassah Medical School, Jerusalem, Israel; <sup>2</sup>Mossakowski Medical Research Centre, Warsaw, Poland; <sup>3</sup>Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel; <sup>4</sup>The Hebrew University School of Medicine, Jerusalem, Israel; <sup>5</sup>The Magda and Richard Hoffman Laboratory of Human Placental Research, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

**Introduction:** Pregnancy complications with a basis of placental dysfunction such as preeclampsia and intra-uterine growth restriction constitute a major cause of mortality and morbidity for mother and child. Their etiology is not fully understood in part because placental disorders are detected at late stages of pregnancy, when early placental progenitors are no longer available for isolation and study. It is therefore of paramount

importance to develop a platform that allows the production of the earliest placental progenitors from differentiated cells. Our research aims to generate stable and fully functional human induced trophoblast stem cells (hiTSCs), from skin cells (fibroblasts), by the transient expression of a small number of master regulatory genes.

**Methods:** By using somatic cell nuclear reprogramming technology utilizing lentiviral vectors with a Tet-on system, we transiently overexpressed in fibroblasts transcription factors which are important in establishment of TSC identity. The resultant colonies were analyzed using qPCR, immunostaining, RNAseq and RRBS in order to assess their gene expression and methylome profiles and confirm TSC identity, while functional experiments, such as differentiation into trophoblast cell subtypes, were also performed.

**Results:** Based on evidence we have collected thus far, our hiTSCs undergo a stable conversion process and are generally indistinguishable from their human blastocyst-derived TSC counterparts. Gene expression and methylomic analyses as well as functional assays such as the formation of trophoblastic lesions in NOD-SCID mice and differentiation into trophoblastic cell types and the formation of organoids indicate fully reprogrammed functional TSCs.

**Conclusion:** These results suggest that stable and functional human TSCs can be produced from differentiated cells and propose a new platform to model placental dysfunction diseases.

## T-179

### Mitochondrial Citrate Carrier Regulates Syncytiotrophoblast Differentiation.

Sarah Wernimont\*,<sup>1</sup> Adam Rauckhorst,<sup>2</sup> Crawford Peter,<sup>1</sup> Eric Taylor.<sup>2</sup>

<sup>1</sup>University of Minnesota, Minneapolis, MN, United States; <sup>2</sup>University of Iowa, Iowa City, IA, United States.

**Introduction:** Throughout gestation, cytotrophoblasts fuse to form syncytia that provide the interface between maternal and fetal circulation. Regulated small molecule transport into and out of mitochondria is increasingly recognized to control cellular differentiation. However, the role of mitochondrial nutrient transport in trophoblasts has not been fully defined. Here, we test the hypothesis that the cytotrophoblast to syncytiotrophoblast differentiation reprograms metabolism and that, in turn, mitochondrial nutrient transport regulates syncytialization.

**Methods:** To study cytotrophoblast differentiation to syncytiotrophoblasts, we used the in vitro BeWo cell model. BeWo cytotrophoblasts treated with forskolin syncytialize and produce HCG, which we measure as a high dynamic range readout of differentiation. By GC-MS, we profiled the metabolome of the cytotrophoblast-syncytiotrophoblast transition at 0, 24 and 48 hours after treatment with forskolin versus vehicle. Based on finding of decreased cellular citrate, we determined RNA and protein expression of the mitochondrial citrate carrier (CIC) during syncytia formation compared to vehicle treated, non-syncytialized cells. Finally, using CRISPR/Cas9 system, we knocked out the CIC and examined syncytialization following forskolin treatment.

**Results:** Metabolomic profiles of cytotrophoblasts and syncytiotrophoblasts differ significantly by principle components and at the level of individual metabolites. With syncytialization, there is a ~80% decrease in the citric acid cycle intermediate, citrate. Citrate is a critical node of metabolic regulation that can influence cellular metabolism and gene regulation. To better understand mechanisms contributing to decreased levels of cellular citrate, we examined expression of CIC expression at the RNA and protein level and found decreased levels with syncytialization. Using CRISPR/Cas9 system, we found that loss of CIC impairs syncytialization.

**Conclusion:** Overall, our data demonstrates distinct metabolomic changes during syncytialization, including, notably, decreased levels of citrate, likely because of decreased mitochondrial citrate carrier expression. Loss of the CIC results in impaired syncytialization, suggesting that mitochondrial nutrient transport regulates syncytialization.

## T-180

### Methylation Analysis in Pathological Placental Samples.

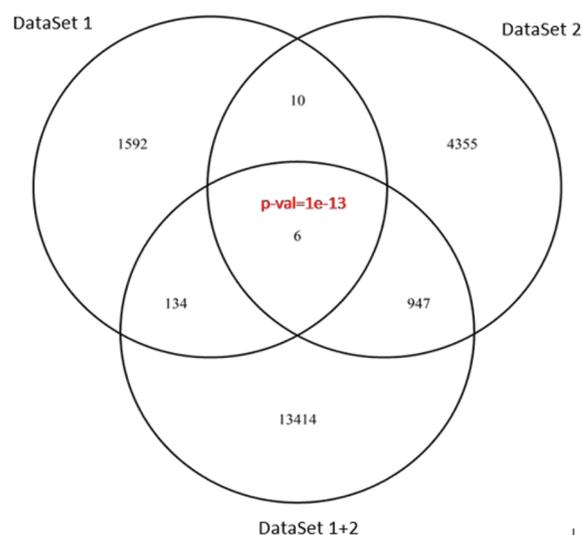
Camino Sm Ruano†, Clara Apicella†, Francisco Miralles\*, Celine Mehats\*, Daniel Vaiman\*. *Institut Cochin, Paris, France.*

**Introduction:** Preeclampsia (PE) and Intrauterine Growth Restriction (IUGR) are major pregnancy pathologies that can impair the baby's future life. DNA Methylation pattern in placentas has already been reported to be altered in case of severe PE and/or IUGR. However, differential methylation analysis can be confounded by many factors, including gestational age and cell composition. Bioinformatic pipelines have been developed to predict covariates from raw data and to use them in regression models to adjust for potential confounding factors. Herein, we aimed to identify true CpGs, linked with the disease status, with minimal influence by any other biological or experimental confounding factors.

**Methods:** DNA Methylation pattern of a total of 31 placental samples (14 ctrl, 12 PE, 3 PE+IUGR and 2 IUGR) was obtained using EPIC Illumina methylation Array (850k CpGs). Using the Planet R package, prediction of variables such as gestational age, ethnicity, cell composition and sample sex were obtained. Minfi and Champ R package were used for removal of CpGs affected by SNPs, present in the sex chromosomes, with low detection value, with a beadcount of <3 in at least 5% of the samples and which behave differently between the two EPIC versions. To determine the main sources of variation, principal component analysis was performed using PCATools. Ward's clustering was used to find clusters of correlation between clinical, predicted covariates and the main Principal Components. Linear regression between each clinical and predicted variables and the total of the 741,184 remaining CpGs was performed in three different sample subsets. Samples coming from only DataSet1 (Early Onset of PE (EOPE), PE+IUGR, Ctrl), only from DataSet2 (Late Onset of PE (LOPE), IUGR and Ctrl samples) and both DataSets together. Anova and post-hoc Dunnett's test was performed to determine the significance between sample status methylation.

**Results:** The removal of all the CpGs influenced by gestational age, cell composition, ethnicity, experiment, age of the mother, parity and birthweight leads to a final total of only 6 CpGs that are correlated to the disease status. These 6 CpGs were found in the three different analyses performed with different samples subsets, improving the significance of these CpGs to a p-value= 1e-13. Gene ontology analysis shows that hypertension is the predominant disease associated with these CpGs (P-value < 0.00075). Moreover, according to the Single Cell Type Atlas (The Human Protein Atlas), these genes are highly expressed in placental cell types, specially Hofbauer cells and fibroblast cells.

**Conclusion:** This strategy allows, despite the limited size of the cohort, to identify in pathological placentas differential methylated CpGs located in genes highly relevant to the placenta pathophysiology. Future studies on meQTL analysis will be developed to determine the role of genetic variants in methylation level.



**T-181****A Potential Role for Calcitonin Gene Related Peptide in Regulating Mitochondrial Function in Endothelial Cells.**

Akansha Mishra, Vipin Alukkal Vidyadharan, Chandra Yallampalli, Madhu Chauhan\*. *Baylor College of Medicine, Houston, TX, United States.*

**Introduction:** Elevated level of soluble tyrosine kinase-1 (sFLT-1) is a suggested cause of oxidative stress and endothelial dysfunction in preeclampsia (PE). Calcitonin gene related peptide (CGRP) is a potent vasodilator known to support vascular adaptation during pregnancy. Circulatory levels of CGRP are lower in PE and blocking function of CGRP results in fetal growth restriction. Therefore, this study was designed to assess if CGRP regulates mitochondrial function in endothelial cells and identify if CGRP effects are altered by sFLT-1 in endothelial cells.

**Methods:** Endothelial cells (HUVEC from ATCC) were cultured in complete endothelial cell growth medium as per manufacturer's instructions. Cells were starved in growth medium containing 2% charcoal stripped FBS for 6 hours, treated with or without peptides ( $10^{-8}$ M) in presence or absence of sFLT-1 for 24 hours and harvested for mRNA extraction using QIAGEN RNA extraction kit. Expression of mRNA for mitochondrial enzyme complexes was assessed by qRT-PCR using gene specific primers and analyzed relative to the expression of GAPDH and 18s. Data was analyzed with Prism GraphPad Software using 1-way ANOVA or unpaired t test.  $P \leq 0.05$  was considered statistically significant.

**Results:** 1) CGRP dose-dependently increases: a) ND1 and ND2 mRNA encoding proteins in mitochondrial complex I, b) Cytochrome b (CYTB) in complex 3,c) Cytochrome c oxidase subunit I (CO1) mRNA in complex 4, and d) ATPase V mRNA in complex 5 ( $P < 0.05$ ); 2) sFLT-1 decreases the expression of ND1 and these decreases are inhibited in presence of CGRP; 3) sFLT-1 shows a tendency to decrease the expression of ND2 in HUVEC which becomes significant in presence of CGRP ( $P < 0.05$ ) and 4) sFLT-1 has no effect on the expression of CYTB, CO1 and ATPase5 in HUVEC cells in presence or absence of CGRP.

**Conclusion:** CGRP mediated increase in mitochondrial enzyme complexes in endothelial cell and rescue of sFLT-1-mediated decreases in ND1, suggest a potential role for CGRP in regulating oxidative stress and ameliorating sFLT-1 induced endothelial dysfunction.

**T-182****Peripheral Blood Mononuclear Cells (PBMCs) Induce Endothelial Dysfunction in Human Umbilical Vein Endothelial Cells (HUVECs) via Proinflammatory Cytokines.**

Aishwarya Rengarajan†, Jason Austin, Amanda Mauro†, Derek Boeldt. *University of Wisconsin Madison, Madison, WI, United States.*

**Introduction:** HUVECs can be used as a model for studying endothelial function in pregnancy and preeclampsia using sustained phase  $Ca^{2+}$  bursting. Sustained  $Ca^{2+}$  bursting contributes to increased production of vasodilator Nitric oxide in the endothelium and hence serves as a tool to assess endothelial function. Decreased  $Ca^{2+}$  bursting is a characteristic feature of PE models, an example of this model being HUVECs treated with inflammatory cytokines demonstrating reduced  $Ca^{2+}$  bursting. However, the effect of PBMCs, whose primary function is to secrete cytokines, has not been studied on HUVEC function. **Hypothesis:** PBMCs contribute to endothelial dysfunction in HUVECs and activated PBMCs will exacerbate endothelial dysfunction.

**Methods:** Confluent HUVECs isolated and pooled from normal pregnant women were loaded with Fura-2 for  $Ca^{2+}$  measurement. HUVECs were treated with ATP ( $100\mu M$ ), washed, PBMCs added, retreated with ATP (30 mins each). PBMCs were isolated using Ficoll-Paque centrifugation from 8 non-pregnant subjects. HUVECs were co-cultured with control or activated PBMCs for 1 hour during imaging. PBMCs were activated using phytohemagglutinin-M  $10\mu g/ml$  prior to co-culture with HUVECs. The number of ATP-stimulated  $Ca^{2+}$  bursts were compared before and after PBMC addition for each experiment. Cytokines were quantified from PBMC culture supernatant using a multiplex assay panel. Statistical comparisons for  $Ca^{2+}$  bursting and cytokine concentrations were performed using rank sum test.

**Results:** Control PBMCs caused a significant decrease in  $Ca^{2+}$  bursting in HUVECs to 78% of pre-treatment value, activated PBMCs resulted in a greater decrease to 73% of pre-treatment value ( $p < 0.001$ ,  $N=8$ ,  $n \geq 4$  for both) in aggregate data. In aggregate, treatment with activated PBMCs resulted in significant decrease in  $Ca^{2+}$  bursting from control ( $p < 0.001$ ,  $N=8$ ,  $n \geq 4$ ). For individual subjects, in PBMCs from  $N=5$  activated was inhibitory vs control ( $p < 0.01$ ), in  $N=2$  activated comparable to control and in  $N=1$  activated was stimulatory vs control ( $p < 0.01$ ). Cytokine concentrations from PBMC conditioned media were 0.37ng/ml (control), 3.2ng/ml (activated) for TNF $\alpha$ ; 0.9ng/ml (control), 8.99ng/ml (activated) for IL-6 ( $p < 0.001$  control vs act for both TNF $\alpha$  and IL-6).

**Conclusion:** The decrease in  $Ca^{2+}$  bursting seen with addition of control/activated PBMCs is comparable to HUVEC models of PE. Activated PBMCs exacerbate  $Ca^{2+}$  bursting as expected when aggregating data from 8 individuals; however, the effect of activated PBMCs to control is individual specific as would be expected with primary cells. PBMCs affect  $Ca^{2+}$  bursting likely via cytokines TNF $\alpha$  and IL-6.

**T-183****Aspirin Resistance Measurements during Pregnancy: The Effect of Aspirin on Platelet Function Compared to Placebo.**

Anadejida J.e.m.c. Landman†, Jeske M. bij de Weg†, Johanna I.p. de Vries, Abel Thijs, Ankie M. Harmsze, Martijn A. Oudijk, Christianne J.m. de Groot, Marjon A. de Boer\*. *1Amsterdam UMC - VUmc, Amsterdam, Netherlands; 2St Antonius Hospital, Nieuwegein, Netherlands; 3Amsterdam UMC - AMC, Amsterdam, Netherlands.*

**Introduction:** Low-dose aspirin has been shown to have variable antiplatelet activity in individual patients and discussion remains on the effectiveness of aspirin 80 mg. The objective of this study is to compare the effect of aspirin 80 mg and placebo on platelet function tests in second and third trimester of pregnancy.

**Methods:** We performed platelet function tests in a subpopulation of the APRIL trial: a randomised double-blinded trial comparing aspirin 80 mg once daily to placebo for the prevention of recurrent preterm birth. Aspirin resistance was measured at 18-22 and 28-32 weeks of gestation with three platelet function tests: VerifyNow®, Chronolog light transmission aggregometry (Chronolog LTA) and serum thromboxane B<sub>2</sub> (TxB<sub>2</sub>). The technician performing the tests was also blinded. The Wilcoxon test was performed to compare the measurements at two time points, and the Mann Whitney U test to compare the means of the groups. Compliance to medication was evaluated by a structured interview on each occasion and by a participant-reported medication diary.

**Results:** In total, 11 women participated in the present study: 6 in the aspirin group and 5 in the placebo group. Treatment groups were comparable with respect to maternal age and body mass index. Platelet function measurements of all participants are shown in Figure 1. The measurements at 18-22 and 28-32 weeks gestation were similar for VerifyNow® ( $p=0.17$ ), Chronolog LTA ( $p=0.83$ ), and TxB<sub>2</sub> ( $p=0.78$ ). Two platelet function tests in the aspirin group showed a markedly reduced platelet activity compared to the placebo group: VerifyNow® ( $p=0.02$ ) and Chronolog LTA ( $p=0.01$ ). The discriminative effect of TxB<sub>2</sub> was less clear ( $p=0.33$ ). There was one participant in the aspirin group without an effect on platelet function, who reported not to be compliant to medication.

**Conclusion:** Aspirin 80 mg has a clear inhibitory effect on platelet function during pregnancy compared to placebo, which is similar in second and third trimester.

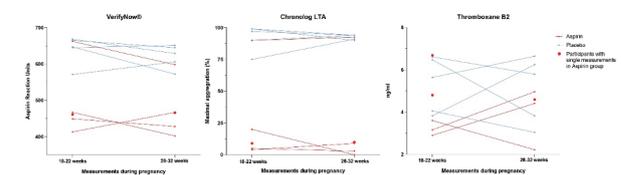


Figure 1 Platelet activity tests during pregnancy

**T-184****Evaluation of the Nutraceutical Conjugated Linoleic Acid for Prevention of Monolayer Breakdown in PE.**

Amanda Mauro†, Derek Boeldt\*. *University of Wisconsin-Madison, Madison, WI, United States.*

**Introduction:** Previous studies have indicated a potential use for the FDA-approved nutraceutical compound t10,c12 conjugated linoleic acid (CLA) as a treatment for preeclampsia (PE), due to its ability to inhibit Src. A key feature of PE is endothelial dysfunction, manifesting in leaky vasculature and impaired vasodilation. We have shown that a mixture of the c9,t11 and t10,c12 CLA isomers (CLA Mix) can block the negative effects of VEGF and TNF $\alpha$  (acting via Src) on Ca<sup>2+</sup> signaling, as a proxy to vasodilator production. We have also found that TNF $\alpha$ , IL-1 $\beta$  and EGF degrade monolayer integrity. To test the ability of CLA Mix to prevent monolayer damage to the endothelium, we subjected human umbilical vein endothelial cells (HUVEC) to CLA Mix pretreatment before screening with a panel of growth factors and cytokines. We hypothesize that CLA Mix will offer protection from the insult of TNF $\alpha$ , IL-1 $\beta$ , and EGF.

**Methods:** Confluent HUVEC were used for monolayer integrity assessment by electrical resistance via the electric cell-substrate impedance sensing (ECIS) system. Serum was withdrawn and cells were given 3 hours to recover. Afterwards, 10 $\mu$ M CLA Mix was added and 30 minutes later growth factors (VEGF, PIGF, bFGF, EGF) and cytokines (IL-6, IL-8, IL-1 $\beta$ , TNF $\alpha$ ) were added at 10ng/mL doses. Data was collected for 24 hours and was analyzed as resistance normalized to control. Statistical analysis was done by student's t-test with n=4 plates (treatments in triplicate).

**Results:** The only increase in monolayer resistance compared to control was for bFGF+CLA Mix, but this was decreased when compared to bFGF alone. For TNF $\alpha$  and PIGF, treatment with CLA Mix was decreased from control but unchanged from the factors alone. EGF+CLA Mix was decreased compared to control and to EGF alone. IL-1 $\beta$  + CLA Mix was decreased compared to control, but briefly increased compared to IL-1 $\beta$  alone. Complete results are shown in the table below.

Results for CLA Mix pretreatment with growth factor and cytokine treatment on monolayer integrity.						
Factor	+CLA Mix vs control	Time (h)	p-value	+CLA Mix vs factor alone	Time (h)	p-value
VEGF	-	-	N.S.	-	-	N.S.
PIGF	↓	11-22	<0.05	-	-	N.S.
EGF	↓	3-24	<0.05	↓	10-24	<0.05
bFGF	↑	11-23	<0.05	↓	1-7, 10-22	<0.05
IL-6	-	-	N.S.	-	-	N.S.
IL-8	-	-	N.S.	-	-	N.S.
IL-1 $\beta$	↓	4-24	<0.05	↑	6-11	<0.05
TNF $\alpha$	↓	5-24	<0.05	-	-	N.S.

**Conclusion:** Largely CLA Mix pretreatment mirrored the response of the factors on their own. While CLA Mix did not improve monolayer integrity with TNF $\alpha$ , IL-1 $\beta$  or EGF treatment, it did not worsen conditions for any of the factors assessed. When taken together with previous studies showing definitive benefits of CLA Mix treatment for Ca<sup>2+</sup> signaling, CLA Mix may be helpful in less severe cases, or earlier in the disease progression, where vasodilator production needs to be improved but the monolayer has yet to be damaged; and CLA Mix will do no additional harm. However, it is unlikely to be effective when the endothelial damage has progressed and is in need of repair.

**T-185****Syncytiotrophoblast-Enriched Extracellular Vesicles from Normal and Preeclamptic Pregnancies Have a Different Impact on Nitrate Stress in Human Umbilical Vein Endothelial Cells.**

Roberto Esteban Villalobos-Labra†, Floor Spaans, Tamara Sáez, Anita Quon, Christy-Lynn Cooke, Sandra T Davidge. *University of Alberta, Edmonton, AB, Canada.*

**Introduction:** Preeclampsia (PE) is a pregnancy disorder that includes new-onset hypertension after 20 weeks of gestation and end-organ dysfunction. Women with PE present with vascular endothelial dysfunction, contributing to the development of hypertension. It is thought that the PE placenta may contribute to endothelial dysfunction by releasing higher levels of syncytiotrophoblast-derived extracellular vesicles (STBEVs) in the maternal circulation. STBEVs from normal pregnancies (NP) at high concentrations (200  $\mu$ g/mL) were shown to impair *ex vivo* vascular/endothelial function and increase nitrotyrosine levels, a marker of nitrate stress that is associated with endothelial dysfunction, in endothelial cells. However, although recent literature shows PE-STBEVs differ in composition from NP-STBEVs, the effects of NP- versus PE-STBEVs on endothelial function are still unknown. Thus, we aimed to assess the uptake of NP- and PE-STBEVs by human umbilical vein endothelial cells (HUVECs) and their impact on nitrotyrosine levels, as a marker of endothelial dysfunction.

**Methods:** Placenta-enriched extracellular vesicles (STBEVs) were collected from placental perfusions (according to Dragovic *et al.*; *Methods*; 2015) and pooled from n=3 NP or PE placentas. HUVECs were isolated from umbilical cords from normal pregnancies (n=5). HUVECs were incubated with dyed STBEVs (80  $\mu$ g/mL for 1 hr, stained with carboxyfluorescein succinimidyl ester) to assess STBEV uptake. In addition, HUVECs were incubated with NP or PE-STBEVs at increasing concentrations (0, 1, 10, 100, and 200  $\mu$ g/mL for 24h) to evaluate nitrotyrosine levels by immunofluorescence staining. Data were analyzed using two-way ANOVA with Sidak's multiple comparisons test.

**Results:** Both NP- and PE-STBEVs were located inside the HUVECs. Nitrotyrosine levels increased with NP-STBEVs at 100 and 200  $\mu$ g/mL compared to untreated cells (1.56 $\pm$ 0.12 and 1.52 $\pm$ 0.10 fold, respectively; p<0.05), while PE-STBEVs did not induce any changes in nitrotyrosine levels (interaction: p<0.0001, Figure 1).

**Conclusion:** Our data showed that NP- and PE-STBEVs were internalized by HUVECs. In contrast to our expectations, only NP-STBEVs induced nitrate stress, which suggests NP- and PE-STBEVs differentially impact endothelial function. Further studies are necessary to evaluate the effects of NP- vs. PE-STBEVs on other markers, such as oxidative stress, which may influence endothelial function.

**T-186****Src Inhibition Offers Monolayer Support in an In Vitro Model of Endothelial Dysfunction.**

Amanda Mauro†, Derek Boeldt\*. *University of Wisconsin-Madison, Madison, WI, United States.*

**Introduction:** Preeclampsia (PE) is a hypertensive disorder of pregnancy. Currently no treatments to prevent PE or to stop PE progression exist. The etiology of PE is unknown, but endothelial dysfunction is known to be one of the main features. Mediators of endothelial dysfunction include growth factors and cytokines, acting via signaling pathways to break down endothelial connections. We have evaluated the effects of growth factors (VEGF, PIGF, bFGF, EGF) and cytokines (TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-8) on monolayer integrity in endothelial cells, finding that TNF $\alpha$ , IL-1 $\beta$ , and EGF caused insult to the monolayer, while bFGF increased monolayer integrity. As these factors activate Src and MEK/ERK, whether directly or indirectly, we hypothesize that the Src inhibitor PP2 and the MEK/ERK inhibitor U0126 will prevent injury from TNF $\alpha$ , IL-1 $\beta$ , and EGF insult.

**Methods:** Human umbilical vein endothelial cells (HUVEC) were grown to confluence on the electric cell-substrate impedance sensing (ECIS) system for measurement of monolayer integrity by electrical resistance. Serum was withdrawn and cells had 3 hours to recover before PP2 and U0126 (10 $\mu$ M) were added, and 30 minutes later growth factors and

cytokines (10ng/mL) were added. Data collection continued for 24 hours. Data was analyzed as resistance normalized to control with n=4 plates (treatments in triplicate). Student's t-test was used for statistical analysis. **Results:** In all cases except for bFGF (N.S.), U0126 led to significant decreases in resistance compared to control 5-24 h (p<0.01). The results for all factors + PP2 are shown in the table below. Most notably, for TNFa + PP2 resistance was significantly decreased from control 5-14 h (p<0.05), with resistance ultimately recovering to control levels. For IL-1b, PP2 did not change resistance and continued to be decreased from control 4-24 h (p<0.05). For IL-6, PP2 increased resistance from control 10-22 h (p<0.05) and from IL-6 alone 5-22 h (p<0.05). For IL-8, PP2 increased resistance compared to control and IL-8 alone 5-25 h (p<0.05).

Results for PP2 + growth factor and cytokine panel for monolayer integrity assessment.						
Factor	+PP2 vs control	Time (h)	p-value	+PP2 vs factor alone	Time (h)	p-value
VEGF	-	-	N.S.	-	-	N.S.
PIGF	-	-	N.S.	-	-	N.S.
EGF	-	-	N.S.	-	-	N.S.
bFGF	↑	18-24	<0.05	↓	2-24	<0.05
IL-6	↑	10-22	<0.05	↑	5-22	<0.05
IL-8	↑	5-25	<0.05	↑	5-25	<0.05
IL-1b	↓	4-24	<0.05	↓	4-24	<0.05
TNFa	↓	5-14	<0.05	-	-	N.S.

**Conclusion:** With the exception of bFGF, U0126 was injurious to the monolayer. PP2 broadly offered support to the monolayer, buffering bFGF and boosting IL-6 and IL-8. Both IL-1b and TNFa led to reductions in monolayer resistance. In the case of TNFa this was prevented with PP2. This is notable, showing potential for Src inhibition to prevent the greatest insult. Src inhibition therefore offers a potential strategy for stabilization of the endothelium, and could serve as part of a therapeutic strategy for treating endothelial dysfunction in PE.

### T-187

#### Hydroxychloroquine Effect on Healthy and Activated Fetoplacental Endothelial Cells *In Vitro*.

Maja Gajic†, Christian Wadsack\*, Mila Cervar-Zivkovic\*, Karoline Mayer-Pickel\*. *Medical University of Graz, Graz, Austria.*

**Introduction:** Preeclampsia (PE) is characterized by insufficient placental perfusion, followed by the production of pro-inflammatory cytokines and anti-angiogenic factors. PE can increase maternal and/or fetal morbidity and mortality. Moreover, it has been shown that PE has a long-term negative effect on offspring. The only currently available treatment for PE is delivery. We suggest hydroxychloroquine (HCQ) as a potential drug for PE. HCQ is an anti-inflammatory drug improving endothelial homeostasis in lupus. However, the effect of the drug on placental vasculature is still unknown. We aim to investigate the effect of HCQ on health fetal endothelial cells as well as in a model of inflammatory-induced endothelial activation at the fetal side of the human placenta.

**Methods:** Primary fetoplacental arterial endothelial cells (fpECAs) isolated from healthy placentas (N=7) were exposed to a pro-inflammatory environment (TNF- $\alpha$ , IL-6 and IL-1 $\beta$ ). Pharmacological (10 ng/ml, 10 ng/ml, 1 ng/ml) concentrations of cytokines in the presence or absence of HCQ (1  $\mu$ g/ml) were used. Subsequently, gene expression of cytokines (IL-8 and IL-6), leukocyte adhesion molecules (ICAM-1, VCAM-1 and selectin E) and angiogenic mediators (placenta growing factor (PIGF) and endoglin (ENG)) were quantified by qPCR. Moreover, protein concentrations of secreted IL-8 and PIGF were evaluated by ELISA, while cell abundance of ICAM-1 and VCAM-1, as well as phosphorylation of nuclear factor  $\kappa$ B (NF $\kappa$ B), was determined by Western Blot analysis. Statistical analysis was done in GraphPad Prism 9.0.1 by use of repeated-measure one way ANOVA with Sidak multiple comparisons and two-Tailed Student t-test where applicable.

**Results:** Upregulation (P<0.001) in gene expression, after challenging fpECAs with the cytokines mix, was observed in all molecules, except

IL-6, PIGF and ENG. However, after adding HCQ expression of specific genes were unchanged. The same pattern was noticed on protein levels for IL-8, ICAM-1 and PIGF. Strikingly, VCAM-1 protein expression significantly (P<0.01) decreased, after treatment with HCQ. The phosphorylation of NF $\kappa$ B was significantly higher in inflammatory condition (P<0.01), but not affected by HCQ. Treatment of the cells with HCQ alone did not differentiate from control.

**Conclusion:** This work shows that HCQ alone does not negatively affect fpECAs. Our finding suggests that HCQ does not have any negative effect on placental vascularization. Yet, HCQ failed to lower induced inflammation, with exception of VCAM-1. This suggests a limited anti-inflammatory effect on fpECAs, in case of cytokine-induced inflammation, notable only on VCAM-1 protein levels in the cell, possibly independent of the NF $\kappa$ B pathway.

### T-188

#### Syncytiotrophoblast Derived Extracellular Vesicles Aberrantly Express HLA DR in Preeclampsia.

Chiara Tersigni,<sup>1</sup> Donatella Lucchetti,<sup>2</sup> Rita Franco,<sup>1</sup> Alessandro Sgambato,<sup>2</sup> Giovanni Scambia,<sup>1</sup> Nicoletta Di Simone.<sup>1</sup> <sup>1</sup>Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy; <sup>2</sup>Università Cattolica del Sacro Cuore, Rome, Italy.

**Introduction:** The lack of expression of Human Leukocyte Antigens (HLA) class II in trophoblast tissues is one of the escape mechanisms of the semiallogeneic fetus from maternal immune system recognition and rejection. In the pathogenesis of preeclampsia (PE), reduced tolerance from maternal immune system towards paternal-derived antigens during placentation, could be a first trigger leading to poor placentation and clinical PE. We previously observed that syncytiotrophoblast-derived extracellular vesicles (STBEVs) collected by dual placental perfusion aberrantly express HLA-DR. Aim of this study was to assess whether STBEVs isolated from plasma of women with clinical diagnosis of PE carry HLA-DR molecules.

**Methods:** Plasma was collected from 22 women with PE and 22 healthy pregnant women. Circulating STBEVs were isolated by ultracentrifugation (120000 g) and analyzed for the expression of HLA-DR and placental alkaline phosphatase (PLAP), a specific marker of the placenta, by Western blot analysis and flow cytometry.

**Results:** STBEVs (PLAP positive) can be detected in plasma of PE and healthy pregnant women. Levels of circulating STBEVs were significantly higher in PE women than in controls matched for gestational age (P<0.01). Circulating STBEVs positive for HLA-DR were observed in 14 of 22 cases of PE (64%) while no HLA-DR positivity was detected in any of the controls (P<0.01).

**Conclusion:** Aberrant expression of HLA-DR in circulating STEVs is specifically associated with PE. Further studies are required: a) to define the role of aberrant placental expression of HLA-DR molecules in the pathogenesis of PE; b) to evaluate a possible application of detecting circulating HLA-DR positive STEVs in the diagnosis and prediction of PE in the first and second trimester of pregnancy.

### T-189

#### Cell-Free Membrane-Bound and Membrane-Unbound Mitochondrial DNA in Maternal Circulation in Preeclampsia.

Spencer C Cushen†,<sup>1</sup> Contessa A Ricci,<sup>1</sup> Danielle Reid,<sup>1</sup> Jessica L Bradshaw,<sup>1</sup> Talisa Silzer,<sup>1</sup> Blessing Alexandra,<sup>1</sup> Jie Sun,<sup>1</sup> Sabrina M Scroggins,<sup>2</sup> Mark K Santillan,<sup>2</sup> Donna A Santillan,<sup>2</sup> Nicole R Phillips,<sup>1</sup> Styliani Gouloupoulou\*. <sup>1</sup>University of North Texas Health Science Center, Fort Worth, TX, United States; <sup>2</sup>University of Iowa Health Care, Iowa City, IA, United States.

**Introduction:** Cell-free circulating mitochondrial DNA (CFCmtDNA) is a damage-associated molecular pattern (DAMP) that activates Toll-like receptor-9 (TLR-9). Previous studies suggested that CFCmtDNA may be a potential pathogenic trigger or a contributor to the maintenance of preeclampsia. The main objectives of this study were 1) to determine absolute concentrations of CFCmtDNA, in membrane-bound and -unbound states, independent of nuclear DNA (nDNA) changes, in cases with preeclampsia and healthy controls and 2) to implement a penalized



rejection who were matched by year of conception to 51 pregnant recipients with preeclampsia. Unplanned pregnancy was common, with a prevalence of 64% in the acute rejection cohort and 44% in the preeclampsia cohort. Shorter transplant to conception interval (2.37 vs 5.43 years,  $p < 0.001$ ) was identified as a risk factor for acute liver rejection. Elevated liver enzymes (AST, ALT) was universal for rejection but occurred in a minority of those with preeclampsia (18%), who were mainly diagnosed based on elevated blood pressures. Although there were no significant differences in maternal outcomes, there was more graft loss after peripartum acute rejection (41% vs 16%,  $p = 0.017$ ). Acute liver rejection was associated with a higher rate of preterm delivery at  $< 32$  weeks (41% vs. 16%,  $p = 0.04$ ) and was independently associated with neonatal composite morbidity (aOR 5.02 [CI 1.06, 24.8],  $p = 0.04$ ) when compared with pregnancies affected by preeclampsia.

**Conclusion:** Though both rejection and preeclampsia can cause liver damage, liver transplant recipients with preeclampsia presented with hypertension without transaminitis and had better obstetric and graft outcomes. These data highlight the importance of preconception counseling and graft optimization prior to pregnancy to reduce the risks of graft loss, preterm delivery, and neonatal morbidity associated with liver transplant rejection during pregnancy.

### T-192

#### Resolved Low Placentation and Risk of Hypertensive Disorder of Pregnancy.

Henri M Rosenberg<sup>†</sup>, Chelsea Debolt, Minhazur Sarker<sup>†</sup>, Geeta Rao, Jacqueline Roig, Angela Bianco\*. *Icahn School of Medicine Mount Sinai, New York, NY, United States.*

**Introduction:** Evidence suggests that there is a negative correlation between placenta previa and hypertensive disorders of pregnancy (HDP). The pathogenesis of HDP is multifactorial. One theory suggests HDP arise from incomplete spiral arteriole remodeling. Initial implantation of the placenta in the lower uterine segment increases vascularity and may facilitate more complete remodeling. As this remodeling occurs late in the first trimester and early in the second trimester, we hypothesize that resolution of low placentation would continue to have a protective effect against HDP.

**Methods:** This retrospective cohort includes 1207 women delivered at Mount Sinai Hospital from 2015-2019, diagnosed with low placentation ( $n = 650$ ) at mid-trimester anatomy survey with resolution on subsequent ultrasound. Women with normal placentation ( $n = 557$ ) at mid-trimester anatomy survey were randomly selected for comparison. Demographic and neonatal characteristics were compared. Primary outcome was development of HDP. Secondary outcomes included progression to and timing of severe HDP, and development of postpartum HDP. Primary and secondary outcomes were assessed using logistic regression adjusting for age, BMI, race/ethnicity, ASA use, IVF, history of HDP, and gestational age at delivery.

**Results:** Resolved low placentation was associated with increasing age, lower BMI, white race, IVF and posterior placenta ( $p < 0.01$ ). Patients with resolved low vs. normal placentation had similar odds of developing all forms of HDP (9.85% vs. 8.65%  $p = 0.43$ , aOR 1.11, 95% CI 0.76-1.63). Comparing resolved placentation to normal placentation, there was no significant difference in gestational age at diagnosis of HDP (37 +/- 3wk vs. 38 +/- 2wk  $p = 0.38$ ), development of severe HDP (3.85% vs. 3.85%  $p = 0.126$ , aOR 1.07 CI 0.60-1.191), or development of post-partum severe pre-eclampsia (1.69% vs. 1.20%  $p = 0.43$ , aOR 1.31, 95% CI 0.52-3.27).

**Conclusion:** Women with resolved low placentation had similar odds of developing pregnancy-induced hypertensive disorders with no difference in development of severe features or post-partum pre-eclampsia compared to having normal placentation throughout pregnancy. Although the pathogenesis of HDP is complex, the location of placentation at time of spiral arteriole remodeling may not play a role in the protective factor of placenta previa at time of delivery.

### T-193

#### Polymorphism of ESR1 Gene in Pregnants with Hypertension State.

Bakhodir Kurbanov. *Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan.*

**Introduction:** One of the candidate genes that can serve as a risk factor for the development of preeclampsia is the gene for the estrogen receptor alpha (ESR1) The aim of our work is to study the rs2228480 polymorphism of the ESR1 gene in patients with hypertension state.

**Methods:** The study group consisted of only 90 pregnant women, including 40 pregnant women with hypertension and preeclampsia (main group) aged 19-38 years and 50 pregnant women with physiological pregnancy (control group). Methods: The material for the study was DNA samples from pregnant women. Isolation of DNA from blood and PCR analysis were performed with kits of reagents and test systems from Ampli Prime Ribo-prep (LLC Next Bio, Russia). The concentration of the obtained preparation of nucleic acids in the samples was determined spectrophotometrically on a NanoDrop-2000 device (NanoDrop Technologies, USA). Testing of the ESR1 mutation was carried out on a PCR amplifier Corbett research (Corbett, Australia), using the test system of the company (OOO NPF Sintol, Russia) according to the manufacturer's instructions.

**Results:** The prevalence of frequencies of genotypes GG, GA and AA in women in the main group was 48.7%, 11.8% and 1.3%, respectively, while in the control group - 60.5%, 21.0% and 1, 3%, respectively. In the control sample, the distribution of the genotypes of the 2014G>A polymorphism of the ESR1 gene corresponded to the expected one under the Hardy - Weinberg equilibrium (HWE). Expected frequency of distribution of genotypes for HWE in the main group:  $G / G = 36.6$ ;  $G / A = 9.7$ ;  $A / A = 0.6$ . The observed frequency of distribution of genotypes for RHV in the main group:  $G / G = 37$ ;  $G / A = 9$ ;  $A / A = 1$ . When comparing the frequencies of genotypes and alleles of the ESR1 gene polymorphism in the studied groups of patients and controls, we did not find statistically significant differences. In the main group, the frequency of the G allele of polymorphism 2014G>A of the ESR1 gene was 88.3%, in the control group - 85.7%, which also indicated an insignificant association with an increased risk of predisposition to preeclampsia ( $\chi^2 = 0.31$ ;  $P = 0, 28$ ;  $OR = 1.26$ ; 95% CI: 0.56-2.89).

**Conclusion:** Thus, according to preliminary data, the presence of the G allele and the hetero- and homozygous G / A and GG genotypes increases, and the identification of the A allele and the AA genotype decreases the risk of preeclampsia. It is recommended to conduct further studies of the association of ESR 1 gene polymorphism with the development of preeclampsia using a larger sample.

### T-194

#### Hypertensive Disorders of Pregnancy Share Common cfDNA Methylation Profiles.

Jarmila A Zdanowicz<sup>†</sup>, Marialuigia Spinelli, Daniel Surbek, Martin Mueller\*. *University of Bern, Bern, Switzerland.*

**Introduction:** Hypertensive disorders in pregnancy (HDP) including preeclampsia (PE) are associated with an increased risk for long-term cardiovascular disorders for both mother and infant. Increased evidence shows that HDP are vascular disorders with a shared predisposition and DNA methylation is essential. Although cell-free DNA (cfDNA) assessments are routinely used in obstetrics, the detection of methylation patterns in early pregnancy is still unexplored. Our aim was to test cardiovascular predisposition for HDP using cfDNA methylation profiles.

**Methods:** We collected 589 first trimester serum samples in our Liquid Biobank Bern. We identified 11 samples with no chronic hypertension and subsequent PE development. 14 patients had chronic hypertension without subsequent PE development. 422 patients had no chronic hypertension and no subsequent PE development. We matched patients according to known PE risk factors and identified 3 groups ( $n = 5$  each): PE and no chronic hypertension (PE), chronic hypertension and no PE (HT), and control (Ctr). We adopted the process of cfDNA extraction and performed whole-genome cfDNA methylation sequencing in our first trimester samples. We assessed unbiased determination of tissue origins of cfDNA using deconvolution, and identified differentially methylated regions (DMRs)

and annotated genes. We used Student's t tests and one-way analysis of variance and Holm-Sidak test for analysis. We considered  $p < 0.05$  to be statistically significant.

**Results:** Clinical characteristics differed only in PE and chronic hypertension incidence. Birth weight was significantly lower in the PE group. Global cfDNA methylation changes and potential cfDNA methylation origin point towards a homogenous PE/HT group (Fig. 1A+B). We detected 86 DMRs in PE/HT (75 genes) compared to PE/Ctr (DMRs: 139; 75 genes) and HT/Ctr (DMRs: 140; 74 genes) groups (Fig. 2C). Analysis of specific genes point towards an association with cardiovascular disorders (Fig. 2C - red highlighted genes).

**Conclusion:** The cfDNA methylation profile in the first trimester supports the pivotal role of the cardiovascular system in the pathogenesis of HDP.

Figure 1

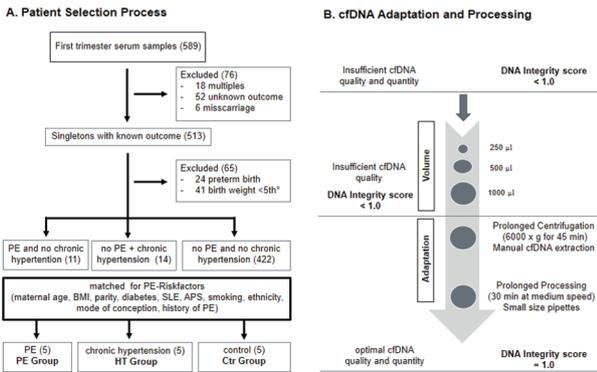
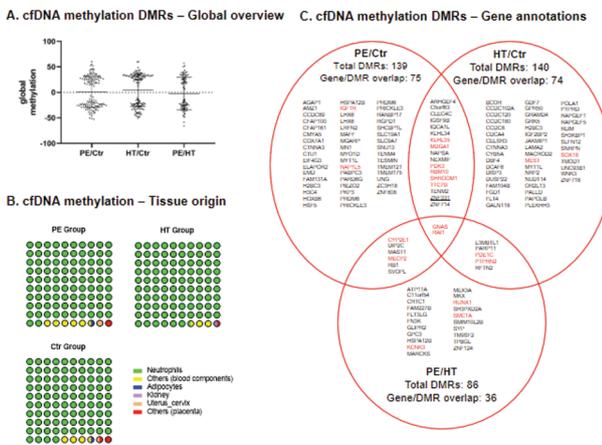


Figure 2



T-195

**Factors Associated with Diagnosis of Gestational Hypertension or Preeclampsia in Women with Gestational Diabetes.**

Vishmayaa Saravanan<sup>†</sup>,<sup>1</sup> Rachel Harrison,<sup>2</sup> Lauren Pavlik,<sup>1</sup> Meredith Cruz,<sup>1</sup> Anna Palatnik.<sup>1</sup> <sup>1</sup>Medical College of Wisconsin, Milwaukee, WI, United States; <sup>2</sup>Advocate-Aurora Medical Group, Chicago, IL, United States.

**Introduction:** Significant gaps remain in understanding the mechanism behind why women with gestational diabetes mellitus (GDM) are at higher risk for developing hypertension of pregnancy. This study aims to identify maternal and pregnancy factors associated with the development of gestational hypertension (gHTN) or preeclampsia (PE) in women with GDM.

**Methods:** This is a retrospective case control study of non-anomalous singleton pregnancies at a single academic center that delivered between 2011-2019 and were diagnosed with GDM via Carpenter-Coustan two-step approach. Cases were defined as women with GDM and gHTN or PE. Controls were defined as women with GDM without gHTN or PE.

Maternal demographics, pregnancy characteristics, and GDM diagnosis and management information were collected. Univariable comparisons of patients' characteristics were conducted using chi-square for categorical data and student t-test for continuous measures. Multivariable logistic regression with backward selection was used to determine which factors had an independent association with a diagnosis of gHTN or PE in women with GDM.

**Results:** The analysis included 937 women, 131 (14.0%) of whom developed gHTN or preE. In univariable analysis women with gHTN or PE had higher early pregnancy body mass index (BMI) ( $35.0 \pm 7.7$  vs.  $31.8 \pm 8.1$  kg/m<sup>2</sup>,  $p < 0.001$ ) and were more likely to have history of chronic hypertension (27.8% vs. 2.7%,  $p < 0.001$ ) (Table 1). In multivariable logistic regression analysis, higher early pregnancy BMI, maternal mood disorder (defined as depression and/or anxiety) and chronic hypertension were significantly associated with higher rates of gHTN or PE (aOR 1.03, 95% CI 1.00-1.06, aOR 2.02, 95% CI 1.21-3.39, and aOR 11.36, 95% CI 5.87-22.00, respectively). Use of pharmacotherapy for GDM management was not associated with higher rates of gHTN or PE.

**Conclusion:** Women with GDM were more likely to develop gestational hypertension or preeclampsia if they had a history of chronic hypertension, diagnosis of mood disorder, or a higher early pregnancy BMI.

Table 1 – Baseline and pregnancy characteristics

	No Gestational Hypertension or Preeclampsia (N=806)	Gestational Hypertension or Preeclampsia (N=131)	p-value
Age (years)	31.7 ± 4.9	32.2 ± 5.7	0.239
<b>Early pregnancy BMI (kg/m<sup>2</sup>)</b>	<b>31.8 ± 8.1</b>	<b>35.0 ± 7.7</b>	<b>&lt;0.001</b>
Nulliparity	275 (34.0%)	43 (32.3%)	0.700
Insurance			
Private	375 (46.5%)	63 (48.1%)	0.488
Public	168 (20.9%)	36 (27.5%)	
None	35 (4.3%)	8 (6.1%)	
Missing	228 (28.3%)	24 (18.3%)	
Maternal race/ethnicity			0.064
White	476 (59.0%)	77 (58.7%)	
Non-Hispanic Black	107 (13.3%)	28 (21.4%)	
Hispanic	83 (10.3%)	11 (8.4%)	
Other	111 (13.8%)	12 (9.2%)	
Missing	29 (3.6%)	3 (2.3%)	
Mood disorder	164 (20.4%)	35 (26.3%)	0.119
Asthma	62 (7.7%)	14 (10.5%)	0.263
<b>Chronic hypertension</b>	<b>22 (2.7%)</b>	<b>37 (27.8%)</b>	<b>&lt;0.001</b>
Marital status			0.168
Married	521 (64.8%)	76 (58.0%)	
Single	217 (26.9%)	44 (33.6%)	
Divorced	23 (2.9%)	6 (4.6%)	
Missing	45 (5.6%)	5 (3.8%)	
Tobacco use	60 (7.5%)	12 (9.1%)	0.819
Gestational weeks at GDM diagnosis	26.6 ± 5.4	26.2 ± 6.0	0.396
Gestational weeks at GDM treatment	39.4 ± 5.7	27.9 ± 5.7	0.076
Pharmacotherapy for GDM	466 (57.8%)	83 (62.4%)	0.281
Medication used for GDM treatment			0.101
Metformin	58 (12.5%)	6 (7.2%)	
Insulin	227 (48.7%)	49 (59.0%)	
Glyburide	181 (38.8%)	25 (30.1%)	
Missing	0 (0.0%)	3 (3.6%)	

BMI=body mass index, GDM=gestational diabetes mellitus. All data presented as N (%) or mean ± standard deviation. Bold indicates statistical significance.

Table 2 – Logistic regression: impact of pregnancy factors on development of gestational hypertension or preeclampsia

	OR	95% CI	aOR*	95% CI
Age (years)	1.02	0.99-1.06	1.03	0.98-1.08
<b>Early pregnancy BMI (kg/m<sup>2</sup>)</b>	<b>1.05</b>	<b>1.02-1.07</b>	<b>1.03</b>	<b>1.00-1.06</b>
Nulliparity	0.93	0.63-1.37	-	-
Insurance				
Private	Ref	Ref	-	-
Public	1.28	0.81-2.00	-	-
None	1.36	0.60-3.07	-	-
Maternal race/ethnicity				
White	Ref	Ref	Ref	Ref
Non-Hispanic Black	1.62	1.00-2.62	1.26	0.65-2.43
Hispanic	0.82	0.42-1.61	0.72	0.30-1.73
Other	0.67	0.35-1.27	1.27	0.62-2.61
<b>Mood disorder</b>	<b>1.40</b>	<b>0.92-2.13</b>	<b>2.02</b>	<b>1.21-3.39</b>
Asthma	1.42	0.77-2.61	0.76	0.30-1.93
<b>Chronic hypertension</b>	<b>13.77</b>	<b>7.80-24.32</b>	<b>11.36</b>	<b>5.87-22.00</b>
Marital status				
Single	Ref	Ref	Ref	Ref
Married	0.72	0.48-1.08	1.01	0.59-1.74
Divorced	1.29	0.50-3.34	1.74	0.55-5.50
Tobacco use	1.23	0.64-2.37	-	-
Gestational weeks at GDM diagnosis	0.99	0.95-1.02	1.01	0.97-1.06
Gestational weeks at GDM treatment	0.96	0.92-1.00	-	-
Pharmacotherapy for GDM	1.23	0.84-1.80	1.19	0.71-2.01

BMI=body mass index, GDM=gestational diabetes mellitus, OR=odds ratio, aOR=adjusted OR, CI=confidence interval. \*Controlled for maternal age, early pregnancy BMI, maternal race/ethnicity, mood disorder, asthma, chronic hypertension, marital status, weeks at GDM diagnosis, and pharmacotherapy for GDM. Bold indicates statistical significance

## T-196

**In Situ Mechanical Characterization Predicts the Developmental Potential of Oocytes and Embryos.**

Oren Wintner\*,<sup>1</sup> Naama Srebnik,<sup>1</sup> Dorit Kalo,<sup>2</sup> Zvi Roth,<sup>2</sup> Amnon Buxboim\*,<sup>1</sup> <sup>1</sup>The Hebrew University of Jerusalem, Jerusalem, Israel; <sup>2</sup>The Hebrew University of Jerusalem, Rehovot, Israel.

**Introduction:** IVF treatments account for 1.7% of live births in the USA alone. Early identification of embryos with high implantation potential is required for avoiding clinical complications to the newborn and to the mother that are associated with multiple pregnancy and for shortening time to pregnancy. We hypothesize that non-invasive in situ measurement of the viscoelastic properties of oocytes during in vitro maturation (IVM) can accurately and robustly predict maturation, fertilization and implantation potential.

**Methods:** We developed the MechanoPLATE - a multiplate-based glass device for performing continuous viscoelastic characterization of oocytes and embryos under optimal culture conditions. Stress-strain relationships are obtained via in situ aspiration creep test measurements that are performed in each well of the MechanoPLATE separately. Accurate mechanical characterization of oocytes and embryos is calculated by fitting the measured compliance to a linear viscoelastic model. In this manner, we measure the mechanical dynamic response of the intact oocyte or embryo to applied forces and independently probe ooplasm mechanics. Applied forces are maintained below a threshold to avoid structural damage and to maintain biological functions. The developmental potential of oocytes and embryos is scored based on the capacity to complete second metaphase (MII) and to reach blastulation.

**Results:** Using a bovine model, we demonstrate a non-invasive rheological characterization, which does not compromise the developmental potential of oocytes and embryos. Surprisingly, we find that the viscoelastic properties of the oocytes change drastically such that GV oocytes behave like a purely elastic solid material that transforms into a fluid-like viscoelastic material after 22 hours of IVM. Moreover, only high quality GV oocytes that complete first meiosis and reach MII soften during IVM whereas poor quality arrested oocytes remain stiff. These mechanical differences that mark a decrease in the developmental potential of oocytes are consistent with a decrease in the reproductive potential as exhibited by aged versus young cows and by oocytes collected from antral follicles during the hot versus the cold season.

**Conclusion:** In situ rheological profiling offers a real-time, non-invasive, and standardized method for evaluating the developmental potential of oocytes and embryos. In addition, viscoelastic measurement inside the MechanoPLATE can be combined with time-lapse imaging for improving the selection of high quality embryos for transfer. Collectively, our work introduces a mechanical assisted reproductive technology that we believe will improve implantation and live birth rates in human IVF.

## T-197

**TOP5300, an Orally Active FSH Receptor Agonist, May Better Treat Specific Infertility Patient Populations.**

Joie Z Guner†, Diana Monsivais, Fabio Stossi, Hannah Johnson, William E Gibbons, Martin M Matzuk, Stephen S Palmer\*. *Baylor College of Medicine, Houston, TX, United States.*

**Introduction:** TOP5300 is an orally active allosteric agonist of the follicle-stimulating hormone receptor (FSHR), effective in human granulosa cells, and safe from unwanted safety concerns in preclinical trials. The present studies were designed to determine whether TOP5300 may enable personalized medicine for discrete populations of women (advanced ovarian reserve [ARA], polycystic ovarian syndrome [PCOS], normal ovarian reserve [NOR]) utilizing controlled ovarian stimulation protocols for treatment of infertility. Our results show TOP5300 delivers a superior FSHR-mediated response in granulosa cells from ARA and PCOS patient populations that was not achieved with recombinant FSH.

**Methods:** Following an approved IRB protocol, 56 patients undergoing IVF consented to provide discarded follicular fluid at the time of oocyte retrieval. Granulosa cells (GC) were isolated, cultured for 7 days to re-sensitize FSHR, and subsequently treated for either 6 hours (gene expression, FSHR membrane localization) or 48 hours (estradiol

with either recombinant follitropin- $\alpha$  (AFP8468A, NHPP; rh-FSH) or with TOP5300 (MW=570; FSHR EC<sub>50</sub>=9nM). Estradiol production in supernatant was measured by ELISA, and RNA was isolated to measure gene expression by quantitative Real Time PCR (TaqMan) and RNAseq (Illumina). Immunofluorescent detection of FSHR with FSHR antibody (Mab105) was co-localized with FOXL2 antibody in GC.

**Results:** GC cultures were successfully established for 70% of all patients: PCOS (Rotterdam criteria), ARA (age>35), and NOR. In addition, viability of GC was consistently above 95%. Both TOP5300 and rh-FSH stimulated estradiol production and steroidogenic gene expression from human GC. The fold increase in estradiol production (48hr) with TOP5300 was often greater than rh-FSH among PCOS and ARA patients, but was heterogeneous within each patient population. TOP5300 induced statistically greater expression of both STAR and CYP19A1 in GC after 6hr of treatment compared to rh-FSH in both ARA and PCOS patients, but not for GC from NOR patients. The intensity of membrane FSHR staining for TOP5300 relative to rh-FSH was greater in ARA and PCOS patients, and similar for TOP5300 and rh-FSH in GC obtained from NOR patients.

**Conclusion:** TOP5300 leads to greater induction of ovarian steroidogenic gene expression and extended presence of the FSHR at the plasma membrane in GC from PCOS and ARA patient populations. One possible explanation for the superior steroidogenic response of TOP5300 relative to rh-FSH in GC from PCOS and ARA may reflect extended presence of FSHR at the plasma membrane of GC. Our results provide the first evidence that an oral FSHR allosteric agonist performs differently in specified infertile patient populations, addressing a therapeutic gap in personalized medicine available for infertility treatments.

## T-198

**Current Practices and Knowledge Surrounding Ovarian Stimulation in Transgender Men.**

Samuel K Yost†, Emily K Kobernik, Molly B Moravek\*. *University of Michigan, Ann Arbor, MI, United States.*

**Introduction:** Assisted reproductive technology (ART) options for transgender men are resource intense, thus optimizing chance of success is paramount. It is controversial whether testosterone should be stopped prior to ovarian stimulation, or duration of potential cessation. Actual US practices are unknown, and there is no uniform training experience. Published data is scarce but indicates low utilization of ART in transgender men desiring genetic children, in part due to concerns over worsening dysphoria during the process. Our objective is to assess current practice patterns in the area of fertility preservation for transgender men undergoing hormonal gender-affirming therapy.

**Methods:** An anonymous 38 question, multiple choice and free response survey assessing demographics, training/education, and individual practices regarding ovarian stimulation in transgender men was distributed to members of the Society for Reproductive Endocrinology and Infertility. Bivariate analysis was performed to compare demographic characteristics, training, education, and practice information between providers with formal training (including fellowship, residency, conferences, or coursework) and those without. Comparisons between continuous variables were performed using Student's T-test or Wilcoxon Rank test, and Chi-square or Fisher's exact test for categorical variables, where appropriate.

**Results:** There were 98 respondents, with 92 responses on provision of ART to transgender men. The majority (n=76, 82.6%) currently provide this service, and the rest (n=16, 17.4%) were interested. The majority (n=68, 75.8%) reported that they do/would have patients stop testosterone completely, for an average of 30 days, prior to starting ovarian stimulation. Of those who recommended complete cessation, 40/68 (58.8%) identified specific concerns about stimulating while on testosterone; most common were oocyte quality (40%) or stimulation success (37.5%). There were no significant relationships between formal training, comfort with counseling and/or stimulation, and cessation of testosterone. The mean comfort levels for providing ART counseling and performing stimulation were each 7.1 on a 0-10 scale, and 35.0% of respondents received some form of formal training.

**Conclusion:** In the absence of relevant data or formal guidelines, most REIs stop testosterone completely prior to ovarian stimulation. Because cessation of hormones and return of menses can significantly increase dysphoria, consideration could be given to maintaining testosterone therapy during stimulation after appropriate counseling. Other areas of variable practice include recommended duration of cessation, mental health consults, or requiring a menses before stimulation. This suggests an opportunity for additional research to determine the optimal approach to ART for transgender men, and subsequent standardization of education in this area.

### T-199

#### Is Conception with Assisted Reproductive Technology Associated with Increased Maternal Psychological Stress in Nulliparous Women?

Amir Lueth\*, Nathan Blue, Robert Silver. *University of Utah, Salt Lake City, UT, United States.*

**Introduction:** Pregnancy achieved through assisted reproductive technology (ART) has been associated with obstetric morbidity, but results are mixed. One potential confounder is the potential stress associated with ART. Thus, we aimed to examine the relationship between maternal stress during pregnancy and ART in a well characterized cohort with prospectively and objectively assessed emotional health.

**Methods:** Secondary analysis of the Nulliparous Pregnancy Outcomes Study Monitoring Mothers-to-be (nuMoM2b), a prospective observational cohort study. Participants were recruited in their first trimester of pregnancy from 8 institutions and had 4 study visits, including at delivery. ART was defined as IVF or intrauterine insemination. For the primary analysis, psychological stress was assessed using the Perceived Stress Scale questionnaire and compared between women who underwent ART and women who conceived naturally. Secondary analyses included comparisons of Edinburgh Postnatal Depression Scale (EPDS) and the State-Trait Anxiety Inventory (STAI-T) scores. Comparisons were made using univariable and multivariable analyses as appropriate.

**Results:** 10,038 women were included in the analysis. 3.9% (n=391) women conceived with ART. Several factors were associated with ART, including maternal age, first trimester bleeding, hyperthyroidism, and inherited bleeding disorders (Table 1). In unadjusted analysis, women who underwent ART were less likely to have high (OR 0.18, 0.06-0.55) or moderate stress scores (OR 0.59, 0.47-0.74). After adjusting for maternal age, education, race, parity, smoking, prior abdominal surgery, 1<sup>st</sup> trimester bleeding and medical diagnoses, differences in stress were no longer different between groups. Analyzing by type of ART did not change the results. In secondary analysis, women who conceived through ART were less likely to have a high STAI-T score (OR 0.65, 0.47-0.89) in the first trimester (Table 2).

**Conclusion:** In this large prospective cohort of nulliparous women, conception with ART was not independently associated with differences in perceived stress scale, STAI-T, or EPDS scores.

### T-200

#### The Influence of Hashimoto Thyroiditis in the Metabolism of Follicle Microenvironment.

Diana C S Bastos†, Maria Isabel Chiamolera\*, Renata E. C. Silva†, Maria C B Souza\*, Roberto A Antunes\*, Marcelo M Souza\*, Ana C A Mancebo\*, Patricia C F Arêas\*, Fernando M Reis\*, Flavia F Bloise\*, Tania M Ortiga-Carvalho\*. <sup>1</sup>Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; <sup>2</sup>Federal University of São Paulo, São Paulo, Brazil; <sup>3</sup>Fertipraxis Centro de Reprodução Humana, Rio de Janeiro, Brazil; <sup>4</sup>Federal University of Minas Gerais, Belo Horizonte, Brazil.

**Introduction:** Hashimoto thyroiditis is the most common autoimmune disease in women of childbearing age. This disease decreases thyroid gland function and negatively affects female fertility, impairing fertilization, embryo quality, and live birth rates. However, the mechanism of this association is still unknown. The autoantibodies, characteristic of this disease, are present in the ovarian follicular fluid. Thus, the ovarian follicle microenvironment may be the answer to how the disease could impact

fertility. This study aimed to investigate the follicular fluid metabolic components from women with Hashimoto thyroiditis undergoing in vitro fertilization and compare it to data from euthyroid patients.

**Methods:** In this study, we collected patients' follicular fluid from two reproductive medicine centers for two years. In total 61 patients were screened, including 38 euthyroid women who were positive for thyroid autoantibodies and 23 negative controls. 210 metabolites were analyzed in the follicular fluid was carried out by the liquid chromatography method coupled to an electrospray ionization mass spectrometer. Multivariate analysis was performed by partial least squares discriminant analysis (PLS-DA). The metabolomic profile of each group was identified using the Metaboanalyst 4.0 software.

**Results:** As a result, we got 15 potential biomarkers, of which 9 were increased and 6 were decreased in the Hashimoto thyroiditis patients' follicular fluid compared with the control group. Among the biomarkers identified, Hashimoto thyroiditis patients showed an increased abundance of amino acids (serine, threonine, histidine, and leucine), carnitines (stearyl carnitine, propionyl carnitine, palmitoyl carnitine, and butyryl carnitine), and phosphatidylcholine, PC(16:0/14:0) compared to control follicular fluids.

**Conclusion:** Our results showed different metabolite concentrations between women with Hashimoto thyroiditis and controls, thus suggesting that the disease changes the follicular fluid metabolic profile and the ovarian follicle microenvironment. Our finds suggest biological pathways that may help explain how Hashimoto's disease affects female fertility.

### T-201

#### Dose-Dependent Trend toward Increased Menstrual Cycle Length with Chronic Marijuana Use in Rhesus Macaques.

Kimberly Ryan†, Shruthi Mahalingaiah, Lily Campbell, Jon Hennebold, Jamie Lo\*. <sup>1,4</sup>Oregon Health & Science University, Portland, OR, United States; <sup>2</sup>Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>3</sup>Boston University, Boston, MA, United States; <sup>4</sup>Oregon National Primate Research Center, Beaverton, OR, United States.

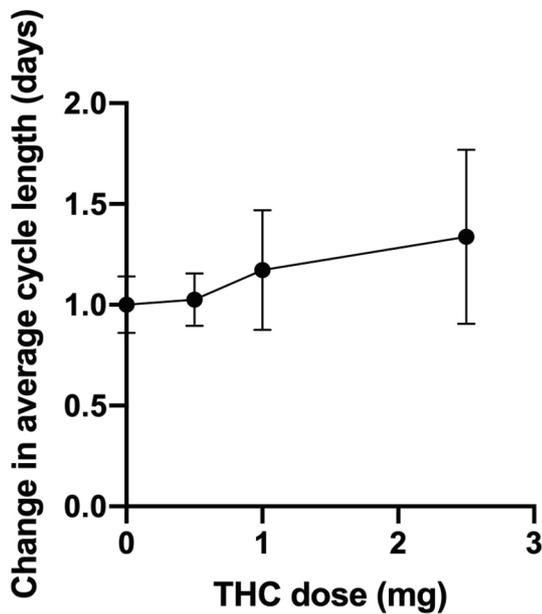
**Introduction:** Despite the increased prevalence of marijuana use in women of reproductive age, there is limited data on its impact on female reproductive health. Results from existing human studies are conflicting and often reflect marijuana use through smoking and not the direct effect of delta-9-tetrahydrocannabinol (THC), the main psychoactive ingredient of marijuana. Our study's objective is to determine the dose-dependent effect of contemporary marijuana exposure on female menstrual cyclicity in a nonhuman primate model.

**Methods:** Adult female rhesus macaques (n=8) with regular menstrual cycle lengths (MCL) were maintained on a standard chow diet with a daily THC edible. All animals were titrated to the maximum dose (2.5mg/7kg/day, equivalent to a heavy medical dose in humans) of THC over 4 months. THC dosing was increased every month to replicate established medical marijuana acclimation guidelines. MCL for up to 4 cycles was recorded before and during THC induction. Serum collected on menstrual cycle days 1, 3, and 5 was analyzed for estradiol (E2) and progesterone (P4). Anti-Müllerian hormone (AMH) and prolactin (PRL) assays were performed on day 3 samples.

**Results:** All rhesus macaques in this study were of reproductive age and the majority (7 of 8) of proven prior fertility. No animals had prior exposure to THC or other environmental perturbations. The average MCL was 28-29 [standard deviation (SD) 2.8] days pre-THC and during THC-induction; but, the average MCL was 37.8 [SD 15.8] days at highest THC dosing (Fig 1). There was no significant THC effect on average basal E2, P4, or PRL levels. Average AMH concentrations increased with higher THC dosing. Mean AMH at baseline (pre-THC) was 10.7ng/ml [SD 8.4] compared to 13.2ng/ml [SD 5.5] at 0.5mg/7kg/day, 14.6ng/ml [SD 6.4] at 1mg/7kg/day and 14.0ng/ml [SD 8.0] at 2.5mg/7kg/day of THC.

**Conclusion:** A dose-dependent trend towards increased MCL and AMH levels with increasing THC doses was observed in reproductive-aged rhesus macaques. Unlike smoking marijuana, THC edibles allows the study of the direct effects of THC and avoids the chemicals in smoke that may compound the effect of THC on reproductive characteristics.

Further studies are needed to determine whether the increase in AMH is indicative of other reproductive abnormalities and reduced fecundity, as well as the effect of a longer duration of exposure.



#### T-202

##### Transfer Lift: Early Evaluation of the Optimal Day of Embryo Transfer by Causal Inference.

Yoav Kan Tor†, Zabari Nir†, Matan Gavish\*, Amnon Buxboim\*. Hebrew University of Jerusalem, Jerusalem, Israel.

**Introduction:** The live birth rates of IVF-ET treatments have steadily declined worldwide during the past decade concomitantly with increasing patient's maternal age. Classification algorithms that assess the potential to implant of cleavage-stage embryos based on time-lapse real-time imaging of preimplantation development support extended incubation towards blastocyst transfer. However, optimizing the day-of-transfer with respect to the developmental properties of individual embryos is lacking.

**Methods:** To assess the individual treatment effect of blastocyst transfer with respect to implantation, we trained a causal inference model using a retrospective dataset of time-lapse imaging of freshly transferred Day-3 (2,167 embryos) and Day-5 (778 blastocysts) embryos obtained from four medical centers. Cleavage-stage embryos were characterized by the Transfer Lift, which is a novel developmental property that quantifies the increase (positive Transfer Lift) or decrease (negative Transfer Lift) in the estimated implantation rate of blastocyst-stage versus cleavage-stage transfers.

**Results:** While extended incubation towards blastocyst transfer of most embryos was estimated to increase their implantation rate by up to 30%, it decreases the implantation rate of 8% of the embryos by up to 10%. Complementary to the Transfer Lift, we provide a classifier that predicts blastulation outcome of cleavage stage embryos (66 hours post fertilization) with AUC 0.92, which can be used for de-selecting embryos for blastocyst transfer.

**Conclusion:** Retrospective evaluation of a test-set of 338 cleavage-stage and 150 blastocyst-stage transferred embryos reveals that adjusting the individual day-of-transfer according to the Transfer Lift can improve implantation rate by >30%. Our decision-support tool marks the first utilization of counterfactual inference in reproductive medicine.

#### T-203

##### Novel Compound Heterozygous Variants in PATL2 Associate with Oocyte Meiotic Arrest.

Beatriz Rodriguez-Alonso†, Hakan Cakmak\*, Aleksandar Rajkovic\*. UCSF, San Francisco, CA, United States.

**Introduction:** Oocyte maturation defect is characterized by oocyte meiotic arrest that can occur at different stages of oocyte development, thus impairing fertilization and embryo development. Studies over the past few years have begun to elucidate genetic heterogeneity of this condition. **Methods:** We report the case of a 38-year-old G0 healthy woman with primary infertility who presented for infertility work up with unremarkable pelvic ultrasound, hormone testing and semen analysis. She underwent 3 antagonist IVF cycles with excellent ovarian response. The patient had a total of 64 dominant follicles (between sizes of 13-20 mm) on the day of trigger shot. The patient received Lupron 4 mg and/or hCG up to 5000 IU to induce oocyte maturation. Although the oocyte yield was excellent (total of 89 eggs retrieved), the mature (MII) oocyte rate was very low (2 MII, 6 MI, and remaining was GV or abnormal looking). ICSI was performed to all MII and MI oocysts, and no fertilization was observed. The diagnosis of oocyte meiotic arrest was entertained and whole exome sequencing of the patient revealed two novel compound heterozygous missense variants in the PATL2 gene, c.1418C>T at chromosomal position ch15:44959349 (hg19) and c.16G>A at chromosomal position ch15:44968673. Both variants were confirmed by Sanger sequencing.

**Results:** These variants were not reported previously, and were not present in ClinVar or HGMD databases. The population allele frequency for the c.1418C>T (p.Ser473Leu) heterozygous variant was very low (3 alleles out of >180,000 alleles) in gnomAD, while the c.16G>A (p.Gly6Arg) variant was not reported in the 1000 Genomes, ExAC, or gnomAD population sequencing projects. Given the strong gene-disease but absent variant-disease associations, the variants were both classified as "variant of uncertain significance". The PATL2 gene encodes protein PAT1 homolog 2, a protein highly expressed in immature human oocytes. PATL2 acts as a translational repressor, and when there is a loss-of-function, a downstream protein synthesis is activated leading to oocyte maturation arrest. Variants in this gene are associated with oocyte maturation defect 4, an autosomal recessive disorder characterized by some oocytes exhibiting maturation arrest at GV or MI stage.

**Conclusion:** In women diagnosed with oocyte maturation arrest, genetic testing can avoid subsequent IVF cycles with anticipated lack of fertilization, saving the patients from the financial and emotional hardship of repeated failed cycles. The knowledge of the full spectrum of PATL2 mutations leading to female infertility is necessary for better counseling of women affected with this condition.

#### T-204

##### Oh Boy! Exploring the Effect of Microfluidic Sperm Separation on Embryonic Sex.

Safina Usmani†, Caroline Peschansky†, Sarah Dynia†, Sonia Patel†, Jawaria Amir†, Royi Lynn†, Kayla Vitale†, Lauren Grimm†, Lizabeth Dulle†, Erica Loudon\*, Roohi Jeelani\*, Angeline Beltsos\*. Vios Fertility Institute, Chicago, IL, United States.

**Introduction:** Increased interest in the effects of male factor fertility on embryo development between days 3-5, when paternal gene activation takes place, has led to innovative sperm sorting techniques that are thought to result in higher quality sperm selection for use in in-vitro fertilization (IVF), including microfluidics sperm separation. Previous independent studies have shown inconclusive data on whether or not different sperm preparation techniques truly result in better quality embryos, either morphologically or chromosomally. As new sperm preparation protocols become more widely used, understanding their clinical utility is paramount prior to laboratory use. The purpose of our study is to understand whether or not different sperm preparation techniques result in bias of sex differentiation in developing embryos

**Methods:** Retrospective chart review at a private fertility clinic. All IVF cycles between June 2018 to January 2021 were analyzed and split into two groups: those that had received sperm processing using microfluidics sperm sorting and those that used density gradient centrifugation. Only

cycles resulting in utilizable embryos that underwent preimplantation genetic testing (PGT) were used. Quantitative semen parameters, including concentration and motility, as well as the results of embryonic chromosomal analysis were evaluated. T-tests and chi square analysis were used to analyze the data using SPSS (SPSS Inc., Chicago, IL, USA).

**Results:** A total of 2,390 embryos were analyzed, with 720 embryos included in the microfluidics group and 1670 embryos included in the density gradient control group. The average age of the egg and sperm sources in the microfluidics group was 39.98 and 39.5 years respectively, compared to 39.99 ( $p>0.05$ ) and 37.95 years ( $p<0.05$ ) in the control group. The microfluidics group had an average concentration and total motility of 70.9M and 66% respectively, compared to 72M ( $p>0.05$ ) and 65% ( $p>0.05$ ) in the control group. PGT results showed a euploid rate of 58.05% in the microfluidics group, compared to 52.6% ( $p<0.05$ ). Of the embryos that received a chromosomal sex designation, we found that the microfluidics sperm sorting device resulted in 52.5% of embryos being male, 39.6% female ( $p<0.05$ ), compared to a near even split of 50.2% males and 49.8% females in the density gradient group.

**Conclusion:** Our data suggests that use of microfluidic sperm separation yields more male embryos than female embryos. Interestingly, while not a primary endpoint of our study, we did also find that the microfluidic technique resulted in increased rate of euploidy compared to the traditional density gradient centrifugation. Further studies are warranted to validate these chromosomal sex differences seen in embryos created via different sperm preparation techniques.

### T-205

#### Identifying the Optimal, Multistep and Adaptive Embryo Transfer Strategy for Improving IVF Outcome.

Yoav Kan Tor†, Deborah Wolhandler†, Buxboim Amnon. *Hebrew University of Jerusalem, Jerusalem, Israel.*

**Introduction:** Human IVF technology may require a sequence of fresh and frozen embryo transfer cycles to achieve conception and live birth. Given the finite, often limiting number of high-quality fertilized oocytes retrieved from patients, the multistep decision of how many and which embryos to transfer on each cycle becomes crucial for shortening time to pregnancy while avoiding clinical complications associated with multiple pregnancy. We hypothesize that a multistep, look-ahead approach can identify the optimal embryo transfer strategy and improve IVF outcome.

**Methods:** Using dynamic programming, we developed an online decision-support tool that surveys all possible embryo transfer combinations at each step as defined by the given cohort of a patient's embryos, and scores the expected action outcome (EAO). For each possible action (transfer one, two or three embryos), the EAO scores the potential to conceive combined with penalties due to multiple pregnancy and time to pregnancy by integrating the future actions allowed by all possible strategies. The potential to conceive depends on the assessments of embryo quality and endometrial receptivity - the latter is computationally adjusted in real time based on the outcome of previous transfers. Embryo quality can be assessed using various methods chosen by the user. Here we present machine learning and automated deep learning algorithms that predict the potential to reach important developmental milestones: blastulation, implantation, avoiding 1st trimester miscarriage and live birth. Finally, EAO scoring also allows a physician evaluation of embryo freezing effects and related patient-specific medical risks.

**Results:** The medical utility of our decision-support tool is demonstrated retrospectively using an expansive database of IVF treatments collected from five medical centers. It includes time-lapse videos, maternal information, embryo transfer statistics, chemical and clinical pregnancy measurements, miscarriage and live birth outcome for more 20,000 embryos and 5,400 transfer cycles. For each medical center we identify a distinctive embryo-transfer policy applied to all patients, rather than a patient-specific strategy. We retrospectively quantify the distances between the transfer decisions made by the physicians and the optimal actions defined by the decision-support tool. A negative correlation between these distances and implantation outcome will support accuracy and clinical utility.

**Conclusion:** The multistep embryo transfer decision-making process can be projected onto a graph and solved analytically using dynamic programming. Clinical adoption of established computational tools has the potential to implement a personalized-care approach, shorten time to pregnancy, minimize health risks and improve live-birth rates.

### T-206

#### Does More Really Mean More? Comparing Pregnancy Rates and Number of Gestational Sacs Visible on Ultrasound Following Transfer of 1 vs. 2 PGT-Normal Embryos.

Anisa Hussain†, Abeer Salhia\*, Lauren Grimm†, Jacqueline Sehring†, Jody Esguerra†, Angeline Beltsos\*, Roohi Jeelani\*. *Vios Fertility Institute, Chicago, IL, United States.*

**Introduction:** We investigated whether transferring 2 embryos determined to be euploid by preimplantation genetic testing for aneuploidy (PGT-A) truly resulted in improved clinical pregnancy rate without putting patient health and wellbeing at risk due to multiple pregnancy; would the benefits outweigh the risks?

**Methods:** Patients undergoing day 5 frozen embryo transfer (FET) of 1 or 2 euploid (PGT-normal) embryos were included. All embryos were biopsied and tested with Next Generation DNA Sequencing, frozen, then thawed for transfer. 10-12 days post-transfer serum beta-hCG level was tested, with a level of 5 mIU/mL or greater indicating pregnancy. Following positive pregnancy test, serum beta-hCG level was monitored every other day for 4 days, as the level should double every two days with a clinical pregnancy. 10-14 days after the initial positive test, when accompanied by appropriately-rising serum beta-hCG, an ultrasound was performed to confirm intrauterine pregnancy. Clinical pregnancies were managed at the fertility clinic for approximately 8 weeks, after which care was transferred to an OB/GYN.

**Results:** 173 total cycles were included. In 163 cycles, a single PGT-normal embryo was transferred (single embryo transfer = sET). In the remaining 10 cycles, two PGT-normal embryos were transferred (double embryo transfer = dET). 79% of sETs and 90% dETs resulted in positive serum beta hCG level. Overall clinical pregnancy rate was 60% for sET and 90% for dET. Chi square test determined no significant difference between positive pregnancy test and clinical pregnancy rates in both groups ( $p=.41$  and  $p=.059$ , respectively). For those patients that achieved pregnancy after transfer of 1 or 2 PGT-normal embryos, dET resulted in a significantly higher average number of gestational sacs visible on ultrasound, with an average of .946 gestational sacs for sETs and 1.444 for dETs ( $p<.05$ ).

**Conclusion:** Previous studies suggest that PGT-A significantly increases pregnancy and live birth rates in patients with recurrent pregnancy loss, implantation failure, and advanced maternal age. However, current guidelines do not address whether or not increasing the number of embryos transferred increases the chance of clinical pregnancy. While clinical pregnancy rates and transfer of care to an OB/GYN did not differ between sET and dET, the number of intrauterine pregnancies did increase significantly with dET. When counseling patients about multiple embryo transfer, physicians must bear in mind the risks that come with multiple pregnancy, including preterm delivery, low birth weight, and fetal loss. One must consider if the risks associated with multiple pregnancy are truly warranted when the chance of clinical pregnancy is the same with single and double embryo transfer.

### T-207

#### The Perfect Embryo: The Relationship between BMI and AMH and Resultant Embryo Quality in PCOS Patients.

Jawaria Amir\*,<sup>1</sup> Lauren Grimm†,<sup>2</sup> Caroline Peschansky†,<sup>2</sup> Sonai Patel†,<sup>2</sup> Sarah Dynia†,<sup>2</sup> Safina Usmani†,<sup>2</sup> Royi Lynn†,<sup>2</sup> Erica Louden\*,<sup>2</sup> Angeline Beltsos\*,<sup>2</sup> Roohi Jeelani\*.<sup>2</sup> *<sup>1</sup>Rush University, Chicago, IL, United States; <sup>2</sup>Vios Fertility Institute, Chicago, IL, United States.*

**Introduction:** Polycystic ovarian syndrome (PCOS) is a multifactorial condition that affects 5-10% of reproductive aged women and is a common cause of anovulatory infertility. Anti-Müllerian hormone (AMH), a marker for ovarian reserve, is produced by growing ovarian antral follicles. AMH levels are elevated in women with PCOS in comparison to normo-

ovulatory women, consistent with the increased number of antral follicles in PCOS. Evidence suggests that AMH concentrations are correlated with the degree of ovulatory dysfunction in PCOS patients. PCOS is also associated with obesity where approximately 50% of patients are overweight or obese. While the association between obesity and PCOS is complex, obesity exacerbates many aspects of the phenotype. The relationship between obesity and AMH levels has been studied with conflicting results. While most studies have demonstrated a negative correlation or no correlation, a 2018 study demonstrated a positive correlation between AMH and BMI in non-PCOS patients. We sought to investigate a correlation between AMH and BMI in PCOS patients and whether worsening BMI and elevated AMH levels resulted in poorer embryo quality as seen in embryo grade and preimplantation genetic testing outcomes in this cohort.

**Methods:** A retrospective chart review was performed on a total of 465 patients with a diagnosis of PCOS who underwent IVF at a private infertility center from 2018 to 2020. All cycles from this group were analyzed for blast quality score and preimplantation genetic testing. Logistic regressions to calculate Pearson coefficient correlation were utilized to analyze the data using SPSS 21.0 (SPSS Inc., Chicago, IL, USA).

**Results:** A total of 465 cases were identified, out of which 352 patients made blastocysts for grading and 167 had genetic testing done. Our results indicate there is no significant correlation between BMI and serum AMH levels in women with PCOS ( $r$  squared = 0.00). Similarly, there was no significant correlation when AMH and BMI were used to assess a correlation with Blastocyst Quality (morphology) or euploid embryo rate ( $r$  squared = 0.00). As was expected, there was a slight positive correlation between serum AMH level and euploidy rate ( $r$  squared = 0.02).

**Conclusion:** While previous research has shown discrepancies in whether or not a relationship exists between BMI and serum AMH levels in non-PCOS women, our study shows that among women with PCOS, there is no correlation between BMI, AMH, or embryo quality, both chromosomally or morphologically. While this new information may help to alleviate any concerns in embryo utility for women with PCOS, especially severe PCOS, further research is needed to better understand the clinical outcomes in women with various presentations of PCOS.

### T-208

#### Prevalence of Hirsutism and Polycystic Ovarian Syndrome (PCOS) in Latina/Latinx Females: Findings from the Environment, Leiomyomas, Latinas and Adiposity Study (ELLAS).

Amanda R Schwartz<sup>†</sup>,<sup>1</sup> Anne Waldo,<sup>1</sup> Amanda Manorot,<sup>1</sup> DeBlanc Jennie,<sup>1</sup> Maricella Castillo MacKenzie,<sup>1</sup> Samantha Schon,<sup>1</sup> Donna Baird,<sup>2</sup> Erica E Marsh\*.<sup>1</sup> <sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States.

**Introduction:** The prevalence of PCOS is estimated to be between 6% and 9% in the United States but variations by region and race/ethnicity are not well understood. Although previous literature has demonstrated higher BMI and insulin resistance in Mexican Americans, which may potentiate increased risk for PCOS, the prevalence of hirsutism and PCOS in Latinx women (LX) in the United States remains poorly characterized. The objective of this study is to explore the prevalence of diagnosed PCOS and of PCOS-related clinical findings.

**Methods:** A cross-sectional analysis was performed from a prospective longitudinal cohort study of LX between the ages of 21 and 50 years. All study members were participants in ELLAS, a NIH funded community engaged study. Participants were administered questionnaires collecting demographic information, medical, and reproductive health history including questions pertaining to hirsutism and menstrual cycles. Additionally, fasting blood samples were collected to assess for glucose dysregulation. Statistical analysis was performed using Chi-squared and Wilcoxon rank sum tests.

**Results:** 652 LX have enrolled in ELLAS and 574 completed the first study visit. The average age of the cohort is 37.5 years  $\pm$  6.99 years. 6.2% of women reported a prior diagnosis of PCOS, but only 4.4% reported any prior medical treatment for PCOS. 86 women (15.0%) reported hirsutism

of which 15.1% had a known diagnosis of PCOS. Women with hirsutism were more likely to be born in the United States (16.3 vs 8.1%;  $p$  = 0.015) and have a higher BMI (30.6 vs 28.8;  $p$  = 0.03). They also had longer menses (5.0 vs 4.0 days;  $p$  = 0.004) and cycles that were more likely to be irregular in the past three months (36.0% vs 23.1%,  $p$  = 0.026). Based on hemoglobin A1C values, 96 (20.1%) were prediabetic and 30 (6.3%) were diabetic. Women with previously diagnosed PCOS or both hirsutism and irregular cycles were more likely to be obese (56.7% vs 38.8%;  $p$  = 0.014) and to report a history of infertility (43.3% vs 26.6%;  $p$  = 0.007).

**Conclusion:** While the prevalence of diagnosed PCOS among this cohort of LX was only 6.2%, PCOS-related clinical findings of hirsutism (15.0%) and irregular menstrual cycles (33.7%) were commonly reported. The high prevalence of obesity, glucose dysregulation and infertility in this cohort of largely first generation LX highlights a need for improved preventative and reproductive health care for this group and suggests potential underdiagnosis of PCOS. Further research is needed to better characterize the prevalence and treatment of PCOS and associated comorbidities in LX.

### T-209

#### Towards the Molecular Understanding of PCOS Pathogenesis by RNA-seq Analysis of Multiple Tissues of Two Rat PCOS Models.

Qiong Lin, Joerg Mueller, Martin Fritsch, Ralf Lesche, Jorge Kageyama, Thomas M Zollner. *Pharma R&D, Bayer AG, Berlin, Germany.*

**Introduction:** Polycystic ovarian syndrome (PCOS) is a common endocrine metabolic disorder affecting up to 20% of women in reproductive age. The PCOS patients may have a range of reproductive, metabolic, and endocrine dysfunction with medical comorbidities, including type II diabetes, cardiovascular diseases and psychiatric disorders. While the etiology and pathophysiology of PCOS remains to be elucidated, the androgen excess seen in up to 90% of affected women leads to several, often combined symptoms such as hirsutism, acne, ovarian cysts, infertility and metabolic syndrome.

**Methods:** In this study, we used two rat experimental models of PCOS. High androgen levels were either induced by treatment with dihydrotestosterone (DHT) or the aromatase inhibitor letrozole, to explore the molecular mechanisms of PCOS pathogenesis.

**Results:** RNA-seq based transcriptomic analysis of various tissues in the rat PCOS models revealed changes in important molecular pathways, e.g., ATP metabolic process, energy expenditure. In addition, the dysregulated genes in disease models are shown to be regulated by inflammatory pathways, glucose homeostasis, response to insulin, and regulation of angiogenesis, suggesting a variety of pathway involved in developing PCOS phenotypes. Furthermore, several potential master regulators were identified (which may have tissue specific functions) by combining our data set with publicly available data on tissue specific expression (e.g. GTEx).

**Conclusion:** In summary, by generating transcriptomic maps of two rat models we have improved the molecular understanding of PCOS pathogenesis in these models.

### T-210

#### Identification of Distinct Seminal Plasma Cytokine Profiles Associated with Male Age and Lifestyle Characteristics in Unexplained Recurrent Pregnancy Loss.

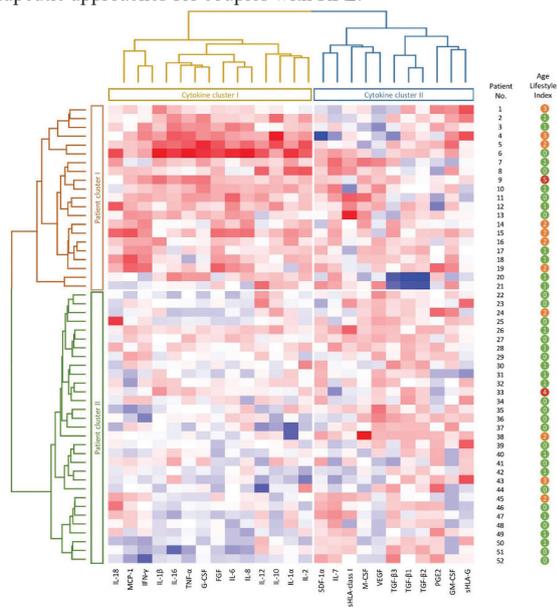
Nadia A. du Fossé<sup>†</sup>, Eileen E.L.O Lashley\*, Els van Beelen\*, Tess Meuleman\*, Saskia le Cessie\*, Jan M.M. van Lith\*, Michael Eikmans\*, Marie-Louise P van der Hooft\*. *Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** Seminal plasma contains a wide range of cytokines, chemokines and growth factors. Part of these signaling molecules assist in inducing a state of active maternal immune tolerance towards the fetus. Disbalances in seminal plasma content may contribute to pregnancy loss. This study investigated cytokine expression profiles in seminal plasma of male partners of couples with unexplained recurrent pregnancy loss (RPL) and the association with clinical and lifestyle characteristics, including smoking, alcohol consumption and Body Mass Index (BMI).

**Methods:** In the seminal plasma of 52 men who visited a specialized RPL clinic the levels of 25 pre-selected cytokines, chemokines and growth factors were measured by Bio-Plex assay or ELISA. Two-way hierarchical cluster analysis was performed. Identified patient clusters were compared on clinical and lifestyle characteristics.

**Results:** Two distinct cytokine expression profiles in the seminal plasma were revealed by cluster analysis (Figure 1). Patient cluster I showed relatively higher levels of pro-inflammatory cytokines, including IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-12, IL-18 and TNF- $\alpha$ , compared to Patient cluster II. Men belonging to Patient cluster I were significantly older and had significantly more lifestyle risk factors compared to men in Patient cluster II.

**Conclusion:** Cluster analysis suggested the existence of a less favorable pro-inflammatory cytokine expression profile, being present in part of men affected by RPL and associated with advanced male age and lifestyle risk factors. These findings may serve as a starting point for further research into underlying mechanisms and ultimately lead to novel diagnostic and therapeutic approaches for couples with RPL.



## T-211

### Membrane Lipid Rich Freezing Medium Improves Pre-Pubertal Testicular Tissue Cryosurvival.

Guruprasad Kalthur\*, Reyon Dcunha, Ananda Hanumappa, Satish K Adiga. *Kasturba Medical College, Manipal, Manipal, India.*

**Introduction:** Cryopreservation of testicular tissue has proven to have potential benefits in artificial reproductive technology (ART), especially in pre-pubertal boys diagnosed with cancer. During the freeze-thaw process, testicular cells are highly prone to undergo damage due to loss of membrane lipids, lipid peroxidation and high oxidative stress. Since preventing damage to the germ cells is very essential for successful generation of spermatozoa, either by *in-vitro* spermatogenesis or by auto-transplantation techniques, an efficient cryopreservation protocol is very critical. However, an optimum protocol for cryopreservation of testicular tissue is still lacking. In this direction, in the present study we aimed at exploring the beneficial effects of few major membrane lipids on prepubertal testicular tissue cryopreservation.

**Methods:** Pre-pubertal male Swiss albino mice were sacrificed and testicular tissue was cut into 3 mm<sup>2</sup> pieces and segregated into 3 groups. The fresh tissue was not subjected to any cryopreservation. Part of tissue was frozen with control freezing medium (5% DMSO, 30% FCS in DMEM/F12 media) and freezing medium with membrane lipids (soy lecithin, phosphatidylethanolamine, phosphatidyl serine and cholesterol). The tissues were placed in the cryovials containing different freezing

medium, transferred to an isopropanol chamber and placed in -80°C freezer for 24h. Samples were later stored in liquid nitrogen until further assessment. The samples were thawed rapidly in a water bath maintained at 37°C for 5 min and subjected to enzymatic digestion (trypsin collagenase: 1 mg/mL). Following digestion, the contents were passed through a 70  $\mu$ m and 40  $\mu$ m cell strainer, and centrifuged at 1100 rpm for 10 min. The cell pellet formed was resuspended in Hanks balanced salt solution (HBSS) containing 10% FCS and different parameters were assessed.

**Results:** Significant increase in viability of germ cells ( $p < 0.05$ ), reduction in  $\gamma$ -H2AX ( $p < 0.001$ ) positive cells and Caspase-3 expression ( $p < 0.05$ ) was observed in the tissue cryopreserved in the membrane lipid rich medium. Freeze-thaw process resulted in a significant increase in malondialdehyde (MDA) level, protein carbonyl content ( $p < 0.0001$ ) and a significant decrease ( $p < 0.001$ ) in the reduced glutathione (GSH) compared to that of fresh tissue. Pre-pubertal tissue cryopreserved in the membrane lipid rich medium showed reduced oxidative stress and significant increase ( $p < 0.05$ ) in the *Gpx4* expression in tissue cryopreserved using membrane lipid rich medium, suggesting the mitigation of oxidative stress generated during freeze-thaw process.

**Conclusion:** Membrane lipid rich medium can enhance the survival of pre-pubertal testicular tissue during cryopreservation and hence has a high translational value in fertility preservation for prepubertal boys undergoing chemotherapy.

## T-212

### Elucidating the Role of SYCP2L in Oocyte Quality and Fecundity in Humans.

Caterina Clementi, Karen Hunter Cohn, Genevieve Galarneau, Piraye Yurttas Beim\*. *Celmatix Inc., New York, NY, United States.*

**Introduction:** Synaptonemal complex protein 2-like (SYCP2L) has been shown in mice to be an oocyte-specific protein that localizes to centromeres of primordial oocytes at the dictyate stage and regulates their survival. Common genetic variants in *SYCP2L* have previously been associated with age of menopause in women by multiple GWAS, however, its role in human reproductive biology remains to be further elucidated.

**Methods:** Our Personalized Reproductive Medicine (PRem) Initiative cohort consists of a centralized repository of biological samples derived from patients undergoing treatment at multiple fertility centers in the United States, linked to detailed reproductive phenotype data. Samples underwent whole genome or exome sequencing. We used Ensembl Variant Effect Predictor to perform the functional annotation of variants with a minor allele frequency  $< 1\%$  in gnomAD located within gene boundaries. For a subset of genes known to regulate ovarian function, we evaluated variants predicted to have high impact effects on protein function.

**Results:** Within our PRem cohort, we identified 9 patients who had rare genetic variants predicted to have a significant functional impact on SYCP2L. We found that, although relatively young (ranging from 26-36 years of age), patients with these rare variants experienced some of the poorest reproductive outcomes within our cohort. All necessitated *in vitro* fertilization (IVF) treatment and most experienced multiple failed IVF cycles. Three patients were never able to conceive with their own oocytes, but were able to achieve a live birth with donated oocytes. For patients who did have successful IVF cycles with their own eggs, the majority of ongoing pregnancies were only achieved with preimplantation genetic testing to screen for euploid embryos.

**Conclusion:** Rare functional variants in *SYCP2L* are associated with reduced fecundity and poor IVF outcomes in women, most likely due to poor oocyte quality. This is consistent with observations that SYCP2L is an oocyte-specific protein known to localize to centromeres during pre-ovulatory meiosis in mice. Notably, several patients with rare functional variants in *SYCP2L* were able to achieve ongoing pregnancies after preimplantation genetic screening of their embryos.

## T-213

**Does Culture Medium Used in IVF-Treatment Impact Post-Implantation Embryonic Growth and Developmental Trajectories with Sex-Specific Modification? The Rotterdam Periconception Cohort.**

Linette van Duijn†, Régine PM Steegers-Theunissen\*, Esther B Baart\*, Sten P Willemsen\*, Joop SE Laven†, Melek Rousian\*. *Erasmus University Medical Centre, Rotterdam, Netherlands.*

**Introduction:** Increasing success rates after *in vitro* fertilization (IVF) can be attributed to several advancements, such improved culture conditions. Culture media are of special interest as supplier of essential nutrients and growth factors, which have previously been shown to impact birthweight. IVF pregnancies are associated with an increased male:female ratio. However, it is unknown if culture media also impact prenatal growth. Therefore, our aim is to study the (sex-specific) impact of two different culture media (SAGE 1-Step and Vitrolife G-1 PLUS) used in IVF treatment on first trimester embryonic growth and development, and fetal outcomes.

**Methods:** In the Rotterdam Periconception Cohort, 879 pregnancies were included; 153 after culture in Vitrolife, 251 after culture in SAGE, and 475 naturally conceived. Serial measurements of crown-rump length (CRL), embryonic volume (EV) and Carnegie stage at 7, 9 and 11 weeks gestational age (GA) were performed offline by using 3D ultrasound and virtual reality techniques. Second-trimester estimated fetal weight (EFW) and birth outcomes, such as weight and gestational age, were retrieved from medical records. Linear mixed models were used to study associations between culture in Vitrolife or SAGE, or natural conception, and first-trimester embryonic growth and development with stratification for fetal sex and adjusted for GA and maternal characteristics. Differences in EFW and birth outcomes were assessed by Kruskal-Wallis tests.

**Results:** Embryos cultured in SAGE grow faster than those cultured in Vitrolife ( $\beta_{EV}$  0.030 (95%CI 0.008-0.052),  $p=0.007$ ). No significant differences were observed regarding CRL and Carnegie stages. After stratification for fetal sex, similar results were observed for male embryos ( $\beta_{EV}$  0.048 (95%CI 0.015-0.081),  $p=0.005$ ). When compared to naturally conceived embryos, embryos cultured in SAGE grow faster ( $\beta_{EV}$  0.033 (95%CI 0.006-0.060),  $p=0.018$ ). This association was most pronounced in male embryos ( $\beta_{EV}$  0.066 (95%CI 0.024-0.108),  $p=0.002$ ). EFW and birth outcomes were comparable between the three groups, except for gestational age, which was shorter in naturally conceived pregnancies than after IVF treatment with culture in either Vitrolife or SAGE (272, 276 and 276 days respectively,  $p=0.001$ ).

**Conclusion:** SAGE culture medium used for IVF treatment accelerates embryonic growth trajectories compared to Vitrolife and natural conceived pregnancies, especially in male embryos. Further research should focus on the scientific and clinical impact of faster embryonic growth as well as on optimising culture medium composition for optimal embryogenesis, fetal development and offspring health during the life course.

## T-214

**Using Serum Metabolomics to Identify Biomarkers of Viable Early, Intrauterine Pregnancy: An Untargeted <sup>1</sup>H NMR-Based Approach.**

Christopher James Hill,<sup>1</sup> Marie Phelan,<sup>1</sup> Andrew Horne,<sup>2</sup> Kristina Gemzell-Danielsson,<sup>3</sup> Nicola Tempest,<sup>1</sup> Dharani Hapangama\*.<sup>1</sup>

<sup>1</sup>University of Liverpool, Liverpool, United Kingdom; <sup>2</sup>University of Edinburgh, Edinburgh, United Kingdom; <sup>3</sup>Karolinska Institutet, Stockholm, Sweden.

**Introduction:** Around 10% of all intrauterine pregnancies are lost in the first trimester. A further 1-2% of pregnancies are located outside the endometrial cavity; these ectopic pregnancies are the leading cause of maternal mortality in the first trimester of gestation. Early miscarriages may also cause significant morbidity when bleeding or infection occurs. The symptoms of miscarriages and ectopic pregnancy are often similar (pain and bleeding), however, such symptoms are also common in viable intrauterine pregnancies (VIUPs). To date, no biomarkers have been identified to differentiate VIUPs from non-viable and ectopic pregnancies.

**Methods:** This is a prospective cohort study that included 332 pregnant women at less than ten weeks of gestation, who attended the early

pregnancy assessment unit (EPAU) at Liverpool Women's Hospital with pain and/or bleeding. Blood samples were collected from the 332 pregnant women prior to final clinical diagnosis of pregnancy outcome. Serum samples were subjected to NMR metabolomics profiling. 1D <sup>1</sup>H-NMR spectra were acquired at 37 °C on a 700 MHz spectrometer. Relative metabolite abundances underwent statistical analysis using MetaboAnalyst 5.0 ( $p$ -value FDR adjusted).

**Results:** Final pregnancy outcomes were as follows: one hydatidiform mole (0.3%), 48 ectopic pregnancies (14.4%), three pregnancies of unknown location (PULs, 0.9%), 78 failed pregnancies of unknown location (FPULs, 23.4%), 47 miscarriages (14.1%), two vanishing twin pregnancies (0.6%) and 153 VIUPs (45.8%). Due to small sample numbers, the hydatidiform mole, PULs and vanishing twin pregnancies were excluded from further analysis. To compare VIUPs to other pregnancy outcomes, ectopic pregnancies, FPULs and miscarriages were grouped together. Univariate analysis of serum metabolite concentrations identified four metabolites (phenylalanine, alanine, glutamate and glutamine) as significantly different in VIUPs compared to other pregnancy outcomes. Multivariate partial least squared discriminant analysis provided only weak correlation between the serum metabolome and pregnancy outcome.

**Conclusion:** This study identifies some serum metabolites associated with VIUPs. The 'metabolite profile' that we have identified could be clinically useful to developed into a diagnostic test that could confirm VIUPs and thus exclude potentially life-threatening pregnancy outcomes in emergency gynaecology.

## T-215

**Endometrial Mitochondrial DNA Secreted in Extracellular Vesicles: A Novel Mechanism Modulating Maternal-Embryo Bioenergetics.**

David Bolumar†,<sup>1</sup> Alicia Amadoz,<sup>1</sup> Inmaculada Moreno,<sup>1</sup> Carlos Marín,<sup>1</sup> Antonio Diez,<sup>1</sup> Jorge Jiménez-Almazán,<sup>1</sup> Carlos Simón,<sup>1,2</sup> Felipe Vilella.<sup>1</sup> <sup>1</sup>Igenomix Foundation/INCLIVA, Paterna (Valencia), Spain; <sup>2</sup>POG Department, University of Valencia, Valencia, Spain.

**Introduction:** Maternal-embryo communication during preconception is key for pregnancy success and offspring health. We revealed a cell-cell communication mechanism in which miR-30d delivered from the endometrium to the pre-implantation embryo via extracellular vesicles (EVs) modifies the embryo transcriptome. Here, we investigated endometrial EV mitochondrial DNA (mtDNA) cargo internalization by the embryo as a potential mechanism by which the maternal endometrium modulates embryo bioenergetics.

**Methods:** Secreted EVs [apoptotic bodies (ABs), microvesicles (MVs) and exosomes (EXOs)] were isolated from endometrial fluid (EF) of fertile donors ( $n = 10$ ) in their receptive phase. mtDNA copy number was quantified in receptive-phase human endometrial biopsies by qPCR ratio of  $\beta$ -ACTIN (nuclear) to ATP8 (mitochondrial) genes, and changes in metabolic gene set were studied using targeted RNAseq (Ion AmpliSeq Transcriptome Human Gene Expression Core Panel). EV DNA cargo tagged with 5-ethynyl-2'-deoxyuridine was followed by confocal imaging after co-incubation of EVs with murine embryos ( $n = 600$ ). ATP levels were assessed by FLASC luciferase reporter system, and mitochondrial membrane potential was evaluated by JC-1 confocal visualization ( $n = 250$  embryos).

**Results:** Receptive-phase endometria had lower mtDNA content together with an upregulation of genes that promote mitophagy in vesicular compartments. Yet, in human EF-derived EVs, MVs were  $11.12 \pm 0.53$ -fold enriched in the 13 mtDNA-encoded genes for electron transport chain complexes, and 7.1-26.7-fold enriched in transcription factor binding sites (TFBSs). Most TFBSs mapped to the mitochondrial genome; some were associated with transcription factors (*SRF*, *GABP*, *E2F4*, *TR4*, *FOXA2*, *FOXA1*, *CTCF*, *GATA2*, *PAX5*) with roles in early embryo development, mitochondrial function and biogenesis. EV DNA transfer and internalization were detected in murine embryos in the cytoplasm and nuclei of trophectoderm cells. Murine embryos incubated with ABs or MVs from human EF maintained their ATP levels, but co-incubation with EXOs reduced the ATP content ( $p < 0.001$ ). Mitochondrial membrane potential did not differ among conditions, but JC-1 patterning indicated EXO co-incubation promoted a reduction in total mitochondrial mass.

**Conclusion:** During the preconception period, endometrial mtDNA content is reduced through mitophagy. Processed mtDNA, both coding and regulatory, is secreted through EVs to the EF and taken up by the embryo, where it modulates embryo energetics to support embryo ATP status. These findings require further investigation. CS & FV contributed equally.

## T-216

### High Cortisol Levels in Endometrium Impair Receptivity While Increased Estrone Levels Could Favor Pregnancy.

Almudena Devesa-Peiro<sup>†,1,2</sup>, Diana Marti-Garcia<sup>†,1</sup>, Elena Labarta,<sup>3,1</sup> Marina Lopez-Nogueroles,<sup>4</sup> Patricia Sebastian-Leon,<sup>1</sup> Patricia Diaz-Gimeno\*.<sup>1</sup> <sup>1</sup>IVI Foundation - Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain; <sup>2</sup>University of Valencia, Valencia, Spain; <sup>3</sup>IVI-RMA IVI Valencia, Valencia, Spain; <sup>4</sup>Analytical Unit Platform, Instituto de Investigación Sanitaria La Fe (IISLAFE), Valencia, Spain.

**Introduction:** Although it is known infertility and Assisted Reproductive Treatments are an important source of stress, the impact of chronic stress on fertility and endometrial function is still controversial. This study evaluates the molecular relationship between both stress-related biomarker metabolites and reproductive hormones measured in endometrium and reproductive outcomes.

**Methods:** Functions in common between receptivity and chronic stress response were consulted in GeneCards, KEGG and Gene Ontology databases for understanding the molecules involved in both processes. The concentrations of prioritized metabolites in common were measured by LC/MS-MS in endometrial biopsies collected at the window of implantation of 76 IVF patients in a hormone replacement therapy cycle and with no uterine pathologies. Correlations between metabolites' concentrations were calculated using Pearson's r; and three concentration cut-offs (first and third quartile and relative maximum) were established to evaluate their relationship with receptivity (defined by the ERA test) and reproductive outcomes using Barnard's test.

**Results:** We identified 118 genes potentially involved in stress and receptivity that were enriched in the steroid hormone biosynthesis pathway (FDR<0.05), concretely in the deviation from progesterone (P4) to estrogens but also in the cortisol to cortisone conversion. Patients with cortisol < 2.18 ng/ml (n=19) and P4 > 40.07 µg/mL (n=9) gave a significantly higher proportion of receptive endometria (78.9% vs 50.9% and 77.8% vs 34.8% respectively; p<0.05); and testosterone > 0.52 ng/ml (n=19) and cortisone > 98.91 ng/ml (n=19) rose the proportion of patients with non-receptive endometria (63.2% vs 35.2% p<0.05 and 78.9% vs 54.4% p=0.06, respectively). Patients with estrone > 21.27 ng/ml (n=4) favored pregnancy (100% pregnancy success vs 40%, p<0.05) and potentially implantation (100% vs 46.7%, p=0.07). P4 and 17OHP4 positively correlated with cortisol (r=0.29 and 0.25, respectively) and androstenedione (r=0.71 and 0.81, respectively), which is a precursor of estrogens; and 17OHP4 also correlated with estrone (r=0.29) (all p<0.05).

**Conclusion:** High endometrial cortisol and cortisone levels potentially reduce endometrial receptivity, giving clues of how chronic stress metabolites could compromise endometrial function. Independently, high estrone levels could promote pregnancy. A reason for caution is the preliminary nature of the study with modest sample sizes.

## T-217

### Mercury Disturb Reproductive Functions of Primary Endometrial Stromal Cells (ESC).

Roberto Gonzalez-Martin<sup>†,1</sup>, Andrea Palomar<sup>†,2</sup>, Silvia Pérez-Deben<sup>†,1</sup>, Alicia Quiñonero,<sup>1</sup> Francisco Domínguez\*.<sup>2</sup> <sup>1</sup>IVI Foundation-RMA Global, Valencia, Spain; <sup>2</sup>IIS La Fe - IVI Foundation, Valencia, Spain.

**Introduction:** Mercury (Hg) exposure has been related to reproductive alterations in both animal models and epidemiological studies (Rzymiski et al., 2015, Ann Agric Environ Med). Increased levels of Hg in endometrium have been associated with pathological conditions (Guyot et al., 2015, Plos One). So far it has been described that exposure to Hg induces oxidative stress and perturbs the structure of the cytoskeleton in Ishikawa cells (Guyot et al., 2015, Plos One). However, the effect of Hg on primary

endometrial stromal cells (ESC) has not been tested yet. This study aims to analyze the effects of in vitro exposure to Hg on ESC viability, ROS production and decidualization capacity.

**Methods:** Primary ESC were isolated by gravity sedimentation from endometrial biopsies collected from healthy oocyte donors, the day of ovarian puncture (n=12). Cell viability of ESC (n=4) exposed to Hg (0-500 nM) up to 72h was measured each 24h using colorimetric MTS assay (Promega). The acute production of cellular Reactive Oxygen Species (ROS) was detected by dichlorofluorescein diacetate (DCFDA) a fluorogenic dye that measures hydroxyl, peroxy and other reactive oxygen species ROS activity within the cell (DCFDA Cellular ROS Detection Assay Kit, Abcam) and then measured with a fluorescence microscopy in ESC (n=4) exposed to Hg (0-500nM) for 24h. For the in vitro decidualization study, ESC (n=4) were pre-treated with Hg (0-500 nM) for 24 h. Then, decidualization was induced with P4+E2 for 8 days in the presence of their respective doses of Hg. Decidualization was checked by prolactin (PRL) secretion in culture media by ELISA (Abnova). Cell integrity was assessed by F-actin immunostaining with Rhodamine-Phalloidin (Abcam), and their proliferative status was checked using Ki67 immunostaining (Merk Millipore).

**Results:** In ESC exposed to Hg (500 nM), cell viability was significantly reduced (p<0.05) at 48 h and 72 h. ROS production was significantly increased (p<0.01) in ESCs exposed to Hg (500nM) for 24h. After 8 days of decidualization, PRL secretion was significantly decreased (p<0.05) at 250nM and 500nM. Accordingly, altered actin cytoskeleton was observed at 250nM and 500nM in a dose dependent manner. There was also a decrease in Ki67 positive cells, indicating a decrease in cell proliferation at 500nM (p<0.01) which reflect alterations in the correct differentiation of these cells.

**Conclusion:** High doses of Hg acutely affect the physiology of endometrial cells by decreasing cell viability, possibly related to an increase of ROS production. At low-medium doses, Hg can act as an endocrine disruptor, inhibiting ESC decidualization and disturbing actin cytoskeleton. Funded by APOTIP/2018/010, PFIS (PI/00009), Miguel Servet Contract (CPII18/00002) and ISCIII FIS project (PI17/00931).

## T-218

### Cabergoline Stimulates Human Endometrial Stromal Cell Decidualization and Reverses Inhibitory Effects of Interleukin-1β In Vitro.

Jie Yu,<sup>1</sup> Sarah L Berga,<sup>2</sup> Qingying Meng,<sup>3</sup> Mingjing Xia,<sup>4</sup> Trudy Kohout,<sup>3</sup> Marcel van Duin,<sup>3</sup> Robert N Taylor\*.<sup>2</sup> <sup>1</sup>Wake Forest School of Medicine, Winston-Salem, NC, United States; <sup>2</sup>University at Buffalo, Buffalo, NY, United States; <sup>3</sup>Ferring Research Institute, San Diego, CA, United States; <sup>4</sup>Emory University School of Medicine, Atlanta, GA, United States.

**Introduction:** Human embryonic implantation is regulated by neuroendocrine hormones, ovarian steroids, growth factors and cytokines. Sympathetic innervation of the uterus also may play a role. We tested the hypothesis that cabergoline (Cb), an agonist of type 2 dopamine receptors (DRD2), could influence endometrial decidualization in vitro.

**Methods:** Immunohistochemistry confirmed the presence of catecholaminergic neurons in human uterine tissue. DRD2 mRNA and protein expression in endometrial tissue and cells were validated by quantitative RT-PCR, cDNA microarrays, RNA sequencing and Western blotting. Isolated human endometrial stromal cells (ESC) were subjected to dose-response and time-course experiments in the absence or presence of decidualizing hormones (10 nM estradiol, 100 nM progesterone and 0,5 mM dibutyryl cAMP). In some cases, interleukin (IL)-1β (0.1 nM) was used as an inflammatory stimulus. Well-characterized in vitro biomarkers were quantified.

**Results:** DRD2 were maximally expressed in vivo in the mid-secretory phase of the cycle and upregulated in ESC in response to decidualizing hormones, as were classical (eg, prolactin) and emerging (eg, VEGF and connexin 43) differentiation biomarkers. Cabergoline augmented biomarker expression, whereas risperidone, a dopamine

receptor antagonist, inhibited ESC differentiation. Cabergoline induced characteristic decidual morphology changes and blocked detrimental effects of IL-1 $\beta$  on decidual cytology.

**Conclusion:** Our results support the hypothesis that dopaminergic neurons modulate decidualization *in situ*. We postulate that dopamine agonists, like Cb, could be developed as therapeutic agents to enhance implantation in couples with inflammation-associated infertility.

### T-219

#### Entosis Occurs in Human Embryo Implantation.

Andrea Palomar<sup>†,1</sup>, Roberto Gonzalez-Martin<sup>†,2</sup>, Stefania Salsano,<sup>2</sup> Silvia Pérez-Debén,<sup>2</sup> Alicia Quiñero,<sup>2</sup> Francisco Domínguez\*.<sup>1</sup> *IIS La Fe - IVI Foundation, Valencia, Spain; <sup>2</sup>IVI Foundation-RMA Global, Valencia, Spain.*

**Introduction:** The incapability of the embryo to clear the luminal epithelial cells to reach the stroma could lead to implantation failures. Although apoptosis is the proposed mechanism for the removal of endometrial epithelial cells (EEC), recent research points out that a non-apoptotic cell-in-cell invasion mechanism named entosis would lead to the clearance of the EEC lining. Emerging evidences in murine models strongly suggest that entosis could drive the first stages of human implantation. Thus, it is proposed that trophoblast cells of human blastocysts could internalize EEC, throughout the activation of Rho-ROCK pathway in EEC. The main objective of this research was to confirm the occurrence of entosis in human embryo implantation and the implication of Rho-ROCK pathway in this mechanism.

**Methods:** Primary human EEC obtained from endometrial biopsies of egg donors attending our clinic were cocultured *in vitro* with human trophoblastic spheroids (JAR) and human aneuploid blastocysts (in suspension and adherent conditions) to assess internalization of EEC inside trophoblastic cells. EEC and trophoblast cells were differentially stained using Cell-Tracker fluorescent probes. Internalization phenomena was evaluated by confocal microscopy analysis and 3D reconstruction models. To study the role of Rho-ROCK pathway on internalization, EEC were treated with 10 $\mu$ M of ROCK inhibitor (Y-27632). Inhibited and control stained EEC were cocultured with JAR spheroids. After 24 hours, JAR spheroids were isolated. Effect of ROCK inhibition was assessed by counting fluorescent signal of EEC and checking gene expression of ROCK1, Vimentin and hCG by qPCR on isolated JAR spheroids.

**Results:** Confocal microscopy analysis and 3D reconstruction models confirmed entosis of EEC by trophoblast cells, meaning that EEC were internalized by JAR spheroids and trophoblast cells of human blastocysts. After the analysis of fluorescent signals associated to EEC in isolated JAR spheroids from cocultures in suspension, we found that inhibition of ROCK in EEC lead to a decrease in EEC internalization. Expression of vimentin in isolated JAR spheroids was also decreased, indicating lower number of EEC internalized.

**Conclusion:** Our data support that entosis occurs in human embryo implantation and Rho-ROCK pathway may be implicated in this process. Up to our knowledge, our study is the first to confirm the occurrence of entosis in human embryo implantation. Support: PFIS (PI/00009), APOTIP/2018/010, Miguel Servet Contract (CPII18/00002) and ISCIII FIS project (PI17/00931).

### T-220

#### In Vitro Decidualization and Molecular Characterization of Murine Endometrial Stromal Cells.

Jungwoo Kim,<sup>1</sup> Yoon Young Kim,<sup>1</sup> Yong Jin Kim,<sup>2</sup> Sung Woo Kim,<sup>1</sup> Hoon Kim,<sup>1</sup> Seung-Yup Ku.<sup>1</sup> *<sup>1</sup>Seoul National University Hospital, Seoul, Korea, Republic of; <sup>2</sup>Korea University Guro Hospital, Seoul, Korea, Republic of.*

**Introduction:** In pregnancy, the decidualization of uterine endometrial cells has a pivotal role in the implantation of a fertilized embryo. Decidualization of endometrium includes drastic functional and morphological changes of endometrial stromal cells and can lead to the establishment of a successful pregnancy. Endometrial decidualization is caused by a complex combination of transcription factors, morphogens, cytokines, cell cycle regulators, and signaling pathways. However,

the deeper knowledge of molecular characteristics and mechanisms of reception of embryo by decidualized endometrial cells were not sufficiently elucidated.

**Methods:** Uterine horns of 6 weeks old female C57BL/6 mice were isolated and the endometrial stromal cells were extracted using collagenase type I (1 mg/ml). Collected cells were expanded and treated under each condition, 1) P4, 2) P4+E2, 3) P4+ cAMP, 4) cAMP, for 14 days. The expression of specific genes and proteins were analyzed using RT-qPCR and immunoblotting.

**Results:** Here, we were focusing on finding the best condition of inducing decidualization in mouse endometrial stromal cells. We found that three important molecules are mainly involved in the induction of decidualization, such as cyclic adenosine monophosphate (cAMP), estradiol (E2), and progesterone (P4). We confirmed that the groups of treatment with P4 and P4+E2 for 14 days show morphological change like decidualization, which are representatively contracted cytosol. Also, we found that forkhead box O1 (FOXO1), which is a well-known transcription factor in the decidualization process, and desmin, which is an intermediate filament expressed for decidualization, are highly expressed in decidualized cells. Most importantly, the expression of a decidual marker, such as decidual prolactin (PRL), was confirmed by western blot analysis. These results indicate that P4 and P4+E2 treatment can stimulate endometrial stromal cells to transform into a decidualization state.

**Conclusion:** These results indicate that our experimental system generates *in vitro* decidualized mouse endometrial cells and can reveal signals or factors increasing proper communications between the embryo and decidualized endometrial cells, even we address the potential methods to cure infertility because of abnormality of endometrial reception and communication network between them (2020R1A2C1010293 and 2020R1F1A1076286).

### T-221

#### Depletion of *Fkbp5* Gene in Mice Protects against Maternal Stress-Induced Age-Related Decline in Live Births.

Monica C Moore<sup>†</sup>, Xiaofang Guo, Nihan Semerci, Asli Ozmen, Kellie Larson, Frederick Schatz, Umith Kayisli, Michael N. Teng, Charles Lockwood\*, Ozlem Guzeloglu-Kayisli\*. *USF Health Morsani College of Medicine, Tampa, FL, United States.*

**Introduction:** FK506-binding protein 51 (FKBP51) is an immunophilin that acts as a co-chaperone with heat shock protein 90 that negatively regulates transcriptional activity of glucocorticoid receptor (GR) and progesterone receptor (PR). FKBP51 levels are increased by stress condition and with advancing age. Our previous studies demonstrate that *Fkbp5* gene depletion was protective against maternal restrained stress-induced preterm birth (PTB) and fetal growth restriction (FGR). The current study investigated whether *Fkbp5* deficiency also affected litter size in maternal age and stress-dependent manners.

**Methods:** Timed-pregnant *Fkbp5*<sup>+/+</sup> wild type or *Fkbp5*<sup>-/-</sup> knockout mice were randomly grouped as placebo (Control; n=86) or maternal restrained stress-administration (1h x3/day, n=54) from embryonic days 8 to 18. Litter size was counted upon delivery and compared on age groups of 1-3 months, 4-6 months, and 7-10 months with a *t*-test with *P* < 0.05 accepted as statistically significant. As an initial study, ovaries from *Fkbp5*<sup>+/+</sup> and *Fkbp5*<sup>-/-</sup> control mice were harvested at 8 weeks of age and serially sectioned and stained with Periodic acid-Schiff (PAS). Follicles were counted according to developmental stage and means were compared for primordial, primary, secondary, antral, and atretic follicles with a One-Way ANOVA test with *P* < 0.05 accepted as statistically significant.

**Results:** Litter size was similar in *Fkbp5*<sup>+/+</sup> and *Fkbp5*<sup>-/-</sup> mice in all age groups, and follicle numbers were similar in *Fkbp5*<sup>+/+</sup> and *Fkbp5*<sup>-/-</sup> control mice at 8 weeks. Although number of live births per litter was not significantly different in *Fkbp5*<sup>+/+</sup> vs. *Fkbp5*<sup>-/-</sup> mice of 1-3 months of age group (Mean  $\pm$  SEM: 7.13  $\pm$  2.32 vs. 5.5  $\pm$  4.04; *P* = 0.15) or 4-6 months of age group (8.43  $\pm$  1.81 vs. 6.85  $\pm$  2.27; *P* = 0.13), number of live births per litter was significantly higher in *Fkbp5*<sup>+/+</sup> vs. *Fkbp5*<sup>-/-</sup> mice of 6-10 months of age groups (7.45  $\pm$  1.79 vs. 5.18  $\pm$  2.89; *P* < 0.01). Interestingly, administration of maternal stress significantly reduced live births per litter in *Fkbp5*<sup>+/+</sup> mice (maternal restrained stress-induced *Fkbp5*<sup>+/+</sup> Stress vs.

*Fkbp5*<sup>+/+</sup>-control: 5.22 ±2.68 vs. 7.45 ±1.79,  $P < 0.05$ ), but not in *Fkbp5*<sup>-/-</sup> mice (maternal restrained stress- *Fkbp5*<sup>-/-</sup> vs. *Fkbp5*<sup>-/-</sup>-control: 5.18 ±2.89 vs. 5.94 ±2.49;  $P = 0.47$ ) at 6-10 months.

**Conclusion:** Significant reduction in live births in stress-induced *Fkbp5*<sup>+/+</sup> mice with advanced age and the concurrent absence of this reduction in *Fkbp5*<sup>-/-</sup> mice suggest that critical role of FKBP51 in mediating combined action of stress and advancing maternal age, which require further studies to explain the FKBP51 mediated mechanism(s).

## T-222

### Single-Cell RNA Sequencing of Ovaries Reveals Transcriptional Networks Underlying Follicular Quiescence Regulated by Mullerian Inhibiting Substance.

Marie-Charlotte L Meinsohn,<sup>1</sup> Hatice Duygu Saatcioglu,<sup>1</sup> Lihua Zhang,<sup>1</sup> Maeva Chauvin,<sup>1</sup> Nicholas Nagykeri,<sup>1</sup> Esther Oliva,<sup>2</sup> Patricia Donahoe,<sup>1</sup> David Pépin.<sup>1</sup> <sup>1</sup>Massachusetts General Hospital - Harvard Medical School, Boston, MA, United States; <sup>2</sup>Massachusetts General Hospital, Boston, MA, United States.

**Introduction:** Women are born with a limited number of primordial follicles which constitute their ovarian reserve. The activation of a primordial follicle is an irreversible process that leads to either ovulation or atresia. Mullerian inhibiting substance (MIS/AMH), produced by granulosa cells of growing follicles, is an important regulator of ovarian reserve maintenance by providing negative feedback to primordial follicle. We hypothesized that MIS inhibits folliculogenesis by imposing a quiescent state on granulosa cells.

**Methods:** Mice were injected with AAV9-MIS, or empty vector control at postnatal day 1 and euthanized at day 6, and the ovaries were dissociated to perform single-cell RNA sequencing (inDROP). We catalogued a cell atlas of the neonatal ovary along with gene expression signatures uniquely associated with MIS treatment and confirmed these cell-specific signatures by qPCR and in situ hybridization

**Results:** We first confirmed expression of the MIS receptor (MIS2) in pregranulosa and granulosa cells in mouse ovaries by RNAish. In these cells, we identified a unique cell-specific signature induced by MIS. By comparing gene expression in quiescent and activated follicles in the control to that of granulosa cells treated by MIS we defined a quiescent gene signature involving important pathways of stemness, immediate-early genes, and cytokine signaling. Interestingly, treatment with MIS resulted in a preantral follicle maturation defect in which oocyte development occurred without concomitant expansion and differentiation of granulosa cells. This observation was supported by the modest transcriptional perturbation associated with MIS in Oocytes, suggesting the inhibition of folliculogenesis is primarily driven by inhibition of granulosa cell differentiation.

**Conclusion:** Treatment with MIS imposed a quiescent cell state in granulosa cells of primordial follicles that led to a profound suppression of ovarian activity independently of oocytes maturation.

## T-223

### Chronic Interferon Gamma Expression Drives Manifestation of Ovarian Dysfunction.

Enitome E Bafor†,<sup>1</sup> Megan M Hess,<sup>1</sup> Julio C Valencia,<sup>1</sup> Loretta Smith,<sup>1</sup> John Fenimore,<sup>1</sup> Rebecca Erwin-Cohen,<sup>1</sup> Michael Sanford,<sup>1</sup> Bérénice A Benayoun\*,<sup>2</sup> Howard A Young\*.<sup>1</sup> <sup>1</sup>National Cancer Institute, Frederick, MD, United States; <sup>2</sup>University of Southern California, Los Angeles, CA, United States.

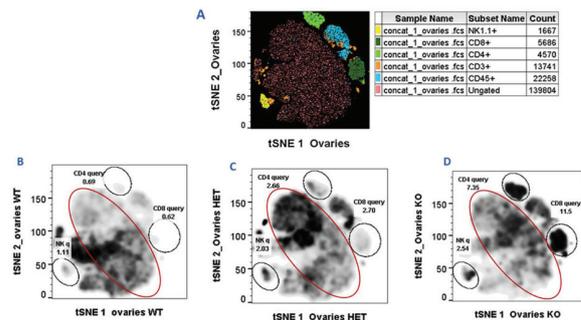
**Introduction:** An increasing population of women suffer from ovarian dysfunction. Better understanding of mechanisms underlying the ovarian dysfunction pathogenesis using newer animal models is imperative for better therapies, ovarian longevity and reproductive success. A mouse model previously generated in our laboratory, with a 162 nt AU-rich element (ARE) replaced with random nucleotides in the 3' untranslated region (3'UTR) of the interferon gamma (IFN $\gamma$ ) gene, displays chronic circulating serum IFN $\gamma$  levels. Homozygous ARE-deletion mice (ARE<sup>-/-</sup>) exhibit varied autoimmune diseases and fail to reproduce. We hypothesized that the persistent IFN $\gamma$  levels result in autoimmune ovarian

dysfunction. As a first step, we sought to identify the prevailing female reproductive pathology which we present as a novel mouse model of IFN $\gamma$ -induced ovarian dysfunction.

**Methods:** Estrous synchronization was done before experiments and cycle stages determined. Histopathological and immunohistochemistry evaluation were performed with flow cytometric analysis. ELISA assays for hormone analysis was also done. A minimum of 5 mice per genotype was utilized. Data were analyzed using either one-way ANOVA/Dunnett post-hoc or unpaired Student t-test.

**Results:** The ARE<sup>-/-</sup> mice had decreased estrous cycles ( $P < 0.0001$ ; 0-24h) and ovulation rates evidenced by few corpora lutea ( $P = 0.06$ ). Elevated serum progesterone with decreased follicle stimulating and luteinizing hormone ( $P = 0.036$  and  $0.043$  respectively) contributed to the reduced ovulatory rate with elevated primary follicles. CD8a+ cells were concentrated in ovarian stroma, theca and corpus luteum indicating increased ovarian cell cytotoxicity. This was supported by high phenotypic expression of CD8+ ( $P = 0.033$ ) and CD4+ T cells with increased effector and cytolytic phenotypes. Reduced NK1.1+ cells ( $P = 0.026$ ) with increased effector functions were seen. Double negative T cells (DNT) were decreased ( $P = 0.035$ ) suggesting DN/Treg suppression.

**Conclusion:** Taken together, the evidence shows that chronic IFN $\gamma$  expression drives ovarian dysfunction causing infertility/sub-fertility. We also present a novel mouse model of IFN $\gamma$ -induced ovarian dysfunction. **Figure 1.** tSNE flow cytometry plots of ovarian T and NK cell expression. (A) Concatenated cells (B) Wildtype; (C) Heterozygous (D) Homozygous".



## T-224

### Fetal Immune Development in Mice Is Promoted by Maternal Microchimeric Cells.

Christopher Urbschat†,<sup>1</sup> Steven Schepanski,<sup>1</sup> Maria E. Solano,<sup>1</sup> Ina A. Stelzer,<sup>2</sup> Nicole Fischer,<sup>1</sup> Denise Ohnezeit,<sup>1</sup> Victor Puelles,<sup>1</sup> Kristin Thiele,<sup>1</sup> Petra C. Arck\*.<sup>1</sup> <sup>1</sup>University Medical Center Hamburg-Eppendorf, Hamburg, Germany; <sup>2</sup>Stanford University School of Medicine, Stanford, CA, United States.

**Introduction:** During mammalian pregnancies, maternal immune cells transfer from mother to fetus in very low frequencies. Once residing in fetal tissues, they are termed maternal microchimeric cells (MMc). MMc cellular subsets include mainly T, B and myeloid cells and can be detected in fetal immune organs. It has been proposed that MMc enhance neonatal immunity, but insights into the underlying mechanisms are still unknown. To address this gap in knowledge, we developed a mouse model in which offspring are significantly devoid of MMc. Using this model, we investigated functional pathways through which MMc affect fetal immune development.

**Methods:** Since MMc are comprised of T and B cells, we used reciprocal allogeneic mating strategies between *Rag2*<sup>-/-</sup> $\gamma$ <sup>-/-</sup> and wild type male and female mice. *Rag2*<sup>+/-</sup> $\gamma$ <sup>+/-</sup> offspring from *Rag2*<sup>-/-</sup> $\gamma$ <sup>-/-</sup> females were termed MMc<sup>low</sup> and reciprocally MMc<sup>+</sup> when born to wild type mothers. Tissue clearing and lightsheet microscopy (LSM) was performed to localize MMc in fetal bone marrow. Additionally, MMc, fetal bone marrow and thymic cells were characterized upon magnetic activated cell separation, followed by flow cytometry. Bone-marrow derived hematopoietic stem cells (HSC) were assessed for differentially methylated promoter regions.

**Results:** MMc<sup>low</sup> and MMc<sup>+</sup> offspring did not differ with regard to reproductive fitness (implantation, fetal losses, placental function, weight gain), gene imprinting or neonatal microbiome. MMc could be localized in fetal bone marrow using LSM. Significant differentially methylated promoter regions could be identified among HSC between MMc<sup>pos</sup> vs MMc<sup>low</sup> offspring. Moreover, MMc<sup>low</sup> offspring exhibited significantly higher frequencies of myeloid precursor cells, however, lymphoid precursors were decreased. These findings are accompanied by significantly decreased frequency of monocytes and CD8 T cells.

Adoptive immune cell transfer into knockout dams significantly restored levels of MMc and the frequency of monocytes in MMc<sup>low</sup> offspring.

**Conclusion:** We here provide evidence for the functional impact of MMc on neonatal immunity. MMc favour the differentiation of HSC towards monocytes in fetal bone marrow via epigenetic pathways, which yields to an enhanced immunity towards early life pathogen challenges.

## T-225

### Uncomplicated Oocyte Donation Pregnancies Display Elevated CD163 Positive Type 2 Macrophage Load in the Decidua, Which Is Associated with Fetal-Maternal HLA Class II Mismatches.

Xuezi Tian†, Kaveri T.S. Aiyer†, Hanneke M. Kapsenberg\*, Dave L. Roelen\*, Marie-Louise van der Hoorn\*, Michael Eikmans\*. *Leiden University Medical Center, Leiden, Netherlands.*

**Introduction:** The embryo of an oocyte donation (OD) pregnancy is completely allogeneic to the mother, which may lead to a bigger challenge for the maternal immune system to tolerize the fetus compared to autologous pregnancies. Decidual macrophages may be essential in maintaining a healthy pregnancy. In addition, macrophages can be classified into different categories based on phenotype and characteristics, in which type 2 macrophages are thought to exhibit immune suppressive activity. We hypothesized that the quantity and composition of decidual macrophages are different between uncomplicated OD pregnancies and non-OD in vitro fertilization (IVF) pregnancies, and that these differences in macrophages are related to fetal-maternal incompatibility.

**Methods:** This retrospective case-control study included patients who delivered at the Leiden University Medical Center between January 1st 2006 and July 1st 2016. A total of 42 pregnancies were enrolled in this study, conceived by uncomplicated singleton OD pregnancies (n=25) or non-OD IVF pregnancies (n=17). Placentas were collected and immunohistochemically stained for CD14 (pan-macrophage marker) and CD163 (type 2 macrophage marker). The extent of staining in both the decidua basalis and the decidua parietalis was quantitated by digital image analysis software. To assess HLA mismatching, maternal and fetal DNA was typed for HLA-A, -B, C, -DRB1, and -DQB1.

**Results:** A significantly lower percentage of CD14 positive staining was observed in the decidua basalis of OD pregnancies compared to non-OD IVF pregnancies (p=0.030). In the parietalis, OD pregnancies demonstrated a higher percentage of CD163+ staining (p=0.040) and higher CD163/CD14 ratio (p=0.032) compared to non-OD IVF. The OD group was separated into a syngeneic group (number of mismatches lower than half of the antigens per HLA locus) and an allogeneic group (number of mismatches higher than half of the antigens per HLA locus). Significant differences of CD163+ and CD163/CD14 ratio were found in the decidua parietalis when comparing the HLA-class-II-allogeneic OD group with the non-OD IVF group (p<0.05). This difference was not found for the HLA-class-II-syngeneic OD group.

**Conclusion:** Healthy OD pregnancies show a higher CD163 positive cell fraction within the total macrophage population in the decidua compared to non-OD IVF pregnancies, which may suggest that a local type 2 macrophage-related mechanism is needed to compensate for the higher fetal-maternal HLA mismatch load in OD pregnancies.

## T-226

### RNA Sequencing of the Fetal Inflammatory Response Syndrome Type I and Type II.

Robert Para†, Roberto Romero\*, Derek Miller†, Jose Galaz†, Bogdan Done, Azam Peyvandipour, Meyer Gershater†, Li Tao†, Douglas Ruden\*, Jenna Isherwood, Roger Pique-Regi\*, Adi L Tarca\*, Nardhy Gomez-Lopez\*. <sup>1</sup>Wayne State University SOM, Detroit, MI, United States; <sup>2</sup>Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, United States.

**Introduction:** Fetal Inflammatory Response Syndrome (FIRS) is a state of fetal systemic inflammation associated with neonatal morbidity and mortality. FIRS is classified as Type I, characterized by acute inflammatory responses in the fetal circulation and placenta, or Type II, indicated by chronic inflammation. FIRS Type I and Type II are considered distinct syndromes; yet, the molecular mechanisms underlying these fetal inflammatory responses are not well understood. Herein, we investigated the cord blood transcriptome of preterm neonates diagnosed with FIRS Type I or Type II.

**Methods:** Cord blood was obtained from preterm neonates with i) FIRS Type I (n = 11), diagnosed by cord blood interleukin (IL)-6 levels > 11 pg/mL and acute funisitis; ii) FIRS Type II (n = 6), diagnosed by cord blood CXCL10 levels > 82.34 pg/mL, IL-6 < 11 pg/mL, and chronic placental inflammation; and iii) controls (n = 22) without inflammation. Cytokine concentrations were evaluated in fetal plasma. Cord blood transcriptomes were obtained using RNA sequencing. Differentially expressed genes between groups were identified and used to infer perturbed upstream regulators, KEGG pathways, and gene ontology categories. The contribution of immune cell subsets was assessed by integrating cord blood mRNA changes with single-cell signatures. Perturbations of cell- and tissue-specific signatures derived from the Human Gene Atlas were also quantified.

**Results:** Neonates with FIRS Type I had elevated systemic concentrations of inflammatory cytokines (IL-6, IL-8, IL-1β, TNF), whereas those with FIRS Type II showed elevated levels of CXCL10 alone. The FIRS Type I transcriptome was characterized by the upregulation of innate immune pathways and downregulation of adaptive immune responses compared to controls. In contrast, the FIRS Type II transcriptome showed differential expression of HLA genes, yet was largely indistinguishable from controls. PCA analysis using single-cell RNA sequencing-derived signatures revealed that FIRS Type I samples form a cluster distinct from those of FIRS Type II or controls, most strongly driven by monocyte and T-cell signatures. Consistently, using Human Gene Atlas-derived signatures, FIRS Type I was characterized by elevated bone marrow/myeloid/monocyte signatures together with a decreased dendritic cell signature compared to controls.

**Conclusion:** The FIRS Type I cord blood transcriptome is associated with significant alterations indicative of a severe acute inflammatory response, whereas that of FIRS Type II shows only mild differential expression of chronic inflammatory genes. These findings indicate that FIRS Type I and FIRS Type II are driven by distinct immune mechanisms.

## T-227

### Recurrent Stillbirth Due to Chronic Histiocytic Intervillositis: Discovery of a Purified Maternal Alloantibody against Placental Protein.

Emily F Cornish<sup>1</sup>, Thomas McDonnell<sup>2</sup>, David Williams\*. <sup>1</sup>EGA Institute of Women's Health, University College London, London, United Kingdom; <sup>2</sup>Faculty of Engineering Science, University College London, London, United Kingdom.

**Introduction:** Chronic histiocytic intervillitis (CHI) is a rare placental disorder characterised by histiocyte accumulation in the intervillous space. CHI is associated with severe fetal growth restriction, prematurity, intrauterine death and a high recurrence rate (up to 80%). The pathogenesis is unknown, but coexistence with autoimmune disease, complement C4d deposition in affected placental tissues and modest clinical benefit from maternal immunosuppression suggest an alloimmune "placental rejection" aetiology. The diagnosis can only be made through postpartum

histology. There are currently no clinical biomarkers or treatments proven to consistently predict or prevent recurrence of CHI in a subsequent pregnancy.

**Methods:** This was a case-control study designed to investigate whether women with recurrent CHI had altered antibody reactivity to placental antigen, compared with women who had uncomplicated pregnancies. Following ethical approval (London, 19/LO/0105), 22 women affected by CHI were recruited through a Facebook support group and via a Patient Engagement Day held at UCL. Serum samples were collected from a subset of women with  $\geq 2$  stillbirths due to histologically-proven CHI ( $n=6$ ) and controls with  $\geq 2$  healthy pregnancies ( $n=6$ ). Western blots were performed using lysed human syncytiotrophoblast cell lines (JAR and JEG-3), probed with affinity-purified IgG from participants and exposed using chemiluminescence. Immunoprecipitation with patient-derived whole IgG and mass spectrometry analysis were used to elute and identify the putative antigen from trophoblast cell lysate.

**Results:** Chemiluminescence revealed bands corresponding to a 55-60kDa protein in both JAR and JEG-3 cell lysate in all six cases of CHI. No bands were identified in the control participants. Mass spectrometry (MALDI-QTOF) analysis of immunoprecipitation eluate generated credible candidates with high sequence coverage, including the complement inhibitor C4-binding protein. Initial Western blot experiments using membranes containing recombinant C4BP showed reactivity to C4BP in a subset of participants ( $n=3$ ) with a particularly aggressive disease phenotype.

**Conclusion:** These results demonstrate that women with recurrent stillbirth due to CHI have circulating antibodies against proteins in trophoblast cell lines. This provides a biologically plausible mechanism for immune complex formation and complement dysregulation at the feto-placental unit, leading to compromised fetal growth and adverse perinatal outcomes. Further characterisation of this antibody in affected women could lead to identification of a novel biomarker for CHI and translate into discovery of targeted maternal immunosuppressive therapy to prevent recurrence.

#### T-228

##### Utilizing Primary HLA-G+ EVT and EVT-Like Cell Lines to Study Maternal Fetal Interactions.

Sarika Kshirsagar,<sup>1</sup> Tamara Hagen,<sup>2</sup> Tamara Tilburgs\*,<sup>2,3</sup> <sup>1</sup>Harvard University, Cambridge, MA, United States; <sup>2</sup>Cincinnati Childrens Hospital, Cincinnati, OH, United States; <sup>3</sup>University of Cincinnati College of Medicine, Cincinnati, OH, United States.

**Introduction:** During pregnancy invading extravillous trophoblasts (EVT) play a key role in placental development, uterine spiral artery remodeling and prevention of a detrimental maternal immune responses to foreign placental and fetal antigens. Failure of these processes are suggested to play a role in development of pregnancy complications, but very little is known about the underlying mechanisms. EVT express human leukocyte antigen-C (HLA-C), a highly polymorphic antigen that can elicit maternal immune responses, and HLA-G which has been associated with immune tolerance. Thus far, EVT have been difficult to study due to the low EVT numbers that can be obtained, the lack of proliferative capacity, and limited survival *in vitro*. Moreover, HLA-G and HLA-C orthologues are only present in humans, chimpanzees, and gorillas. Thus further studies on human HLA-G+/C+ EVT are essential to understand their role in placental tolerance and inflammation.

**Methods:** Here we present validated methods to purify and culture primary HLA-G+/C+ EVT from 1<sup>st</sup> trimester placental villi as well as the placental disk and chorionic membrane at healthy term pregnancy. In addition, we established highly proliferative HLA-G and HLA-C expressing EVT-like cell lines using a modified protocol first published by Okae et al., 2018.

**Results:** Characterization of HLA-G+/C+ EVT from term pregnancy compared to 1<sup>st</sup> trimester, revealed their unique phenotypes, gene expression profiles and capacity induce regulatory T cells during co-culture assays. Furthermore, essential clinical variables such as gestational age and fetal sex significantly influenced EVT biology and function. The newly established HLA-G+/C+ EVT-like cell lines expressed a

large number of EVT specific proteins (HLA-G, HLA-C, PDL1, B7H3) and gene transcripts (CD9, PDL2, HIF1A, TGF $\beta$ ) while some other EVT-specific markers were not expressed (EGFR1, ITGA5, EBi3). This suggests the EVT-like cell lines resemble EVT but may require additional signaling to fully differentiate into EVT. Indeed switching the EVT-like cell lines from a collagen IV matrix to a fibronectin matrix significantly increased their EGFR1 and ITGA5 protein expression levels. This demonstrates that the EVT-like lines are responsive to their environment and have the capacity to further differentiate.

**Conclusion:** These methods and approaches form a solid basis for further investigation of the role of HLA-G+/C+ EVT in the development of detrimental placental inflammatory responses associated with pregnancy complications including spontaneous preterm delivery and preeclampsia.

#### T-229

##### Ozone Therapy and Pulsed Electro-Magnetic Field (PEMF) Could Improve Female Reproductive Potential.

Zaher Merhi\*,<sup>1</sup> Subasinghe Ashini R Dias†,<sup>2</sup> Daniella Emdin,<sup>3</sup> Lisa Bosman,<sup>3</sup> Andre Hugo Smith.<sup>3</sup> <sup>1</sup>SUNY Downstate Health Sciences University, Brooklyn, NY, United States; <sup>2</sup>Seton Hall University, South Orange, NJ, United States; <sup>3</sup>HOCATT, South Africa, South Africa.

**Introduction:** Women with severely diminished ovarian reserve (DOR) who had repeated failed IVF cycles and women with persistently thin endometrial lining thickness (EMT) during frozen embryo transfer (FET) cycle have limited treatment options with a large majority of patients resorting to using donor oocytes and gestational carrier. Data in animal studies suggest that Ozone Therapy (OT) and PEMF are emerging as potential therapeutic adjuncts for female reproduction. The objective of this study was to determine whether OT+PEMF *in vivo* administration via the HOCATT machine, that uses the CO<sub>2</sub>/Carbonic Acid modality, improves the outcome in women undergoing IVF/FET and to assess the effect of OT *in vitro* on human granulosa cell (GC) function.

**Methods:** The study was approved by IRB and participants were consented. Experiments: **#1**) Women ( $n=15$ ) with severe DOR underwent: 1<sup>st</sup> IVF cycle (Cycle 1), followed by OT (transdermally and vaginally) +PEMF administration for 3 weeks, followed by a 2<sup>nd</sup> IVF cycle (Cycle 2) using the same protocol as Cycle 1. **#2**) Participants ( $n=3$ ) had a persistently thin EMT ( $<5$  mm) despite the intake of most medical agents known to improve EMT, making them unable to have an FET. They underwent OT+PEMF for 3 weeks after which EMT was compared. **#3**) Participants ( $n=6$ ) who underwent oocyte retrieval had their GCs split equally and cultured with OT or placed on room temperature (control); after which PCR was performed for genes involved in steroidogenesis: aromatase (CYP19A1), Side-Chain Cleavage (SCC), StAR, and 3 Beta-hydroxysteroid dehydrogenase (HSD). Data were presented as mean $\pm$ SEM. Paired *t*-test was used to compare data.

**Results: #1**) Cycles 1 and 2 had no significant difference in the # of days of stimulation ( $p>0.05$ ), dose of medications used ( $p>0.05$ ), # of oocytes retrieved ( $1.1 \pm 0.4$  vs.  $1.5 \pm 0.3$ , respectively;  $p=0.3$ ) or peak E2 levels ( $539.5 \pm 96.1$  vs.  $591.5 \pm 102.6$  pg/mL, respectively;  $p=0.6$ ). The # of embryos formed in Cycle 2 was significantly higher than the # of embryos formed in Cycle 1 ( $1.2 \pm 0.3$  vs.  $0.4 \pm 0.4$ , respectively;  $p=0.02$ ). **#2**) All participants reached an EMT of approximately 7 mm. Following FET, participant A is currently in the 2<sup>nd</sup> trimester of pregnancy; participant B became pregnant and had a missed abortion; and participant C did not get pregnant. **#3**) OT caused a significant 5 times increase in CYP19A1 and 50% significant decrease in SCC ( $p<0.05$  for both).

**Conclusion:** OT+PEMF, both of which have vasodilatory, anti-inflammatory, and anti-oxidant actions, could improve EMT and could improve the # of formed embryos without increasing the # of oocytes retrieved, suggesting improvement in oocyte quality. OT alters genes involved in steroidogenesis in particular aromatase.

**T-230****Establishing Human Trophoblast Stem Cell Derived Organoids to Model Early Maternal-Fetal Interactions.**

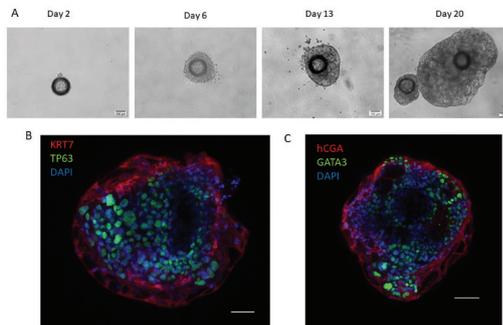
Jie Zhou, Yuchen Tian, Kylie J Dahlgren, Mark J Messler, Sehee Choi, Laura C Schulz, Toshihiko Ezashi, Bret D Ulery, R Michael Roberts, Danny J Schust. *University of Missouri, Columbia, MO, United States.*

**Introduction:** Derivation, culture and differentiation conditions for human trophoblast stem cells (hTSCs) were first reported in 2018 [Okoe, 2018]. These hTSCs can be differentiated into the major trophoblast subpopulations of the placenta including cytotrophoblast cells (CTB), extravillous cytotrophoblast cells (EVT) and syncytiotrophoblast (STB). The development of 3D trophoblast organoids to model early maternal-fetal interactions during human pregnancy [Turco, 2018] have been hindered by the inside-out (STB on the inside, undifferentiated cells and CTB on the outside) morphology of the generated structures.

**Methods:**  $5 \times 10^5$  hTSCs (C27) cells were incubated with collagen IV pre-treated polystyrene beads in supplemented medium at 37°C for 4 hours, then were transferred to low attachment cell culture plates and cultured for an additional 20 days in a series of differentiation-inducing media. Media was changed every 48 hours for the first 6 days then every 3-4 days after that. Organoids were fixed on day 20 for standard immunohistochemistry.

**Results:** The collagen-coated beads were covered with cells within 24 hours of incubation and organoids grew continuously throughout culture to an average size of 400  $\mu$ m (Fig. 1A). On day 20 of culture, the surface cells of the organoids express cytokeratin-7 (KRT7, a pan-trophoblast marker) and hCG $\alpha$  (a syncytiotrophoblast cell maker) while the central cells of the organoids expressed the cytotrophoblast markers GATA3 and TP63 (Fig. 1B, 1C).

**Conclusion:** Using collagen-coated beads, we have successfully established human trophoblast organoids characterized by inner undifferentiated and CTB

**T-231****Extracellular Matrix Hydrogels from Decellularized Endometrium Promote Tissue Regeneration and Fertility Restoration in a Murine Model of Endometrial Damage.**

Sara López-Martínez†, Adolfo Rodríguez-Eguren†, Lucía de Miguel-Gómez†, Amparo Faus, Emilio Francés-Herrero†, Antonio Pellicer\*, Hortensia Ferrero\*, Irene Cervelló\*. *1IVI Foundation - IIS La Fe, Valencia, Spain; 2University of Valencia, Valencia, Spain; 3IVIRMA, Roma, Italy.*

**Introduction:** The finding of an effective therapy for endometrial injury such as Asherman's syndrome and endometrial atrophy remains as an outstanding issue in reproductive medicine. Extracellular matrix (ECM) hydrogels made from decellularized tissues are promising biocompatible materials for tissue regeneration. We previously reported the development of a porcine endometrial ECM hydrogel (EndoECM) (López-Martínez et al., 2021). Here, we investigated its potential alone or supplemented as a treatment for endometrial repair in a murine model of endometrial damage.

**Methods:** Endometrial injury was induced by injection of ethanol in uterine horns of C57BL/6 mice (n=33). After four days, three therapies were injected: (a) saline (negative control), (b) biotin-labelled EndoECM or (c) biotin-labelled EndoECM plus basic fibroblast growth factor, insulin-like growth factor I and platelet-derived growth factor

(EndoECM+GF). Endometrial regeneration and fertility were evaluated after two weeks. Trichrome staining was performed to assess endometrial area, collagen and number of glands using ImageJ/QuPath software (n=3 saline, n=4 EndoECM, n=4 EndoECM+GF mice with a damaged/treated horn and a non-injured horn). Number of fetuses 10 days after natural mating was counted (n=4 saline, n=9 EndoECM, n=9 EndoECM+GF, one or both damaged/treated horns). The estrous cycle was monitored and considered in the analyses. Kruskal-Wallis with Dunn's multiple comparisons test was applied for statistics.

**Results:** All mice presented cyclicity confirming a normal ovarian function not disrupted by ethanol. Both treatments showed an increased number of glands compared to saline solution (31.7±6.26, 58.2±16.53 and 53.5±10.92 glands/mm<sup>2</sup> in saline, EndoECM (P=0.07) and EndoECM+GF (P=0.08)), and a non-significant increase in endometrial thickness. All groups showed a low incidence of fibrosis, yet collagen deposition was significantly higher in EndoECM compared to EndoECM+GF (90.7±3.21 and 82.7±4.86%, P<0.05). Ethanol caused a significant disruption of fertility in saline group (no pregnancy) compared to non-injured horns (80% pregnancy rate, 3.8±2.60 fetuses/horn), which was not significantly improved by EndoECM (8.6%, 0.7±2.00) (P<0.05). By contrast, EndoECM+GF showed an improved trend of fertility (41.7%, 1.6±2.35) with no significant difference compared to non-injured horns.

**Conclusion:** This study suggests for the first time how EndoECM hydrogels alone or supplemented with growth factors can be used to develop novel approaches to treat endometrial pathologies. Funding: ACIF/2017/118; FPU19/04850; FPU18/06327; PI17/01039; PROMETEO/2018/137; CP19/00149.

**T-232****Potential Molecules and Pathways Involved in Ovarian Rescue by Bone Marrow Derived Stem Cells in Human Ovarian Tissue.**

Anna Buigues†, Maria Marchante†, Patricia Diaz-Gimeno, Jessica Martínez, Antonio Pellicer\*, Sonia Herraiz. *1IIS La Fe - IVI Foundation, Valencia, Spain; 2University of Valencia - IVI Foundation, Valencia, Spain; 3IVI-RMA Rome, Rome, Italy.*

**Introduction:** Ovarian rescue to increase reproductive outcomes is an important strategy for treating women with diminished ovarian reserves. Previous studies suggest that bone marrow derived stem cells (BMDSC) have regenerative properties in both human and mouse impaired ovaries. Moreover, BMDSC autologous ovarian transplantation improved ovarian reserve biomarkers in patients with poor ovarian response (POR) allowing pregnancies. Thus, we aimed to identify proteins and pathways that may be involved in the regenerative effects of BMDSC in ovaries to inform future experiments aimed at optimizing clinical application of BMDSC therapy.

**Methods:** Eight ovarian cortex biopsies from POR women were xenografted into NOD/SCID ovariectomized females (n=4). A week later, after xenograft vascularization, animals were allocated into two experimental groups, receiving an intravenous injection of saline (control group) or  $1 \times 10^6$  human BMDSC (BMDSC group). Two weeks after, ovarian xenografts (control n=3; BMDSC n=4) were recovered to establish their proteomic profiles by SWATH. A penalized linear regression model using elastic net was used to identify differently expressed proteins (DEP) among control and BMDSC xenografts.

**Results:** Of the 1,224 proteins quantified by SWATH, 22 were differentially expressed in the BMDSC grafts compared to controls. Discriminant analysis and heatmap considering these DEP showed a clear separation between groups (Figure 1A-B). These proteins were involved in KEGG pathways related to ovarian function, such as FoxO signaling pathway, regulation of actin cytoskeleton, protein processing in endoplasmic reticulum and apoptosis (Figure 1C). Moreover, 8 of the DEP (such as TIPS and CATS) were previously described as proteins whose levels change in plasma with aging.

**Conclusion:** This study shows proteomic changes induced by BMDSC in human ovarian tissue and proposes specific pathways and molecules that may be involved in ovarian rescue induced by these adult stem cells. Of them, TIPS and CATS could be interesting targets to address in

future studies because their expression significantly change in the wave of aging that occurs at the age of 34, when ovarian aging starts. Support: PROMETEO/2018/137

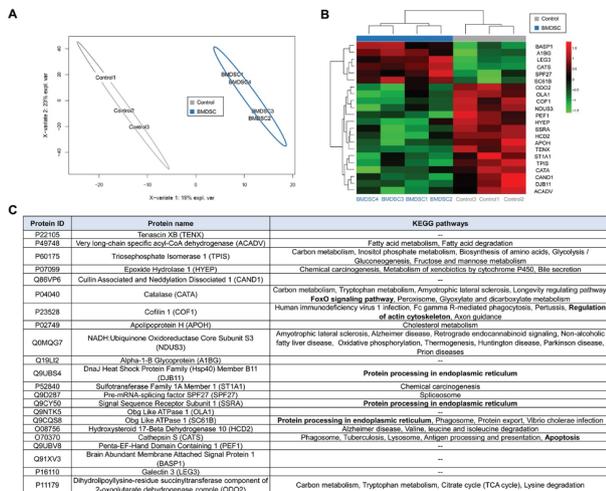


Figure 1. Proteomic changes induced by BMDS in human ovarian tissue. Discriminant analysis (A) and heatmap (B) showing a clear separation between groups. (C) Differentially expressed proteins between control and BMDS-treated xenografts

T-233

Stillbirth Rates Are Falling Faster Than Perceived.

Roshan Selvaratnam†, 1,2 Daniel Rolnik\*, 1 Mary-Ann Davey\*, 1,2 Euan M Wallace\*, 1,2 The Ritchie Centre, Monash University, Melbourne, Australia; 2Safer Care Victoria, Melbourne, Australia.

**Introduction:** Rates of stillbirth are falling across high-income countries despite significant changes in maternal characteristics over time. However, in view of the increasing number of women with risk factors for stillbirth, it might have been expected that the rate of stillbirth would have increased over time. We sought to quantify how the changing stillbirth risk profile of women is affecting the interpretation of the stillbirth rate.

**Methods:** A retrospective, population-based cohort study in Victoria, Australia on all births ≥28 weeks’ gestation from 1983 to 2018. Changes in maternal characteristics over time were assessed. A multivariable logistic regression model was designed for stillbirth based on maternal characteristics in 1983-87 and used to calculate individual predictive probabilities of stillbirth from the regression equation. The number of expected stillbirths per year as a result of the change in maternal demographics was then calculated, assuming no changes in care and in the associations between maternal characteristics and stillbirth over time.

**Results:** There were 2,419,923 births at ≥28 weeks’ gestation between 1983 and 2018 in Victoria, Australia. Compared to 1983-87, there were more women of older age groups giving birth, more nulliparous women, more Indigenous women and women born in Oceania, Asia, and Africa, more multiple pregnancies, and more women with pre-existing diabetes in 2014-18. Despite this, the rate of stillbirth fell from 5.42 per 1,000 births in 1983 to 1.72 per 1,000 births in 2018 (p-value < 0.001). Applying the multivariable logistic regression equation derived from 1983-87 to each year, had there been no changes in care or in the associations between maternal characteristics and stillbirth, the rate of stillbirth would have increased by 12%, from 4.94 per 1,000 in 1983 to 5.54 per 1,000 in 2018 as a result of the change in maternal characteristics.

**Conclusion:** There has been a substantial reduction in late pregnancy stillbirth in Victoria over the last 35 years. This reduction has been achieved despite a demographic shift toward greater numbers and proportions of childbearing women with a higher stillbirth risk profile than previously. Had the risk associated with maternal characteristics not changed and care not improved, the rate of stillbirth would be expected to have increased by 12%. This suggests that the impact of long-term efforts to reduce stillbirth has been underestimated and that the contributions improved care have made to outcomes are larger than recognised.

T-234

Resilience, Coping Styles and Cognitive Appraisal Moderate Disaster Effects on Maternal Posttraumatic Stress: The Fort McMurray Wood Buffalo Wildfire Study.

Barbara Verstraeten†, 1 Guillaume Elgbeili, 2 Ashley Hyde†, 1 Suzanne King\*, 2,3 David Olson\*. 1 University of Alberta, Edmonton, AB, Canada; 2Douglas Mental Health University Institute, Montreal, QC, Canada; 3McGill University, Montreal, QC, Canada.

**Introduction:** Women are more susceptible to posttraumatic stress disorder. Mitigating stress in pregnant women is important for their own well-being, and to protect the unborn child from the effects of prenatal stress. Disaster stress can be measured as objective hardship (degree of disaster exposure), subjective distress (emotional response intensity) and cognitive appraisal of the event. We postulate that resilience, positive coping styles and cognitive appraisal would moderate effects of objective hardship on posttraumatic stress (PTS) symptoms in mothers 10 and 24 months after the 2016 Fort McMurray Wood Buffalo wildfire in Alberta, Canada.

**Methods:** 200 women who were pregnant or within 6 months of conception during the fire were recruited 10.3 ± 4.0 months after the disaster. They completed the Impact of Event Scale-Revised (IES-R) for PTS symptoms, Connor-Davidson Resilience Scale for resilience and Brief COPE for coping strategies at recruitment. At 24 months post-fire (24MPF), 109 women (54.5%) completed the McMurray Objective Maternal Stress Scale and indicated their cognitive appraisal of the disaster (very negative to very positive). Of these, 75 (37.5%) also returned the 24MPF IES-R. Repeated measures, correlation and multiple linear regressions with interaction terms to test moderation of the relationship between objective hardship and PTS symptoms were performed, with p<0.05 considered significant.

**Results:** PTS symptoms did not significantly decrease between recruitment and 24MPF (p=0.26). High levels of objective hardship, dysfunctional coping, and negative cognitive appraisal correlated with higher PTS symptoms at recruitment and 24MPF (p<0.001). High dysfunctional coping at recruitment (n=75, p=0.03) and high emotion-focused coping at 24MPF (p=0.02) associated with more severe PTS symptoms. High levels of resilience protected against PTS symptoms at low, but not high, degrees of hardship at recruitment but not 24MPF (p=0.01). While a negative cognitive appraisal was associated with more PTS symptoms at increasing levels of hardship, neutral or positive cognitive appraisal protected women against PTS symptoms at 24MPF (p=0.001) with increasing magnitude of effects the more severe the objective hardship.

**Conclusion:** The persistence of PTS symptoms two years after a natural disaster is concerning. Attitudes and personality play an important role in the development of enduring post-disaster PTS symptoms. The ability to positively reframe a tragedy is protective towards the continuation of symptoms, even at high levels of objective hardship. Supporting resilience, reframing and positive coping styles may be beneficial for maternal mental health and child development.

T-235

Roles of Maternal and Fetal Vascular Pathology in a Case-Control Study of Autism.

Christine Chen, 1,2 Jillamika Pongsachai, 1,2 Jennifer S Feng†, 1,2,3 Joan Krickellas, 1,2 Sadia F Chowdhury†, 1,2,3 Adwoa Nantwi†, 4 Sylvia Dygulski, 1,2 Hannah Bromberg, 1,2 Ruchit Shah, 1,5 Michael Joyce, 1 Mehrin Jan, 1,2 Serena Chen, 1,2 Beata Dygulaska, 2 Carolyn Salafia\*, 1,2,5 1Placental Analytics, New Rochelle, NY, United States; 2NYPBMH, Brooklyn, NY, United States; 3CUNY Hunter College, New York, NY, United States; 4NYU CGPH, New Rochelle, NY, United States; 5Institute of Basic Research, Staten Island, NY, United States.

**Introduction:** Oxidative stress pathways have been identified as abnormal in children with autism spectrum disorder (ASD); placental evidence of maternal and/or fetal vascular pathology (MVP, FVP) such as infarcts and vascular thromboses have not been studied in ASD in a low risk community based population.

**Methods:** A community hospital based sample with universal placental examination was searched for those births followed to at least age 2 years

at our institution. Billing codes were searched for diagnoses related to autism spectrum disorder (ASD) among the patient population of the Department of Pediatrics Developmental Pediatrics group. At least 2 diagnoses related to ASD as per Newschaffer et al were required to be considered an ASD case. Controls were selected from the next infant born of same gender, gestational age +/-2 weeks, and season of birth +/-2 weeks. Maternal uteroplacental vascular pathology (UVP) considered both acceleration of villous maturation and lesions such as infarct and thrombosis; fetal vascular pathology (FVP) was coded to distinguish terminal villous lesions (avascular villi, stromal karyorrhexis and erythrocyte fragmentation) and mural thrombi embedded in the walls of muscular placental vessels. Contingency tables considered  $p < 0.05$  significant.

**Results:** There was no association of maternal UVP lesions and ASD ( $p = 0.17$ ). 27 (21%) of ASD had villous fibrosis, hypovascularity and increased syncytial knotting compared to 14% of controls ( $p = 0.09$ ). There was no association of ASD with presence, number, size or location of placental infarcts ( $p = 0.45$ ). Chorionic or fetal stem mural thrombi were seen in 18 (13.7%) ASD and 50 (12%) of controls. However, fetal vascular pathology confined to terminal villi was seen in 12 (9%) of ASD cases and 19 (4.5%) of controls.

**Conclusion:** Gross placental destructive lesions of uteroplacental vascular thrombosis and infarct were not associated with ASD in the population based cohort. Among lesions of the fetal placental vasculature, mural thrombi have been linked to neurodevelopmental impairment. However, this study finds no link to ASD, but rather lesions of the finer terminal villous capillary network. We have elsewhere reported a significant increase in terminal villous hemosiderin in placentas of newborns with an older sibling with ASD. These findings in a population of sporadic ASD provide additional evidence for capillary level vascular fragility to be a significant pathology in both genetic and sporadic ASD.

#### T-236

##### The Contribution of Social and Environmental Determinants of Health to Racial Differences in Preterm Birth Risk.

Julia J Brittain†,<sup>1</sup> Shawn J Latendresse,<sup>2</sup> Timothy P York\*,<sup>3</sup> <sup>1</sup>University of Richmond, Richmond, VA, United States; <sup>2</sup>Baylor University, Waco, TX, United States; <sup>3</sup>Virginia Commonwealth University, Richmond, VA, United States.

**Introduction:** Preterm birth is one of the most persistent health disparities in medicine. Preterm birth is more prevalent in Black versus White American women and contributes to 3.4 times more Black American infant deaths. Multiple lines of evidence indicate that social inequities play a significant role in racial health disparities, including preterm birth. Differential access/exposure to social and environmental determinants of health (SEDHs) is known to correlate broadly with self-identified race (Race), yet little is known about how these exposures contribute to racial disparities in preterm birth risk. The aim of this research was to investigate whether and to what extent SEDHs in five broad domains mediated the relationship between Race and gestational age at birth (GA).

**Methods:** Data on SEDHs and birth outcomes was obtained from the Pregnancy, Race, Environment, Genes (PREG) study (PI: York), a prospective cohort of 177 women (50.3% Black American) in Richmond, Virginia. At up to four study visits, extensive measures were collected by questionnaire on pre-pregnancy SEDH, pregnancy-specific environmental exposures and medical history. Variables reflecting five empirically supported domains of SEDH were extracted, each with racially disparate links to preterm birth, including economic stability, education, social and community context, health and health care, and neighborhood/built environment. The internal consistency of instruments were assessed by Cronbach's alpha and factor analyses, both exploratory and confirmatory approaches, were performed to validate instruments by each self-identified racial group. A mediation model approach (mediation R package v. 4.5) was used to test the hypothesis that the direct association of Race on GA can be indirectly accounted for, at least in part, by variables from the five SEDH domains.

**Results:** The screened variable list consisted of 31 variables representing the five SEDH domains. The variable set considered for mediation analysis

was identified from those estimated by linear regression to have a direct effect on GA at a  $Pval < 0.10$  ( $N = 12$ ). Race was estimated to explain 6.91% of the inter-individual variability in GA ( $Pval < 0.001$ ). Mediation model results identified three variables with an indirect effect including: 'education level' ( $Pval = 0.022$ ), 'someone close in trouble with the police' ( $Pval = 0.022$ ), and 'length of time at current job' ( $Pval = 0.036$ ). **Conclusion:** Current research on racial disparities in birth outcomes is limited by the availability of information obtainable by medical records abstraction. This study identified specific variables that mediated the influence of Race on GA. Further studies in larger samples should seek to identify other variables that account for race-specific preterm birth risk.

#### T-237

##### Exposure to Consumer Product Chemicals and Changes in Plasma Oxylinins in Pregnant Women.

Barrett M Welch†,<sup>1</sup> Alexander P Keil,<sup>2</sup> Paige A Bommarito,<sup>1</sup> Thomas J van t Erve,<sup>1</sup> Leesa J Deterding,<sup>1</sup> Jason G Williams,<sup>1</sup> David E Cantowine,<sup>3</sup> Thomas F McElrath,<sup>3</sup> Kelly K Ferguson\*,<sup>1</sup> <sup>1</sup>NIH/NIEHS, Research Triangle Park, NC, United States; <sup>2</sup>UNC, Chapel Hill, NC, United States; <sup>3</sup>Harvard Medical School, Boston, MA, United States.

**Introduction:** Exposure to consumer product chemicals during pregnancy may increase susceptibility to pregnancy disorders by influencing maternal inflammation. However, effects on specific inflammatory pathways have not been well characterized. Recently, we observed that oxylinins, an important class of lipid mediators of inflammation, are associated with adverse fetal growth outcomes. In this study, we aimed to determine the association between plasma oxylinins and urinary biomarkers of three classes of consumer product chemicals among pregnant women.

**Methods:** Data come from a pilot study of 90 pregnant women nested within the LIFECODES cohort. Maternal plasma and urine were collected at three prenatal visits. Plasma was analyzed for 61 oxylinins, which were grouped into biosynthetic pathways defined by upstream: 1) fatty acid precursor: linoleic, arachidonic, docosahexaenoic, or eicosapentaenoic acid; and 2) enzyme pathway: cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP). Urine was analyzed for 12 phthalate, 12 phenols, and 9 organophosphate ester (OPEs). Linear mixed effect models were used for single-pollutant analyses, while the novel quantile g-computation was used to examine the joint effect of class-specific chemical mixtures.

**Results:** Single-pollutant and mixture analyses showed urinary biomarkers of consumer product chemicals were most consistently associated with oxylinins produced from arachidonic acid by LOX or COX enzymes. The LOX-derived oxylinins, including a variety of hydroxyeicosatetraenoic acids (HETEs), were most strongly and positively associated with phenol biomarkers. Mixture analyses showed that at mid-pregnancy a quartile increase in all 7 phenols was associated with a 72% increase (95% confidence interval [CI]: 7%,178%) in 5-HETE, an important pro-inflammatory oxylinin. The HETE oxylinins were also positively associated with monoethyl phthalate, but not associated with other phthalate or OPE metabolites. In addition, the COX-derived oxylinin prostaglandin E2 (PGE2) was inversely associated with several chemicals from each class. Single-pollutant models showed a doubling of the OPEs diphenyl phosphate and bis(1,3-dichloro-2-propyl) phosphate was associated with 28% lower (95%CI: -42%,-12%) and 24% lower (95%CI: -38%,-6%) in PGE2, respectively.

**Conclusion:** We estimate that elevated phenol, phthalate, and OPE exposures are associated with changes in inflammation-related oxylinins, but that the direction of association varies between oxylinins based upon the biosynthetic pathway of production. These findings provide insight into impacts of environmental chemicals on specific pathways of maternal inflammation in pregnancy.

## T-238

**Recent Trends in Gestational Diabetes Based on Maternal Age, Race/Ethnicity, and Pre-Pregnancy Weight Categories.**

Darios Getahun,<sup>1</sup> Michael J Fassett,<sup>2</sup> Morgan R Peltier,<sup>3</sup> Chantal C Avila,<sup>4</sup> Xia Li,<sup>4</sup> Vicki Y Chiu,<sup>4</sup> David A Sacks.<sup>5</sup> <sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Los Angeles, CA, United States; <sup>3</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>4</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States.

**Introduction:** The objective of this study was to characterize the recent trends in gestational diabetes (GDM) by maternal age, race/ethnicity, and pre-pregnancy BMI in a large integrated healthcare system.

**Methods:** This population study used electronic health records of pregnancies delivered at the Kaiser Permanente Southern California healthcare system between 2009-2018 (n=379,866). GDM diagnosis was made using Carpenter-Coustan thresholds. We first examined the annual rate of GDM, followed by the adjusted trends by comparing rates in 2009-2010 vs 2017-2018. Adjusted relative risks were used to examine race/ethnicity, age, and BMI disparities in GDM diagnosis.

**Results:** GDM prevalence decreased from 10.8% in 2009-2010 to 9.8% in 2017-2018, an adjusted relative decrease (ARD) of 9%. The prevalence was highest in Asian/Pacific Islanders (17.2%), followed by Latinas (11.8%), Blacks (7.6%), and Whites (7.1%). Among Whites, GDM prevalence decreased from 7.5% (2009-2010) to 6.3% (2017-2018); ARD:25% (95%CI:18-31%), and this was driven by a decrease in the 30-34 age group (ARD:35%, 95%CI:25-43%). Among Blacks, the prevalence decreased from 7.8% (2009-2010) to 6.8% (2017-2018); ARD:24% (95%CI:11-35%), and this was largely driven by a decrease in the <30 age group (ARD:39%, 95%CI:20-53%). Among Latinas, the prevalence decreased from 12% (2009-2010) to 10.5% (2017-2018); ARD:23% (95%CI:19-26%), and this was largely driven by a decrease in the <30 age group (ARD:26%, 95%CI:19-31%). In contrast, among Asian/Pacific Islanders, the decrease was limited to women aged 30-34 years from 16.1% (2009-2010) to 14.8% (2017-2018), ARD:15% (95%CI:4-25%). Among Whites and Latinas normal, overweight, and obese women had significant decreases in GDM prevalence over time. GDM prevalence decreased in obese Blacks and normal weight Asian/Pacific Islanders.

**Conclusion:** This study shows a higher GDM prevalence rate among Asian/Pacific Islanders. The prevalence rate decreased in all racial/ethnic groups over the study period. The observed decrease among Blacks and Latinas was largely driven by a decrease at younger maternal age group and among Whites and Asian/Pacific Islanders among women aged 30-34 years. Substantial variance in the prevalence rate of GDM was observed across pre-pregnancy BMI categories.

## T-239

**Design and Methods of the Apple Women's Health Study: A Digital Longitudinal Cohort Study.**

Shruthi Mahalingaiah,<sup>1</sup> Victoria Fruh,<sup>1</sup> Erika Rodriguez,<sup>1</sup> Sai Charan Konanki,<sup>1</sup> Jukka-Pekka Onnela,<sup>1</sup> Alexis de Figueiredo Veiga,<sup>1</sup> Anne Marie Z Jukic,<sup>2</sup> Kelly K Ferguson,<sup>2</sup> Donna D Baird,<sup>2</sup> Allen J Wilcox,<sup>2</sup> Curry L Christine,<sup>3</sup> Suharwardy Sanaa,<sup>3</sup> Fischer-Colbrrie Tyler,<sup>3</sup> Agrawal Gracee,<sup>3</sup> Brent A Coull\*,<sup>1</sup> Russ B. Hauser\*,<sup>1</sup> Michelle A Williams\*.<sup>1</sup> <sup>1</sup>Harvard T.H. Chan School of Public Health, Boston, MA, United States; <sup>2</sup>National Institute of Environmental Health Sciences, Research Triangle Park, NC, United States; <sup>3</sup>Apple Inc., Cupertino, CA, United States.

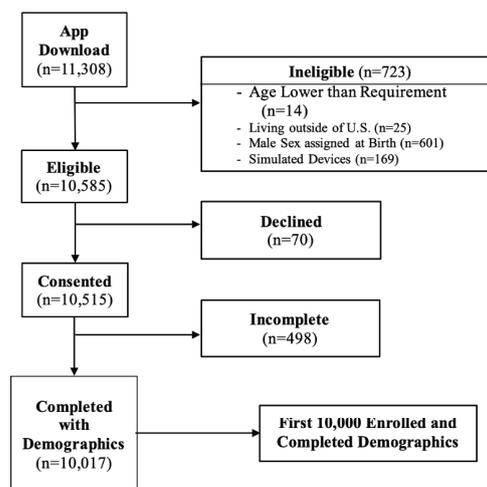
**Introduction:** The Apple Women's Health Study (AWHS) Cohort was designed to gain a deeper understanding of the relationship between menstrual cycles, health, and behaviors. This abstract describes the design and methods of the ongoing AWHS and provides demographic characteristics of the first 10,000 participants who meet inclusion criteria and the subset who responded to the 6-month follow-up.

**Methods:** This is a mobile-application-based longitudinal cohort study with survey and sensor-based data. Eligibility criteria include having

ever menstruated, installing the Apple Research app on iPhone with iOS version 13.2 or later, living in USA, age of 18 years or older in most states (19 years old in Alabama and Nebraska, 21 years old in Puerto Rico), proficiency in written and spoken English, sole user of an iCloud account or iPhone, and providing informed consent to participate in the study.

**Results:** We collected data from the 10,000 participants who responded to the demographics survey upon enrollment between November 14, 2019 and May 15, 2020. Participants were asked to complete a monthly follow-up through November 2020. Mean age at enrollment was 34 years old (+/- standard deviation 10.3). Race/ethnicity was representative of the U.S. population (69% White), while education was above average (51% had graduated college or beyond). Participant geographic distribution included all U.S. states and Puerto Rico. Seventy percent reported use of an Apple Watch, and 26% consented to sensor-based data collection. The majority of participants (54%) did not continue after enrollment. Non-White participants were slightly more likely to drop out than White participants; those remaining at 6 months were otherwise similar in demographic characteristics to the original enrollment group.

**Conclusion:** Because the AWHS can capture time-varying demographic, lifestyle, and behavioral data that is built into Research app that can interface with multiple data sources, it has strong potential to contribute to much new knowledge about long overlooked and understudied women's health issues.



## T-240

**Does Fetal Placental Weight Ratio Impact Childhood Growth of Term Newborns in a Low-Risk Community Based Setting?**

Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulska,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>5</sup> Michael Joyce,<sup>1</sup> Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jil-lamika Pongsachai,<sup>1,2</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*.<sup>1,2,6</sup> <sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New Rochelle, NY, United States; <sup>5</sup>Institute of Basic Research, Staten Island, NY, United States; <sup>6</sup>Institute for Basic Research, Staten Island, NY, United States.

**Introduction:** In a cohort based in the mid-last century, fetal placental weight ratio impacted childhood growth and blood pressure at age 7 years. We explore in a community based low risk modern cohort whether the fetal placental weight ratio varies with childhood growth centiles at 1 and 2 yrs of life.

**Methods:** Fetal placental weight ratio was calculated for 899 term singleton liveborns delivered between January 2010 and March 2015, and compared using Spearman's correlation to weight and length centiles calculated for measures at birth and ages 1 and 2 years.

**Results:** There were no correlations between the fetal placental weight ratio and weight and length centiles calculated at birth, or at ages 1 or 2

years. Similar lack of association was found in males compared to females. However, there was a significant association of fetal placental weight ratio with both birth and Year 1 growth centiles that was unique to Caucasian infants ( $p$ 's =0.04-0.015 v.  $p>0.20$  in other race/ethnicities). This effect was found to be due to associations of fetal placental weight ratio with growth centiles in female Caucasian infants.

**Conclusion:** We have previously demonstrated significant differences between associations of fetal placental weight ratio in males as compared to females. We speculated that males are less responsive than females to prenatal "cues" signaled by the placenta to modulate fetal growth. Our data suggest that this female-specific "responsiveness" to intrauterine environmental cues has effects on childhood growth that persist to at least age 2 years. This may carry implications for sensitivity of female children to environmental cue in early life that contribute to developmental programming.

#### T-241

##### Early Childhood Growth in Autism Spectrum Disorders: A Case Control Study.

Mehrin Jan,<sup>1,2</sup> Serena Chen,<sup>1,2</sup> Christine Chen,<sup>1,2</sup> Jillamika Pong-sachai,<sup>1,2</sup> Jennifer S Feng†,<sup>1,2,3</sup> Joan Krickellas,<sup>1,2</sup> Sadia F Chowdhury†,<sup>1,2,3</sup> Adwoa Nantwi†,<sup>4</sup> Sylvia Dygulska,<sup>1,2</sup> Hannah Bromberg,<sup>1,2</sup> Ruchit Shah,<sup>5,1</sup> Michael Joyce,<sup>1</sup> Beata Dygulska,<sup>2</sup> Carolyn Salafia\*,<sup>1,2,5</sup>  
<sup>1</sup>Placental Analytics, New Rochelle, NY, United States; <sup>2</sup>NYPBMH, Brooklyn, NY, United States; <sup>3</sup>CUNY Hunter College, New York, NY, United States; <sup>4</sup>NYU CGPH, New York, NY, United States; <sup>5</sup>Institute for Basic Research, Staten Island, NY, United States.

**Introduction:** Early growth in children subsequently diagnosed with autism spectrum disorders (ASD) is infrequently studied in low risk community based samples. We here compare change in centiles for weight and length to age 2 years in ASD versus gestational age, sex, and season of birth matched controls.

**Methods:** A community hospital based sample with universal placental examination was searched for those births followed to at least age 2 years at our institution. Billing codes were searched for diagnoses related to autism spectrum disorder (ASD) among the patient population of the Department of Pediatrics Developmental Pediatrics group. At least 2 diagnoses related to ASD as per Newschaffer et al were required to be considered an ASD case. Controls were selected from the next infant born of same gender, gestational age +/-2 weeks, and season of birth +/- 2 weeks. 165 ASD and 617 controls were included in the current analysis. Due to nonnormal distribution of growth variables, non-parametric tests were used.

**Results:** At birth, a trend to increased head circumference centile ( $p=0.06$ ) was observed but no other differences between cases and controls. While mean weight centile at Year 1 decreased 5% from birth in ASD (compared to an increase of 5% in controls), there was a broad range of weight centile changes that precluded statistical significance. No differences in growth centiles at ages 1 or 2 years were observed between ASD cases and controls. No sex differences in birth or age 1 or 2-year growth centiles were found in controls. but ASD males tended to reduced head circumference and length centile (each  $p=0.08$ ).

**Conclusion:** In this large population sample of ASD with matched controls, little difference in childhood growth measures, either as absolute values or as rates of change, are seen in ASD cases. Our data suggest reduced growth in ASD males, but further studies are required to confirm and examine the implications of reduced growth in ASD males.

#### T-242

##### Periconceptional Maternal Obesity and Underweight Have a Negative Impact on Post-Implantation Embryonic Growth and Developmental Trajectories: The Rotterdam Periconception Cohort.

Linette van Duijn†, Melek Rousian\*, Joop SE Laven\*, Régine PM Steegers-Theunissen\*. Erasmus University Medical Centre, Rotterdam, Netherlands.

**Introduction:** Globally, the prevalence of extremes in body mass index (BMI) have risen rapidly over the past few decades. Besides numerous non-communicable diseases, these extremes are also associated with

severe pregnancy complications. Although complications are generally detected in the second and third trimester of pregnancy, most of them originate in the periconception period, during which embryogenesis takes place. Moreover, fetal sex also impacts pregnancy course and outcome. Therefore, our aim is to study (sex-specific) associations between periconceptional maternal BMI and embryonic growth and morphological development.

**Methods:** A total of 884 women with singleton pregnancies were selected from the Rotterdam Periconception Cohort, comprising 15 women with underweight, 483 with normal weight, 231 with overweight and 155 with obesity. Longitudinal three-dimensional ultrasound examinations were performed at 7, 9 and 11 weeks of gestation for offline measurements to estimate embryonic growth, i.e., crown-rump length (CRL), embryonic volume (EV), and morphological development, i.e., Carnegie stages, using virtual reality techniques. Linear mixed models were used to study associations between maternal periconceptional BMI and first-trimester growth and developmental trajectories, and stratified for fetal sex. Adjustments were made for gestational age and maternal characteristics.

**Results:** Compared with pregnancies of normal weight women, a negative trend in embryonic growth was observed in pregnancies of obese women ( $\beta_{EV}$  -0.03 (95%CI -0.06, 0.00,  $p=0.086$ ), whereas embryonic growth and morphological development in overweight women were comparable. Maternal underweight was associated with faster morphological development ( $\beta_{Carnegie}$  0.78 (95%CI 0.25, 1.31),  $p=0.004$ ). Moreover, after stratification for fetal sex, female embryos of underweight women grow and morphologically develop faster than those of normal weight women ( $\beta_{EV}$  0.13 (95%CI 0.03, 0.23),  $p=0.008$ ;  $\beta_{Carnegie}$  1.39,  $p<0.001$ ), whereas female embryos of obese women grow slower ( $\beta_{EV}$  -0.05 (95%CI -0.09, -0.01),  $p=0.027$ ).

**Conclusion:** Periconceptional maternal underweight is associated with faster morphological development and embryonic growth, especially in females. In contrast, female embryos of obese women grow slower than those of women with normal weight. These results may be explained by sex specific adaptation to the prenatal environment. Future research should focus on strategies for personalisation of optimal preconceptional maternal weight in order to reduce BMI-related pregnancy complications and improve the health of future generations.

#### T-243

##### Knowledge May Not Be the Barrier to Care for Pregnant Women with Opioid Use Disorder: The ACOG District II Education Bundle.

Neil Seligman,<sup>1</sup> Kathleen Dermady,<sup>2</sup> David Garry,<sup>3</sup> Leah Kaufman,<sup>2</sup> Darcy Dreyer,<sup>4</sup> Marilyn Kacica,<sup>5</sup> Cassie Leonard,<sup>6</sup> Kelly Gilchrist,<sup>7</sup> Christa Christakis.<sup>7</sup> <sup>1</sup>Univ. of Rochester, Rochester, NY, United States; <sup>2</sup>Upstate Medical Center, Syracuse, NY, United States; <sup>3</sup>Stony Brook Medicine, Stony Brook, NY, United States; <sup>4</sup>March of Dimes, Rochester, NY, United States; <sup>5</sup>NYS DOH, Albany, NY, United States; <sup>6</sup>Hudson Headwaters Health Network, Queensbury, NY, United States; <sup>7</sup>ACOG District II, Albany, NY, United States.

**Introduction:** Opioid use disorder (OUD) is one of the most important public health issues affecting pregnant women in the 21st century yet 62% of Ob/Gyns in New York State feel there is not adequate training and resources in their area/region to manage pregnant women with OUD. In 2017, ACOG District II (DII) convened a multidisciplinary task force to create a comprehensive education "Bundle" to increase awareness of evidence-based guidelines. The objective was to assess the effectiveness of the ACOG DII OUD in Pregnancy Bundle to improve provider knowledge.

**Methods:** From 5/2018-9/2019, the Bundle was presented on request by a task force member. A pre- and post-presentation test containing 8 items assessing basic knowledge of OUD management and 2 items assessing knowledge about local hospital policies was administered using Mentimeter (Stockholm, Sweden). Baseline site characteristics were described. Test performance was assessed pre- and post-presentation.

**Results:** The bundle was presented at 8 hospitals. Most (88%) were community hospitals and only 2 had an Ob/Gyn residency. Delivery volume ranged from 454-8,389 births/yr with most (63%) having >1,000 births/yr. 101 and 66 respondents answered the pre- and post-presentation questions respectively. Of the 8 basic knowledge items, the average correct

response rate was 71% (range 42% to 91%) pre-presentation and increased by 12% (range -7% to 41%) to 83% (range 65% to 95%) post-presentation. The greatest improvement was in knowledge of recommended OUD screening methods. Only minimal (<10%) improvement was observed in 5 of 8 items (63%). Most respondents (67% to 77%) were either unsure or stated no specific protocols exist for screening and referral to treatment for OUD in pregnancy at their hospital. 90% were motivated by the presentation to educate themselves about current hospital screening policies.

**Conclusion:** Basic knowledge of OUD was generally good with only modest improvement (12%) post-presentation but, we are optimistic that 90% of those individuals are motivated to educate themselves. Awareness, and/or lack, of policies appears to be the biggest challenge to caring for pregnant women with OUD. Optimal care of these patients must address complexities not common to other chronic medical conditions; efforts should be targeted toward ensuring readiness for every patient in every clinical setting focusing on best practices such as SBIRT, access to medication-assisted treatment, and CAPTA legislation requirements to name a few.

### T-244

#### Trends in Emergency Department Visits among Reproductive Age Women in the United States, 2006-2018.

Marissa S Weiss,<sup>1</sup> Li Jiang,<sup>1</sup> Courtney Townsel,<sup>1</sup> Martina T Caldwell,<sup>2</sup> Dee Fenner,<sup>1</sup> Erica E Marsh\*.<sup>1</sup> <sup>1</sup>University of Michigan, Ann Arbor, MI, United States; <sup>2</sup>Henry Ford Health System, Detroit, MI, United States.

**Introduction:** Emergency Department (ED) utilization continues to increase with a 21.5% volume change from 2006 to 2018. In 2018 alone, there were 143.4 million ED visits in the United States, with reproductive age women (15-44 years old, per CDC definition) comprising 23.5% (n=33,669,997) of all visits. To better understand reasons for high utilization in this patient population, we investigated trends in ED utilization for both OBGYN-specific and non-OBGYN diagnoses over a 13-year period.

**Methods:** The Nationwide Emergency Department Sample, Healthcare Cost and Utilization Project was queried for all ED visits in reproductive aged women from 2006 to 2018. The most frequent non-OBGYN diagnoses (by ICD9-CM and ICD10-CM codes), as well as the most common OBGYN-related diagnoses, were identified. National estimated numbers of ED visits, demographic factors, admission data, and hospital characteristics were explored for each diagnosis.

**Results:** The number of ED visits among reproductive age women increased by 11.6% from 2006-2018 (30,180,837 vs 33,669,997;  $P<.01$ ). The most common primary diagnoses for non-OBGYN related visits were: Abdominal pain (1,985,523 visits in 2018), acute upper respiratory tract infection (1,508,558), urinary tract infection (1,249,307), chest pain (1,200,426), and headache (710,478). The most frequent OBGYN diagnoses were: Unspecified pregnancy related conditions (773,682 of visits in 2018), hemorrhage in early pregnancy (558,425), disorders of menstruation and other abnormal bleeding (414,551), pelvic and perineal pain (314,124), and excessive vomiting of pregnancy (298,117). The number of visits for the most common OBGYN diagnoses also increased during this 13-year period (2,110,889 vs 2,358,899;  $P<.01$ ) and in 2018 only 23,951 (1%) of these visits resulted in inpatient admission.

**Conclusion:** Reproductive age women currently comprise 23.5% of all ED visits, with an 11.6% increase volume over the past 13 years. While these visits are most commonly for non-OBGYN related diagnoses, a significant percentage are for OBGYN complaints. The five most common OBGYN-related diagnoses represented 7% of all visits, though this may be an underestimate since abdominal pain diagnoses often have OBGYN etiologies. These diagnoses include common complications of the first trimester of pregnancy and general gynecologic issues typically managed in the outpatient setting. High ED utilization for these conditions, coupled with low rates of admission, suggest an opportunity to optimize outpatient management for common OBGYN conditions to prevent further overburdening of the ED system.

### T-245

#### Comparison of Ovarian Aging Markers Does Not Reveal Differences between Black and White Women.

Hannah Anvari†, Kara Goldman\*, Mary Ellen G. Pavone\*, Jian-Jun Wei\*, Melissa Simon\*, Francesca Duncan\*. Northwestern University, Chicago, IL, United States.

**Introduction:** The weathering hypothesis is a framework positing that non-Hispanic Black (NHB) women experience decreased health outcomes due to cumulative social and economic disadvantage. Higher allostatic loads - or wear and tear - from lifetime exposure to stress in a race-conscious society can also accelerate aging. Whether racial disparities exist with respect to ovarian aging has not been systematically evaluated.

**Methods:** Ovarian aging is associated with decreased numbers of follicles, increased stromal inflammation and fibrosis. To determine if an association between race and ovarian aging exists, we used fixed human ovarian cortex tissue from a research archive established by the Oncofertility Consortium. Samples were from age-matched NHB and non-Hispanic white (NHW) women (2, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 18, 20, 22, 28, 29, 34 years, N=23 NHB and N=52 NHW) undergoing ovarian tissue cryopreservation (OTC) for hematologic malignancies, solid tumors, or hematologic, gynecologic and rheumatologic disorders. We counted follicles (primordial, primary, secondary, early antral) on H&E stained sections, and reported average follicle number per ovarian area. We further constructed an ovarian tissue microarray (TMA) with tissues from these participants to evaluate differences in stromal markers by histochemical analysis and immunohistochemistry (collagen, vimentin, alpha-smooth muscle actin, desmin, CD68, CD163, and CD34). Picrosirius Red (PSR), specific for collagen I and III fibers, was used to evaluate ovarian fibrosis. We quantified the area positive for PSR staining and reported percent fibrotic area relative to area of ovarian core. We then compared the profiles of 80 fibroinflammatory cytokines in follicular fluid (FF) from the Northwestern University Reproductive Tissue Library (RTL) using cytokine antibody arrays. FF samples were collected from age-matched NHB and NHW women (22, 23, 30, 32, 35, 36, 37, 38, 40, 41, 43, 44 years, N=9 NHB and N=8 NHW) undergoing oocyte retrieval for infertility diagnoses (unexplained, social, diminished ovarian reserve, or polycystic ovarian syndrome).

**Results:** We observed no significant differences in average follicle number in any class by race, but there was a tendency for follicle counts to be higher in NHW females aged 6-9, 10-14, 15-18, 19-22, and 23-34 years. PSR staining for collagen was highest among NHB and NHW females aged 2-11 years relative to 11-16 or 17-34 years, but levels did not vary by race. Analysis of other stromal markers is ongoing. Levels of fibroinflammatory cytokine profiles of FF did not differ among age-matched NHB and NHW females.

**Conclusion:** Our results do not reveal obvious racial disparities in ovarian aging markers, however our population included females with oncologic diagnoses that may not be representative of the general population, therefore future studies are warranted.

### T-246

#### Characterization of Human Uterine Leiomyoma-Derived Exosomes and Its Impact on Endometrium.

Antonia Navarro, Maria Victoria Bariani†, Hang-Soo Park†, Ayman Al-Hendy\*. University of Chicago, Chicago, IL, United States.

**Introduction:** Uterine fibroids (UF) are the most common benign pelvic tumors in women of reproductive age, they cause heavy menstrual bleeding (HMB), leading to severe anemia and subsequent major negative effects on quality of life. The aim of this study is to investigate the effects of human uterine leiomyoma smooth muscle cells (HULM) and human uterine smooth muscle cells (UTSM)-derived exosomes on growth and angiogenesis of the human endometrium-related endothelial cells. In addition, to characterize the miRNA profiling of HULM- and UTSM-derived exosomes using NGS.

**Methods:** We isolated and characterized HULM- and UTSM-derived exosomes. Then, we performed in vitro studies treating human endometrial microvascular endothelial cells (HEMEC) with HULM- and UTSM-derived exosomes to understand the molecular mechanisms by which

uterine fibroids mediates their proliferative and angiogenic properties that ultimately cause HMB. We also performed RNA-seq to obtain the miRNA signature of HULM- and UTSM-derived exosomes. We treated HEMEC cells with HULM- and UTSM-derived exosomes at several time points and different exosome concentrations. Then, we measured cell proliferation via BrdU incorporation assay; we checked mRNA expression of proliferation and angiogenic markers such as PCNA, C-Myc and VEGFA by real-time PCR. Additionally, we performed tube formation assay and RNA-seq of HULM- and UTSM-derived exosomes.

**Results:** We observed that HULM-derived exosomes cocultured with HEMEC increased cell proliferation by 60% ( $P < 0.01$ ), and this increase is more significant culturing it for 48 hrs. We confirmed that this effect is associated with the upregulation of specific markers for proliferation (PCNA and C-Myc) and angiogenesis (VEGFA). HEMEC cells treated with HULM-derived exosomes showed increased in tube formation length by tube formation assay. MiRNA profiles of HULM- and UTSM-derived exosomes demonstrated that 84 miRNA were significantly downregulated and 71 significantly upregulated in HULM-derived exosomes compared to UTSM-derived exosomes. Interestingly, some of the upregulated miRNAs are associated with endometrial receptivity and implantation.

**Conclusion:** Our study suggests that HULM-derived exosomes have strong paracrine effects on human endometrial microvascular endothelial cells, likely containing bioactive factors that enhance endometrial proliferation and angiogenesis, which eventually cause HMB. Further characterization of these factors can lead to novel druggable therapeutic targets to improve health care for women with uterine fibroids associated heavy menstrual bleeding.

Keywords (3): uterine fibroids, exosomes, endometrium

#### T-247

##### Higher Prevalence of SGA in Highly Vulnerable Women the Mothers of Rotterdam Study.

Kajal SC Mohabier, Hanneke JP de Graaf, Eric AP Steegers, Loes CM Bertens. *Erasmus University Medical Center, Rotterdam, Netherlands.*

**Introduction:** Rotterdam, the second largest city of the Netherlands, with many deprived neighborhoods and considerable inequalities in perinatal health. Socioeconomic disadvantage (low SES) is associated with ill health and exposure to chronic stress. Stress on its own also affects ill health. These factors make people with low SES more vulnerable for adverse outcomes. In case of pregnancy, both the health of the mother and the child is affected by the circumstances, making these women highly vulnerable. Objective: To examine perinatal outcomes in highly vulnerable pregnant women within the Mothers of Rotterdam (MoR) study compared to the Netherlands in general.

**Methods:** The MoR- study is a pragmatic prospective cohort study evaluating social care and health outcomes for highly vulnerable pregnant women and their offspring.

Vulnerability was assessed by filling out a checklist, and eligibility was defined as: at least 3 problems from a total of 47 problems divided over at least 2 problem domains (i.e. pregnancy, residence, administration and finance, work and education, parenting, health, social functioning, safety and crime). Records on maternal and neonatal outcomes were obtained from obstetric care professionals. Outcome measures were prevalence rates of preterm birth, small for gestational age (SGA) and perinatal mortality. Crude prevalence rates (per 1000) and 95% confidence intervals (95%CI) were calculated for the MoR-group and the Netherlands. Data from the national birth registry (2015-2018) was used to calculate prevalence rates for the Netherlands. Subsequently, all prevalence rates were standardized for maternal age, parity, and living in a deprived neighbourhood, using direct standardization.

**Results:** 235 women were included in the study and the crude prevalence rates were 47.21 [19.98;74.44] for preterm birth, 220.78 [167.29;274.27] for SGA, and 4.34 [-4.16;12.86] for perinatal mortality. The prevalence rate of SGA was higher in the MoR-group compared to the Netherlands (table 1). The prevalence rates for preterm birth and perinatal mortality were lower in the MoR-group, but with overlapping 95%CI (table 1). Direct standardization did not change the comparison between the MoR-group and the Netherlands.

**Conclusion:** SGA was more often found in the group of highly vulnerable pregnant women compared to the Netherlands. Although preterm birth and perinatal mortality was not more prevalent in the MoR-group, awareness of social risk factors in pregnancies is important to anticipate on adverse outcomes.

	Mothers of Rotterdam	The Netherlands
Preterm birth	47.21 [19.98 ; 74.44]	69.01 [68.40 ; 69.62]
Small for gestational age	220.78 [167.29 ; 274.27]	106.28 [105.54 ; 107.03]
Perinatal mortality	4.34 [-4.16 ; 12.86]	7.17 [6.96 ; 7.37]

#### T-248

##### Association between SARS-CoV-2 Infection and Adverse Pre- and Postnatal Outcomes by Severity of Illness.

Michael J Fassett\*,<sup>1</sup> Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> David A Sacks,<sup>4</sup> Jiaxiao Shi,<sup>5</sup> Vicki Y Chiu,<sup>5</sup> Morgan R Peltier,<sup>6</sup> Darios Geta-hun,<sup>7</sup> <sup>1</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente West Los Angeles Medical Center; Keck School of Medicine, Los Angeles, CA, United States; <sup>5</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>6</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>7</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.

**Introduction:** To examine the association between SARS-CoV-2 infection during pregnancy and adverse pre-and postnatal outcomes by severity of illness.

**Methods:** A retrospective cohort study using data on 29,323 pregnant women extracted from the KPSC electronic health records between 04/6/2020-12/31/2020. SARS-CoV-2 infection was ascertained by PCR-based test that was universally offered to all women. Outcomes were ascertained from the EHRs. Diagnosis of acute respiratory disease (ARD) at the time of, or soon after COVID-19 diagnosis was used as a proxy for disease severity. Multivariable logistic regression was used to estimate the odds ratios (OR).

**Results:** Women with ARD related to COVID-19 were at significantly increased risk for preeclampsia (adj.OR: 1.95, 95%CI:1.08, 3.53), preterm birth (PTB) (adj.OR:2.00, 95%CI:1.08-3.71) especially spontaneous PTB (adj.OR:2.33, 95%CI:1.06- 5.08), and ICU admission (adj.OR:40.25, 95%CI:14.71-110) than women who tested positive but did not have acute respiratory disease related to COVID-19. Offspring of women with ARD were at increased risk of abnormal fetal heart rate pattern (adj.OR:1.82, 95%CI:1.16-2.86), congenital anomalies (adj.OR:10.64, 95%CI:4.75-23.81), NICU admission (adj.OR:2.47, 95%CI:1.42-4.30), and RDS (adj.OR:2.37, 95%CI:0.95-5.90) compared with those infected with SARS-CoV-2 but without respiratory illness. Women with COVID-19 exposure but without ARD were only at increased risk for Apgar score <7 (adj.OR:1.79, 95%CI:1.07-3.00) and neonatal SARS-CoV-2 infection (adj.OR:8.16, 95%CI:4.51-14.77), compared with women without COVID-19 diagnosis.

**Conclusion:** Most adverse pregnancy outcomes associated with SARS-CoV-2 are associated with the respiratory disease caused by the virus. However, even in the absence of maternal ARD, SARS-CoV-2 infection still increased the risk of a low Apgar score. This suggests that there are clinical as well as subclinical associations of SARS-CoV-2 infection with risks of adverse pregnancy outcomes.

**T-249****Association between SARS-CoV-2 Infection and Adverse Pre- and Postnatal Outcomes by the Trimester of Diagnosis.**

Darios Getahun<sup>1</sup>, Lawrence D Lurvey,<sup>2</sup> David Braun,<sup>3</sup> Sacks A Sacks,<sup>4</sup> Alex Fong,<sup>5</sup> Neha Trivedi,<sup>6</sup> Jiaxiao Shi,<sup>7</sup> Vicki Y Chiu,<sup>7</sup> Morgan R Peltier,<sup>8</sup> Michael J Fassett.<sup>9</sup> <sup>1</sup>Kaiser Permanente Southern California; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States; <sup>2</sup>Kaiser Permanente West Los Angeles, Los Angeles, CA, United States; <sup>3</sup>Kaiser Permanente Los Angeles, Los Angeles, CA, United States; <sup>4</sup>Kaiser Permanente Southern California; Keck School of Medicine, Pasadena, CA, United States; <sup>5</sup>Kaiser Permanente Irvine Medical Center, Irvine, CA, United States; <sup>6</sup>Kaiser Permanente San Diego Medical Center, San Diego, CA, United States; <sup>7</sup>Kaiser Permanente Southern California, Pasadena, CA, United States; <sup>8</sup>NYU-Long Island School of Medicine, Mineola, NY, United States; <sup>9</sup>Kaiser Permanente West Los Angeles Medical Center; Kaiser Permanente Bernard J. Tyson School of Medicine, Pasadena, CA, United States.

**Introduction:** To examine how the association between SARS-CoV-2 infection during pregnancy and adverse pre-and postnatal outcomes is affected by timing of diagnosis.

**Methods:** A retrospective cohort study was conducted using data from 29,323 pregnant women extracted from the Kaiser Permanente Southern California electronic health records (04/06/2020-12/31/2020). SARS-CoV-2 infection was ascertained from PCR-based test results that was universally offered to all women. Outcomes were ascertained from the EHRs. Multivariable logistic regression was used to estimate the odds ratios (OR).

**Results:** The incidence of COVID-19 diagnosis during the study period was 4% (1,185/29,323 singleton pregnancies). Women who tested positive for SARS-CoV-2 infection in their 1<sup>st</sup>/2<sup>nd</sup> trimester were at risk of GDM (adj.OR:1.37, 95%CI:1.02-1.86), preterm birth (PTB) (adj. OR:1.81, 95%CI:1.28-2.57), and stillbirth (adj.OR:3.13, 95%CI:1.27-7.75), compared to those who tested negative in all trimesters. The risk of PTB among those infected in 1st or 2nd trimester was largely driven by indicated PTB (adj.OR:2.15, 95%CI:1.35-3.43). Compared to those who tested negative in the 3rd trimester, women who tested positive in their third trimester were at significantly increased risk of ICU admission (adj.OR: 8.64, 95%CI:3.80-19.68). All neonates born to Covid-19 positive mothers were at increased risk for Apgar score <7 (adj.OR:1.96, 95%CI:1.09-3.55) and to test positive for SARS-CoV-2 infection (adj.OR: 10.59, 95%CI:5.84-19.18) after the first day of life, compared to neonates born to women without Covid-19 diagnosis.

**Conclusion:** Women who tested positive for SARS-CoV-2 infection during the 1<sup>st</sup>/2<sup>nd</sup> trimester are at increased risk for PTB and stillbirth. The higher risk of GDM associated with SARS-CoV-2 infection in early trimester of a pregnancy highlights the importance of antenatal counseling and testing for glucose intolerance during pregnancies of infected women.

**T-250****Increased Fetal Demise/Stillbirth within a USA COVID-19 Epicenter.**

Ryan Zahn<sup>†</sup>,<sup>1</sup> Hadeer Eltahan<sup>†</sup>,<sup>1</sup> Debra Heller\*,<sup>2</sup> Nichole Cerone<sup>†</sup>,<sup>1</sup> Themba Nyirenda\*,<sup>1</sup> Judy Urgo\*,<sup>1</sup> Emre Kayaalp\*,<sup>1</sup> Manuel Alvarez\*,<sup>1</sup> Stacy Zamudio\*,<sup>1</sup> Abdulla Al-Khan\*,<sup>1</sup> <sup>1</sup>Hackensack University MC, Hackensack, NJ, United States; <sup>2</sup>Rutgers University, Newark, NJ, United States.

**Introduction:** The Covid-19 pandemic has been associated with an increase in rates of fetal demise and stillbirth. Recent reports implicate both direct biological effects related to SARS-CoV-2 infection, as well as those related to economic and social disruption with subsequent impacts on medical care availability and quality. By May 2020 there subjectively appeared to be a rise in fetal demise/stillbirth at our Institution, located within an outbreak epicenter (Bergen County NJ USA). We tested this hypothesis by comparing data from fetal demise/stillbirths in 2018, 2019 and 2020.

**Methods:** Cases were defined as a 2nd (14<sup>+</sup>-27<sup>+</sup> wks GA) or 3rd trimester (28 wks) fetal demise (less than 24<sup>+</sup> wk) or stillbirth (greater than 24 wk). The EMR was abstracted for demographic data, maternal pre- and pregnancy-associated co-morbidities, diagnoses and placental pathology.

Placental pathological analyses were performed by one expert placental pathologist; she was among the first to document COVID-19 effects in the placenta (DH). There were 53 (2018), 61 (2019) and 79 (2020) cases investigated. Rates of fetal demise by year were compared using Poisson regression. Demographic and clinical variables were compared using parametric (ANOVA) and non-parametric approaches (Kruskal-Wallis, Chi Square) as appropriate.

**Results:** The 2020 fetal demise/stillbirth rate (1.43%) was elevated relative to 2018 (0.84%) and 2019 (0.91% P<0.001). The relative risk (RR) for 2020 demise is 1.77 (95% CI 1.23, 2.54) compared with 2018 and 1.64 (95% CI 1.14, 2.35) with 2019. Rates of demise among pre-viable fetuses (14-23<sup>+</sup> wks) did not differ across years. Late 2nd trimester stillbirth at GA 24<sup>+</sup> - 27<sup>+</sup> wks increased 5-fold in 2020 (rate = 0.12% P<0.01) relative to 2018 (0.03%) and 2019 (0.02%). Third trimester (>28<sup>+</sup> wks) stillbirth rates at 0.22% (2018), 0.13% (2019) and 0.20% (2020) did not differ. Likely cause of death was identified in ~65% of cases for each year. Placental pathology contributory to mortality was identified in >50% of all cases (p=NS between years). However no specific diagnosis or category of placental pathology differed between years. Only 3/79 of the 2020 cases had SARS-CoV-2 infection as a preceding or concurrent diagnosis.

**Conclusion:** Our tertiary care Institution, averaging 6000 births/yr, and located within a pandemic epicenter March-June 2020, experienced an increase in fetal demise/stillbirth rates during the COVID-19 pandemic. This was driven by late 2nd trimester intrauterine deaths. Our current data do not support a direct association with SARS-CoV-2 infection. Social and economic disruption, with consequent effects on health care access/quality may be contributory.

**T-251****SARS-CoV-2 Colonization of Maternal and Fetal Cells of the Human Placenta Promotes Alteration of Local Renin-Angiotensin System.**

Sonam Verma<sup>†</sup>, Ebony B Carter, Indira U Mysorekar\*. *Washington University SOM, St. Louis, MO, United States.*

**Introduction:** The pandemic caused by the Beta-coronavirus SARS-CoV-2 has wreaked havoc in people of all ages. The rate of severe COVID-19 infection appears to increase the risk of adverse pregnancy outcomes in pregnant women such as preeclampsia, but the mechanism by which this difference occurs remains unclear. Here, we explored the pathophysiology of SARS-CoV-2 at the human maternal-fetal interface in pregnant women who tested positive for the virus.

**Methods:** Placental tissues were acquired at delivery from pregnant women who tested positive for SARS COV2 as well as sera pre- and post delivery. RNA In situ hybridization and Immunohistochemistry was used to localize the viral RNA or Spike protein in placental tissue as well as ACE receptor to determine impact of viral infection. The choriocarcinoma cell line, JEG3, was treated with recombinant Spike protein or a chimeric virus, VSV-SARSCoV2-S and viral impact on ACE2 was measured via Western Blotting. Finally, sera from COVID19+ and - women was analyzed for markers associated with preeclampsia.

**Results:** We show that SARS-CoV-2 colonizes multiple compartments at the human maternal-fetal interface. Viral colonization was highest in the maternal decidua, fetal villous and extravillous trophoblasts, Hofbauer cells, and in placentas delivered prematurely. We localized SARS-CoV-2 to cells expressing Angiotensin-converting enzyme 2 (ACE2), the cellular receptor for the SARS-CoV-2 Spike (S) protein and demonstrate that infected placentas had significantly reduced ACE2. ACE2 is a key enzyme in the circulating renin-angiotensin system (RAS), promoting Angiotensin-II binding to its receptor AT<sub>1</sub>R. To uncover whether viral infection alters the RAS in placenta, we infected human trophoblasts with either recombinant S protein or a live modified virus with a vesicular stomatitis viral backbone expressing S protein. In both cases, cellular ACE2 decreased while AT<sub>1</sub>R increased with concomitant increase in the soluble variant of VEGFR1 (soluble fms-like tyrosine kinase-1, sFlt1), an antagonist of angiogenesis. Furthermore, viral infection decreased pro-angiogenic factors, AT<sub>2</sub>R and Placental growth factor, which competitively binds to sFlt1. Finally, we demonstrate that sera from SARS-CoV-2

infected pregnant women had elevated levels of sFlt1 and Angiotensin II type 1-Receptor Autoantibody (AT<sub>1</sub>-AA) prior to delivery, both signatory markers of preeclampsia.

**Conclusion:** In sum, we demonstrate that SARS-CoV-2 colonizes ACE2-expressing fetal trophoblasts, stromal cells, and macrophages in the placenta and that SARS-CoV-2 infection in pregnant women correlates with dysregulation of the normal placental RAS. As RAS regulates blood pressure, SARS-CoV-2 may thus increase adverse hemodynamic outcomes such as preeclampsia in pregnant women.

## T-252

### COVID-19 in Pregnancy: Placental Histopathology Demonstrates Evidence of Acute Infection.

Courtney Olson-Chen\*, Ponnilla Marinescu†, Stefanie Hollenbach, Eva K Pressman, Philip Katzman. *University of Rochester, Rochester, NY, United States.*

**Introduction:** COVID-19 infection in pregnancy has been associated with placental features of vascular malperfusion; however, COVID-19 may also contribute to placental inflammation and infection. We sought to further investigate placental histopathology in pregnant patients diagnosed with COVID-19, focusing on acute infection.

**Methods:** This is a retrospective cohort study of pregnant subjects diagnosed with COVID-19 from March 1, 2020 to February 15, 2021 in a quaternary care center. The electronic medical record was used to identify COVID-19 infection details and placental histopathology results. Descriptive statistics were performed for the collective cohort. We also compared the placental pathology findings in cases of COVID-19 diagnosed on admission to the hospital for delivery with findings in cases of COVID-19 diagnosed earlier in the pregnancy.

**Results:** There were a total of 141 cases of COVID-19 in pregnancy with 106 deliveries having occurred at the time of the study. Placental tissue was submitted for histopathologic evaluation after delivery in 59 (55.7%) subjects. COVID-19 diagnosis occurred at admission for delivery in 33 (55.9%) cases, and 30 (50.8%) subjects were symptomatic at diagnosis. Of the 59 placentas evaluated, 31 (52.5%) were found to have evidence of acute infection, either acute subchorionitis or acute chorioamnionitis. Those with COVID-19 infection diagnosed on admission for delivery were 3.8 times more likely to have evidence of acute placental infection as compared to those diagnosed earlier in the pregnancy (95% CI 1.3, 11). The average gestational age at COVID-19 diagnosis was 35 weeks in those with acute placental infection compared to 31.5 weeks in those without acute placental infection ( $P=0.01$ ). Notably, no subjects with evidence of acute infection on histopathology had clinical evidence of chorioamnionitis.

**Conclusion:** Despite lack of clinical symptoms of chorioamnionitis, placental histopathology demonstrates evidence of acute infection in patients with COVID-19 in pregnancy. Placental infection is more common in subjects with a recent COVID-19 diagnosis. Histopathologic evaluation in cases of COVID-19 should be encouraged to further characterize markers of inflammation and infection and to better understand the potential impact of such placental pathology on fetal and neonatal well-being.

## T-253

### Characteristics, Risk Factors, and Outcomes for Pregnant and Postpartum Patients with COVID-19 Disease.

Felicia LeMoine†, Kaitlyn Taylor†, Elizabeth Sutton\*. <sup>1</sup>LSUSOM OBGYN Residency, Baton Rouge, LA, United States; <sup>2</sup>Woman's Hospital, Baton Rouge, LA, United States.

**Introduction:** Since declaration as a global pandemic on March 11, 2020, SARS-CoV2 infection, or coronavirus disease 2019 (COVID-19), has been confirmed in over 16 million people within the United States with a 1.8% mortality rate as of December 2020. Over 47,000 of said cases have been confirmed among pregnant women, emphasizing the importance of studying the effects of COVID-19 in this population. The aim of this study is to describe the demographics, risk factors, and outcomes of pregnant

and postpartum patients (i.e., delivery within 6 weeks from initial positive SARS-CoV2 nasopharyngeal PCR test) with a history of SARS-CoV-2 infection/COVID-19 disease.

**Methods:** We performed a retrospective chart review of electronic health records for pregnant and postpartum patients with positive SARS-CoV2 nasopharyngeal PCR testing at Woman's Hospital in Baton Rouge, Louisiana between March 11, 2020 to December 3, 2020. Demographic, pre-existing medical and obstetrics characteristics along with both obstetrical and neonatal outcomes were assessed. Patients were grouped according to severity of disease course (asymptomatic-mild versus severe-critical). Chi-square tests and ANOVA were used to compare categorical and numerical demographic, medical, and obstetrical characteristics as well as obstetrical and neonatal outcomes between groups.

**Results:** A total of 131 pregnant (n=124) and postpartum (n=7) patients with positive SARS-CoV2 testing were included, of which 96 (73.2%) were asymptomatic or experienced mild COVID-19 disease and 35 (26.7%) experienced severe or critical COVID-19 disease. Patients with severe-critical COVID-19 disease were more likely to be African American ( $p = 0.03$ ), have obesity ( $p < 0.01$ ), use Medicaid insurance ( $p < 0.01$ ), be multiparous ( $p=0.01$ ), have the diagnosis of gestational diabetes in a previous and/or current pregnancy ( $p=0.02$  and  $0.04$ , respectively). Patients with severe-critical disease were more likely to deliver at an earlier gestational age ( $p < 0.01$ ) and have positive SARS-CoV2 testing at the time of delivery ( $p < 0.01$ ). Ninety-nine percent of pregnancies studied resulted in live births. Neonates born to mothers with severe-critical COVID-19 were more likely to be admitted to the neonatal intensive care unit (NICU;  $p=0.05$ ). Overall maternal mortality, attributable to COVID-19 disease, was 2.3% (n=3).

**Conclusion:** Consistent with previous studies, we found that COVID-19 disease in pregnancy is associated with earlier gestational ages at delivery and, presumably, neonates requiring admission to the NICU. Differences in COVID-19 disease among various races and insurance statuses emphasize the importance in addressing underlying disparities in healthcare nationwide.

## T-254

### Placental Abnormalities in COVID-19. Maryland Study Group Report on COVID-19.

Liviu Cojocaru†, Irina Burd\*,<sup>2</sup> Autusa Pahlavan†, Ramya Reddy†,<sup>2</sup> Ozhan M Turan\*,<sup>1</sup> Katelyn Uribe†,<sup>2</sup> Meghna Ramaswamy†,<sup>1</sup> Sifa Turan\*. <sup>1</sup>University of Maryland School of Medicine, Baltimore, MD, United States; <sup>2</sup>Johns Hopkins University, Baltimore, MD, United States.

**Introduction:** SARS-CoV-2 is known to induce systemic coagulation and immune-inflammatory reactions, which can result in exaggerated host response. At the placental level, this can lead to a higher rate of ischemic, thrombotic, or inflammatory changes that are associated with adverse pregnancy outcomes. We aimed to evaluate the rate of histopathologic findings in the placentas of patients with COVID-19.

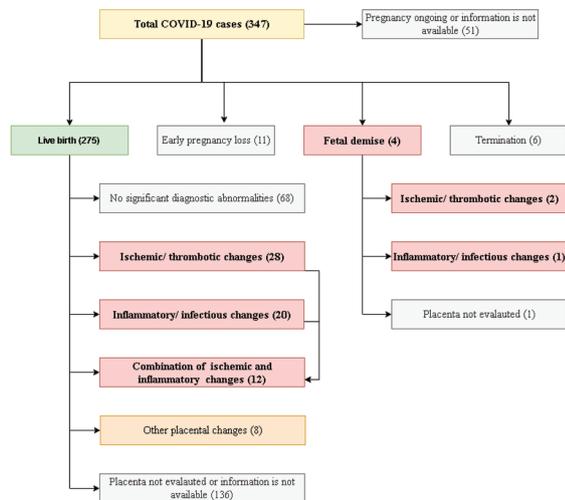
**Methods:** Maryland Study Group is a collaboration of major medical systems (University of Maryland Medical System and Johns Hopkins Health System) in the State of Maryland. We reviewed all patients with COVID-19 from March 2020 until January 2021. Data were recorded in a research electronic data capture registry. Pregnancy outcomes and placental histopathologic reports were analyzed. Pregnancy outcomes included live birth rate, pregnancy losses (<20 weeks), fetal demise (>20 weeks), and fetal growth restriction (estimated fetal weight <10th percentile). Placental histopathologic reports were categorized as no diagnostic abnormalities (NDA), ischemic/thrombotic (I/T) changes, inflammatory/infectious (I/I) changes, and non-specific changes. The rate of I/T and I/I changes were compared with the published historical data (PHD) (12% I/T and I/I in normal live births). Patients with an unknown delivery outcome, early pregnancy losses, terminations, or no pathology report were excluded.

**Results:** Out of 347 cases reviewed, pregnancy outcomes were known in 296 patients (Flowchart). Placental reports were available in 139/275 live birth (51%). I/T changes were seen in 40 (29%) placentas, at a significantly higher rate than PHD ( $p=0.001$ ). I/I changes were seen in 32 (23%)

placentas, which was similar to PHD ( $p=0.06$ ). NDA was reported in 68 cases (43%). There were 14 cases of FGR. Of those, 4 (6%) were in the NDA group and 10 (17%) in placentas with I/T and I/I changes ( $p=0.049$ ). **Conclusion:** Patients with COVID-19 had higher rates of I/T changes that are associated with adverse pregnancy outcomes. Further research is needed to compare the rates of FGR and other pregnancy complications in pregnant women with and without COVID-19. Increased surveillance of pregnant women with COVID-19 should be considered.

#### Flowchart: Placental abnormalities in COVID-19.

Maryland Study Group report on COVID-19.



#### T-255

##### Single-Cell RNA Sequencing of SARS-CoV-2 Cell Entry Factors in the Preconceptional Human Endometrium.

Felipe Vilella\*,<sup>1</sup> Wanxin Wang,<sup>2</sup> Inmaculada Moreno,<sup>3</sup> Beatriz Roson,<sup>1</sup> Steve R Quake,<sup>4</sup> Carlos Simon.<sup>1</sup> <sup>1</sup>Igenomix Foundation INCLIVA, Paterna (Valencia), Spain; <sup>2</sup>Stanford University, Stanford, CA, United States; <sup>3</sup>Igenomix Foundation, Paterna (Valencia), Spain; <sup>4</sup>Stanford University, Stanford, CA, United States.

**Introduction:** ACE2 enzyme serves as SARS-CoV-2 human receptor through binding of the viral S protein and subsequent trimming of S protein between S1 and S2 units by host serine proteases as TMPRSS2, CTSB or CTSL. Here, we aim to investigate the expression of the different cell entry proteins involved in SARS-CoV-2 infection in the different cell types of the human endometrium throughout the menstrual cycle with specific focus on the preconceptional endometrium using single-cell RNAseq (scRNAseq).

**Methods:** Human endometrial cells isolated from endometrial biopsies obtained from 27 donors across the menstrual cycle. After tissue dissociation, single cell capture was performed on Fluidigm C1 system ( $n=2,149$  cells) or Chromium 10x system (Chromium Next GEM Chip G, 10x Genomics) ( $n=71,032$  cells) followed by reverse-transcription, cDNA generation and library construction. Barcoded libraries were sequenced in pair-end reads on Nextseq (Illumina) for the C1 dataset or Novaseq (Illumina) for the 10x dataset. Data pre-processing, quality filtering, and statistical analyses were performed using custom Python, R, and Java scripts.

**Results:** ScRNAseq analysis showed no significant expression of ACE2 in stromal or unciliated epithelial cells in any phase of the menstrual cycle. TMPRSS2 was expressed in epithelial cells during the early proliferative and mid-secretory phases. Interestingly, expression of NRPI, CTSB and CTSL was observed in both stromal and epithelial cells across all phases of the menstrual cycle, and CTSB was the most abundant of these genes. In the mid-secretory phase, we detected coexpression of ACE2 and TMPRSS2 in 0.07% of luminal epithelial cells and coexpression of ACE2 and NRPI in 0.10% of stromal cells and 0.08% of luminal epithelial cells. No cells simultaneously expressed ACE2, NRPI and TMPRSS2 at the time of embryo implantation.

**Conclusion:** Our findings at the single-cell level imply low efficiency of SARS-CoV-2 infection in the endometrium, especially in the preconceptional period. However, NRPI is high in stromal and decidual cells independent of the cycle phase, suggesting an infectivity potential during pregnancy. These findings will facilitate preconception risk assessment in asymptomatic carriers.

#### T-256

##### Stress Decreases Host Viral Resistance and Increases Covid Susceptibility in Embryonic Stem Cells.

Daniel A Rappolee\*,<sup>1</sup> Mohammed Abdulhasan\*,<sup>1</sup> Ximena Ruden\*,<sup>1</sup> Benjamin Rappolee\*,<sup>2</sup> Sudipta Dutta\*,<sup>3</sup> Katherine Gurdziel†,<sup>1</sup> Douglas M Ruden\*,<sup>1</sup> Awoniyi O Awonuga\*,<sup>1</sup> Steven Korzeniewski\*,<sup>1</sup> Elizabeth E Puscheck\*,<sup>1</sup> <sup>1</sup>Wayne State University, Detroit, MI, United States; <sup>2</sup>Reproductive Stress 3M Inc, Grosse Pointe Farms, MI, United States; <sup>3</sup>Texas A&M University, Detroit, TX, United States.

**Introduction:** Two groups have reported that Covid-19 receptors are expressed in human early 1st trimester extra-embryonic membrane cells of yolk sac endoderm and placenta. Thus, the virus may access the early human embryo. We test embryonic stem cells (ESC) here to model the early embryo and assess whether stress affects host virus susceptibility and resistance.

**Methods:** Mouse ESC were cultured for 72hr to maintain normal stemness (NS), and with stemness-maintaining growth factor removed to enable normal differentiation (ND). Alternately, ESC were cultured with doses of positive control hyper-osmotic sorbitol in NS media to test for changes in viral susceptibility. RNA lysates were tested using Illumina NovaSeq6000 RNAseq for changes in gene expression for genes mediating virus uptake (susceptibility). Gene ontology (GO) groups mediating host viral resistance were assessed for stress effects. Thirty-three loading control genes assured correct assignment of cellular gene expression. qRT-PCR was used to confirm expression changes of key genes.

**Results:** Coronavirus receptors TMPRSS2, DPP4, Enpep and Vim increased during stressed ESC culture compared with normal stemness and/or differentiation. The Covid-19 receptor ACE2 was not detected in ESC culture, but ACE2 arises in some human or mouse ESC lines after differentiation to 1st lineage in the early implanting embryo, yolk sac endoderm. TMPRSS2, DPP4, Enpep and VIM are expressed in yolk sac endoderm which arises in stressed ESC. Also, most genes in 4 GO groups mediating host negative regulatory virus interaction - resistance - increase with normal differentiation but decrease during stress.

**Conclusion:** The increase in viral susceptibility gene expression and decrease in viral resistance gene expression in stressed ESC here may also be caused by environmental stresses. Stress may affect viral susceptibility and pathogenesis of ESC and early embryos. It remains to be tested if stress affects human ESC in similar ways. Does Covid-19 infect or kill environmentally stressed ESC and their yolk sac progeny more than unstressed ESC?

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## Regular Members

### Aagaard, Kjersti

Baylor College of Medicine  
Houston, TX  
United States

### Aberdeen, Graham W.

University of Maryland SOM  
Baltimore, MD  
United States

### Abrahams, Vikki M.

Yale University  
New Haven, CT  
United States

### Adams Waldorf, Kristina M.

University of Washington  
Seattle, WA  
United States

### Afshar, Yalda

Pacific Palisades, CA  
United States

### Aghaeepour, Nima

Stanford University  
Palo Alto, CA  
United States

### Ahn, Soo Hyun

Michigan State University  
East Lansing, MI  
United States

### Albrecht, Eugene D.

University of Maryland SOM  
Baltimore, MD  
United States

### Al-Hendy, Ayman

University of Chicago  
Chicago, IL  
United States

### Ali, Ali Farid M.

Ain Shams University  
Cairo  
Egypt

### Allen, Terrence

Duke University Hospital  
Durham, NC  
United States

### Amaral, Lorena M.

University of Mississippi Medical Center  
Jackson, MS  
United States

### Amato, Paula

Oregon Health & Science University  
Portland, OR  
United States

### Anchan, Raymond M.

Sharon, MA  
United States

### Anthony, Russ V.

Colorado State University  
Fort Collins, CO  
United States

### Anumba, Dilly O.

University of Sheffield  
Sheffield  
United Kingdom

### Armant, D. Randall

Wayne State University School of  
Medicine  
San Diego, CA  
United States

### Aronoff, David M.

Vanderbilt University Medical Center  
Nashville, TN  
United States

### Aubuchon, Mira

Missouri Center for Reproductive  
Medicine and Fertility  
Chesterfield, MO  
United States

### Aziz, Natali

Turlock, CA  
United States

### Baart, Esther B.

Erasmus MC, University Medical  
Center  
Rotterdam  
Netherlands

### Babayev, Samir N.

Rochester, MN  
United States

### Bafor, Enitome E.

National Cancer Institute  
Benin City  
Nigeria

### Ballas, Jerasimos

University of California San Diego  
La Jolla, CA  
United States

### Banadakoppa, Manu T.

Pearland, TX  
United States

### Baxi, Laxmi V.

New York, NY  
United States

### Benfield, Rebecca D.

Las Vegas, NV  
United States

### Bennett, Phillip R.

Imperial College London  
London  
United Kingdom

### Berenson, Abbey B.

UTMB  
Galveston, TX  
United States

### Berga, Sarah L.

Jacobs SMBS at University of Buffalo  
Buffalo, NY  
United States

### Bernstein, Ira Mark

University of Vermont COM  
Burlington, VT  
United States

### Bianchi, Diana W.

National Institute of Child Health and  
Human Development  
Bethesda, MD  
United States

### Bierle, Craig J.

Plymouth, MN  
United States

### Bird, Ian M.

University of Wisconsin-Madison  
Madison, WI  
United States

### Bishop, Cecily V.

Oregon National Primate Research  
Center  
Corvallis, OR  
United States

### Blakemore, Karin J.

Johns Hopkins University SOM  
Baltimore, MD  
United States

### Blanchon, Loic

Universite Clermont Auvergne - GReD -  
Medicine School  
Clermont-Ferrand  
France

### Bocca, Silvina M.

The Jones Institute -EVMS  
Norfolk, VA  
United States

### Boeldt, Derek S.

University of Wisconsin-Madison  
Madison, WI  
United States

### Bonney, Elizabeth Ann

University of Vermont  
Winooski, VT  
United States

**Borahay, Mostafa A.**  
Johns Hopkins University  
Baltimore, MD  
United States

**Bougie, Olga**  
Kingston, ON  
Canada

**Bourque, Stephane L.**  
University of Alberta  
Edmonton, AB  
Canada

**Branch, D. Ware**  
University of Utah  
Salt Lake City, UT  
United States

**Braun, Thorsten**  
Charite University of Berlin  
Berlin  
Germany

**Bravo, Maria Cristina**  
University of Vermont  
Colchester, VT  
United States

**Brockway, Heather M.**  
University of Florida College of Medicine  
Gainseville, FL  
United States

**Bruner-Tran, Kaylon L.**  
Vanderbilt University SOM  
Nashville, TN  
United States

**Buhimschi, Catalin S.**  
University of Illinois  
Chicago, IL  
United States

**Bukulmez, Orhan**  
University of Texas Southwestern  
Medical Center  
Dallas, TX  
United States

**Bulun, Serdar E.**  
Northwestern University  
Chicago, IL  
United States

**Burd, Irina**  
Johns Hopkins University  
Baltimore, MD  
United States

**Bush, Mark R.**  
Conceptions Reproductive Associates  
of Colorado  
Littleton, CO  
United States

**Bustillo, Maria**  
Miami, FL  
United States

**Bytautiene Prewit, Egle**  
UT Health San Antonio  
San Antonio, TX  
United States

**Campagnolo, Luisa**  
University of Rome Tor Vergata  
ROMA  
Italy

**Caniggia, Isabella**  
Lunenfeld-Tanenbaum Research  
Institute, Sinai Health System  
Toronto, ON  
Canada

**Carr, Bruce R.**  
UTSW Medical Center  
Dallas, TX  
United States

**Carson, Sandra Ann**  
Alexandria, VA  
United States

**Carvajal, Jorge Andres**  
Pontificia Universidad Catolica de Chile  
Santiago  
Chile

**Catalano, Patrick M.**  
Tufts University School of Medicine  
Boston, MA  
United States

**Catherino, William H.**  
Uniformed Services University  
Bethesda, MD  
United States

**Catov, Janet M.**  
University of Pittsburgh SOM  
Pittsburgh, PA  
United States

**Caughey, Aaron B.**  
OHSU  
Portland, OR  
United States

**Cedars, Marcelle I.**  
UCSF  
San Francisco, CA  
United States

**Cervello, Irene**  
IVI Foundation-IVIRMA  
Valencia  
Spain

**Cha, Dong Hyun**  
Gangnam CHA Hospital  
Seoul  
Korea

**Chambers, Setsuko K.**  
University of Arizona Cancer Center  
Tucson, AZ  
United States

**Chambliss, Linda Ruth**  
Phoenix, AZ  
United States

**Chamley, Lawrence W.**  
University of Auckland  
Auckland  
New Zealand

**Chan, Jessica L.**  
Cedars-Sinai Medical Center  
Los Angeles, CA  
United States

**Chan, Shiao-Yng**  
National University Singapore  
Singapore  
Singapore

**Chanrachakul, Boonsri**  
Samitivej Sukhumvit Hospital  
Bangkok  
Thailand

**Chao, Conrad R.**  
University of New Mexico  
Albuquerque, NM  
United States

**Chattergoon, Natasha N.**  
Oregon Health and Science University  
Portland, OR  
United States

**Chauhan, Madhu Lata S.**  
Baylor College of Medicine  
Houston, TX  
United States

**Chavira Suárez, Erika**  
Universidad Nacional Autónoma de  
México  
Mexico City  
Mexico

**Chen, Chih-Chen**  
E-DA Hospital  
Kaohsiung  
Taiwan

**Chen, Dongbao**  
UC Irvine  
Irvine, CA  
United States

- Chervenak, Frank A.**  
Northwell Health/Lenox Hill  
New York, NY  
United States
- Chishima, Fumihisa**  
Nihon University School of Medicine  
Tokyo  
Japan
- Christians, Julian**  
Simon Fraser University  
Burnaby, BC  
Canada
- Christianson, Mindy S.**  
White Marsh, MD  
United States
- Christman, Gregory M.**  
University of Florida  
Gainesville, FL  
United States
- Ciampa, Erin J.**  
Beth Israel Deaconess Medical Center  
Boston, MA  
United States
- Cilvik, Sarah N.**  
Lewisville, NC  
United States
- Cipolla, Marilyn Jo**  
University of Vermont  
Burlington, VT  
United States
- Collier, Ai-ris Y.**  
Newton Center, MA  
United States
- Connell, Kathleen A.**  
University of Colorado Denver  
Aurora, CO  
United States
- Connor, Kristin**  
Toronto, ON  
Canada
- Conrad, Kirk P.**  
Albuquerque, NM  
United States
- Contag, Stephen A.**  
University of Minnesota  
Minneapolis, MN  
United States
- Cope, Adela**  
Rochester, MN  
United States
- Critchley, Hilary OD**  
Edinburgh  
United Kingdom
- Curtin, William M.**  
Penn State College of Medicine  
Hershey, PA  
United States
- Damewood, Marian D.**  
Dover, PA  
United States
- David, Anna L.**  
University College London  
London  
United Kingdom
- Davidge, Sandra T.**  
Women and Children's Health  
Research Inst. - University of Alberta  
Edmonton, AB  
Canada
- Davis, John S.**  
University of Nebraska Medical Center  
Omaha, NE  
United States
- De Groot, Christianne JM**  
Amsterdam UMC  
Amsterdam  
Netherlands
- DeCherney, Alan H.**  
National Institutes of Health  
Bethesda, MD  
United States
- DeMayo, Francesco J.**  
NIEHS  
Research Triangle Park, NC  
United States
- Denney, Jeffrey M.**  
Winston-Salem, NC  
United States
- Depoix, Christophe L.**  
Universite Catholique de Louvain  
Woluwe-saint-Lambert  
Belgium
- Desoye, Gernot**  
Medical University of Graz  
Graz  
Austria
- Detti, Laura**  
Cleveland Clinic  
Cleveland, OH  
United States
- Di Clemente Besse, Nathalie**  
Arcueil  
France
- Di Simone, Nicoletta**  
Universita' Cattolica del Sacro Cuore  
Rome  
Italy
- Diaz-Gimeno, Patricia**  
IVI Foundation/ Biomedical Research  
Institute La Fe  
Valencia  
Spain
- Dimitriadis, Evdokia**  
The University of Melbourne  
Melbourne, VIC  
Australia
- Dolma, Padma**  
Sonam Norboo Memorial Hospital, Leh  
New Delhi  
India
- Dominguez, Francisco**  
Fundacion IVI/ISSLaFe  
Valencia  
Spain
- Douglas, Natak C.**  
New York, NY  
United States
- Drewlo, Sascha**  
Michigan State University  
Grand Rapids, MI  
United States
- Druzin, Maurice L.**  
Stanford University SOM  
Stanford, CA  
United States
- Ducsay, Charles A.**  
Redlands, CA  
United States
- Dude, Annie**  
Chicago, IL  
United States
- Dudley, Donald J.**  
University of Virginia  
Charlottesville, VA  
United States
- Dumont, Tina**  
Shrewsbury, MA  
United States
- DuPriest, Elizabeth**  
Warner Pacific University  
Portland, OR  
United States
- Edlow, Andrea G.**  
Massachusetts General Hospital  
Boston, MA  
United States

- Einerson, Brett D.**  
University of Utah  
Salt Lake City, UT  
United States
- Elias, Kevin M.**  
Brigham and Women's Hospital  
Boston, MA  
United States
- Elovitz, Michal A.**  
University of Pennsylvania  
Philadelphia, PA  
United States
- England, Sarah K.**  
Washington University SOM  
St. Louis, MO  
United States
- Enninga, Elizabeth Ann**  
Mayo Clinic  
Rochester, MN  
United States
- Eswaran, Hari**  
University of Arkansas COM  
Little Rock, AR  
United States
- Euser, Anna G.**  
University of Colorado School of  
Medicine  
Aurora, CO  
United States
- Fassett, Michael J.**  
Kaiser Permanente West Los Angeles  
Medical Center  
Los Angeles, CA  
United States
- Fazleabas, Asgerally T.**  
Michigan State University  
Grand Rapids, MI  
United States
- Feghali, Maisa**  
University of Pittsburgh/Magee-  
Womens Hospital of UPMC  
Pittsburgh, PA  
United States
- Feng, Liping**  
Duke University Medical Center  
Durham, NC  
United States
- Figuroa, Jorge P.**  
Wake Forest SOM  
Winston-Salem, NC  
United States
- Flores, Idhaliz**  
Ponce Health Sciences University -  
Ponce Research Institute  
Ponce, PR
- Fowler, Paul A.**  
University of Aberdeen  
Aberdeen  
United Kingdom
- Frasch, Martin G.**  
Seattle, WA  
United States
- Frias, Antonio E.**  
Oregon Health & Science University  
Portland, OR  
United States
- Fritz, Rani**  
NYU Winthrop  
Mineola, NY  
United States
- Fuh, Katherine C.**  
Washington University  
St Louis, MO  
United States
- Fujii, Eriko Y.**  
Tokyo  
Japan
- Furukawa, Yuichi**  
OITA University  
Chicago, IL  
United States
- Gammill, Hilary S.**  
Bill & Melinda Gates Foundation  
Bellevue, WA  
United States
- Garcia-Flores, Valeria**  
Wayne State University  
Detroit, MI  
United States
- García-Velasco, Juan A.**  
IVI RMA-Madrid / Rey Juan Carlos  
University  
Madrid  
Spain
- Gargett, Caroline E.**  
Hudson Institute of Medical Research  
Clayton, VIC  
Australia
- George, Erin**  
Philadelphia, PA  
United States
- Getahun, Darios**  
Kaiser Permanente Southern California  
Pasadena, CA  
United States
- Gill, Lisa**  
University of Minnesota  
Minneapolis, MN  
United States
- Gillespie, Shannon L.**  
The Ohio State University  
Columbus, OH  
United States
- Gilner, Jennifer B.**  
Duke University Medical Center  
Durham, NC  
United States
- Girard, Sylvie**  
University of Montreal - Sainte-Justine  
Hospital  
Montreal, QC  
Canada
- Girling, Jane E.**  
University of Otago  
Dunedin  
New Zealand
- Giudice, Linda C.**  
UCSF  
San Francisco, CA  
United States
- Giussani, Dino A.**  
University of Cambridge  
Cambridge  
United Kingdom
- Glantz, John Christopher**  
University of Rochester Medical Center  
Rochester, NY  
United States
- Goetzl, Laura**  
University of Texas, Health Sciences  
Center at Houston  
Houston, TX  
United States
- Goharkhay, Nima**  
Pregnancy Specialty Center of Texas  
Webster, TX  
United States
- Goldsmith, Laura T.**  
Rutgers - New Jersey Med. School  
New York, NY  
United States
- Golos, Thaddeus G.**  
University of Wisconsin-Madison  
Madison, WI  
United States

**Gomez-Lopez, Nardhy**

Wayne State University SOM  
Detroit, MI  
United States

**Gonik, Bernard**

Wayne State University School of  
Medicine  
Detroit, MI  
United States

**Gonzalez, Frank**

University of Illinois at Chicago College  
of Medicine  
Chicago, IL  
United States

**Goshen, Ran**

Rango Science & Medicine Ltd.  
Tel Aviv  
Israel

**Goulopoulou, Styliani**

University of North Texas Health  
Science Center  
Fort Worth, TX  
United States

**Gravett, Michael G.**

University of Washington  
Seattle, WA  
United States

**Gray, Kathryn J.**

Brookline, MA  
United States

**Green, Lucy R.**

University of Southampton  
Southampton  
United Kingdom

**Grobman, William A.**

Northwestern University  
Chicago, IL  
United States

**Grotegut, Chad A.**

Chapel Hill, NC  
United States

**Guarnaccia, Michael M.**

Closter, NJ  
United States

**Guess, Marsha K.**

University of Colorado, Anschutz  
Medical Campus  
Aurora, CO  
United States

**Gunatilake, Ravindu P.**

Glendale, AZ  
United States

**Gupta, Vijayalaxmi G.**

Washington University in St. Louis  
St. Louis, MO  
United States

**Guzman, Edwin Raphael**

Scottsdale, AZ  
United States

**Gyamfi-Bannerman, Cynthia**

Columbia University Irving Medical  
Center  
New York, NY  
United States

**Halvorson, Lisa M.**

Rockville, MD  
United States

**Hannan, Natalie J.**

University of Melbourne  
Heidelberg, VIC  
Australia

**Hapangama, Dharani K.**

University of Liverpool, UK  
Liverpool  
United Kingdom

**Harrington, Whitney**

University of Washington  
Seattle, WA  
United States

**Harrison, Margo S.**

University of Colorado  
Aurora, CO  
United States

**Hartmann, Katherine E.**

Vanderbilt University  
Nashville, TN  
United States

**Hawkins, Shannon M.**

Indiana University School of Medicine  
Indianapolis, IN  
United States

**Heikinheimo, Oskari M.**

Helsinki University Hospital  
Helsinki  
Finland

**Hellwege, Jacklyn N.**

Vanderbilt University Medical Center  
Nashville, TN  
United States

**Helmer, Hanns P.**

Medical University Vienna  
Vienna  
Austria

**Hemmings, Denise G.**

University of Alberta  
Edmonton, AB  
Canada

**Herington, Jennifer**

Vanderbilt University Medical Center  
Nashville, TN  
United States

**Herraiz, Sonia**

Fundación IVI  
Valencia  
Spain

**Hillman, Sara L.**

University College London  
London  
United Kingdom

**Hirota, Yasushi**

The University of Tokyo  
Tokyo  
Japan

**Hirsch, Emmet**

NorthShore University HealthSystem  
Evanston, IL  
United States

**Hobel, Calvin J.**

Cedars-Sinai Medical Center  
Los Angeles, CA  
United States

**Horii, Mariko**

University of California, San Diego  
La Jolla, CA  
United States

**Hornstein, Mark D.**

Brigham & Women's Hospital  
Boston, MA  
United States

**Hou, Zhen**

First Affiliated Hospital, Nanjing Medical  
University  
Nanjing  
Peoples Republic of China

**House, Michael D.**

Tufts Medical Center  
Boston, MA  
United States

**Huang, Gloria**

Yale University School of Medicine  
New Haven, CT  
United States

**Huang, Hong-yuan**

Chang Gung Memorial Hospital  
Kwei-shan, Tao-Yuan  
Taiwan

**Huang, S. Joseph**

Powell, OH  
United States

**Hull, Mary Louise**

University of Adelaide  
North Adelaide, SA  
Australia

**Hurt, K. Joseph**

University of Colorado SOM  
Aurora, CO  
United States

**Illsley, Nicholas**

Hackensack University Medical Center  
Hackensack, NJ  
United States

**Imbar, Tal**

Hadassah - Hebrew Univ. MC  
Jerusalem  
Israel

**Irwin, Juan C.**

UCSF  
San Francisco, CA  
United States

**Ishikawa, Gen**

Bunkyo-ku  
Japan

**Ishikawa, Hiroshi**

Chiba University, Graduate SOM  
Chiba  
Japan

**Ishimoto, Hitoshi**

Tokai University SOM  
Isehara  
Japan

**Itoh, Hiroaki**

Hamamatsu University SOM  
Hamamatsu  
Japan

**Jacobsson, Bo**

Sahlgrenska University Hospital/  
University of Gothenburg  
Gothenburg  
Sweden

**Jansson, Thomas**

University of Colorado Anschutz  
Medical Campus  
Aurora, CO  
United States

**Jellyman, Juanita K.**

California State Polytechnic University  
at Pomona  
Pomona, CA  
United States

**Jeong, Jae-Wook**

Michigan State University  
Grand Rapids, MI  
United States

**Jimenez, Patricia Tereese**

Washington University in St. Louis  
St. Louis, MO  
United States

**Johnson, Donna D.**

Medical University of South Carolina  
Charleston, SC  
United States

**Johnson, Joshua**

University of Colorado School of  
Medicine (AMC)  
Aurora, CO  
United States

**Johnson, Mark Richard**

Imperial College  
London  
United Kingdom

**Jones, Helen**

University of Florida College of  
Medicine  
Gainesville, FL  
United States

**Jonker, Sonnet**

OHSU  
Portland, OR  
United States

**Joshi, Niraj R.**

Michigan State University  
Grand Rapids, MI  
United States

**Joss-Moore, Lisa A.**

University of Utah  
Salt Lake City, UT  
United States

**Kaitu'u-Lino, Tu'uhevaha**

University of Melbourne  
Heidelberg, VIC  
Australia

**Kallen, Amanda N.**

Yale School of Medicine  
New Haven, CT  
United States

**Kalthur, Guruprasad**

Kasturba Medical College, Manipal  
Manipal  
India

**Karumanchi, Ananth**

Cedars-Sinai Medical Center  
Los Angeles, CA  
United States

**Kaufman, David G.**

University of North Carolina at Chapel  
Hill  
Chapel Hill, NC  
United States

**Keelan, Jeffrey A.**

University of Western Australia  
Perth  
Australia

**Kelleher, Meredith A.**

Oregon National Primate Research  
Center  
Beaverton, OR  
United States

**Keller-Wood, Maureen**

University of Florida  
Gainesville, FL  
United States

**Kemp, Matt**

University of Western Australia  
Perth, WA  
Australia

**Khabele, Dineo**

Washington University in St. Louis  
St. Louis, MO  
United States

**Khorram, Omid**

Harbor-UCLA Medical Center  
Torrance, CA  
United States

**Kiesel, Ludwig**

University of Muenster  
Muenster  
Germany

**Kim, J. Julie**

Northwestern University SOM  
Chicago, IL  
United States

**Kim, Jung-Sun**

Samsung Medical Center SKKU SOM  
Seoul  
Korea

**Kim, Tae Hoon**

Michigan State University  
Grand Rapids, MI  
United States

**Kim, Taeho**

Michigan State University  
East Lansing, MI  
United States

**Kim, Yoon Ha**

Chonnam Natl. Univ. Med. School  
Gwangju  
Korea

- Kim, Yoon Young**  
Seoul National University Hospital  
Seoul  
Korea
- Kniss, Douglas A.**  
Hilliard, OH  
United States
- Ko, Nga Ling**  
University of Vermont  
Burlington, VT  
United States
- Kodaman, Pinar H.**  
Yale University School of Medicine  
Orange, CT  
United States
- Kofinas, Alexander**  
Kofinas Perinatal  
New York, NY  
United States
- Kohan-Ghadr, Hamid-Reza**  
Michigan State University  
Grand Rapids, MI  
United States
- Komatsu, Yosuke**  
Gaithersburg, MD  
United States
- Kommagani, Ramakrishna**  
Washington University School of  
Medicine  
St Louis, MO  
United States
- Krause, Bernardo J.**  
Universidad de O'Higgins  
Rancagua  
Chile
- Ku, Seung-Yup**  
Seoul National University Hospital  
Seoul  
Korea
- Kulandavelu, Shathiyah**  
Miami, FL  
United States
- Kumar, Deepak**  
Broadview Heights, OH  
United States
- Kuokkanen, Satu Maarit**  
NYU Langone Reproductive Specialists  
of NY  
Rye, NY  
United States
- Kurlak, Lesia O.**  
University of Nottingham  
Nottingham  
United Kingdom
- Kwak-Kim, Joanne Y.**  
Rosalind Franklin Univ. of Medicine and  
Science  
Vernon Hills, IL  
United States
- Kyathanahalli, Chandrashekara N.**  
NorthShore University Health System  
Evanston, IL  
United States
- LaMarca, Babbette**  
University of MS Medical Center  
Jackson, MS  
United States
- Laughlin-Tommaso, Shannon K.**  
Mayo Clinic  
Rochester, MN  
United States
- Laven, Joop S.E.**  
Div. Reproductive Endocrinology &  
Infertility, Erasmus University MC  
Rotterdam  
Netherlands
- Layman, Lawrence C.**  
Medical College of GA at Augusta  
University  
Augusta, GA  
United States
- Leach, Richard E.**  
Grand Rapids, MI  
United States
- Lee, Eun D.**  
Virginia Commonwealth University  
Richmond, VA  
United States
- Lee, Men-Jean**  
University of Hawaii  
Honolulu, HI  
United States
- Leguizamón, Gustavo F.**  
Buenos Aires  
Argentina
- Leslie, Kimberly K.**  
University of Iowa Hospitals & Clinics  
Iowa City, IA  
United States
- Lesseur, Corina**  
Icahn School of Medicine at Mount  
Sinai  
New York, NY  
United States
- Lessey, Bruce A.**  
Wake Forest Baptist Health  
Winston-Salem, NC  
United States
- Limesand, Sean W.**  
University of Arizona  
Tucson, AZ  
United States
- Liu, Huishu**  
Guangzhou Women & Children Medical  
Center  
Guangzhou  
Peoples Republic of China
- Lo, Jamie**  
Portland, OR  
United States
- Lockwood, Charles J.**  
The University of South Florida Morsani  
College of Medicine  
Tampa, FL  
United States
- Loewendorf, Andrea I.**  
ImmunoVation, Immunology and  
Beyond Consulting  
Stanton, CA  
United States
- Longo, Monica**  
University of Texas Health Science  
Center at Houston  
Houston, TX  
United States
- Loret de Mola, J. Ricardo**  
SIU School of Medicine  
Springfield, IL  
United States
- Ludmir, Jack**  
Thomas Jefferson University  
Philadelphia, PA  
United States
- Lye, Stephen J.**  
Lunenfeld-Tanenbaum Research  
Institute  
Toronto, ON  
Canada
- MacPhee, Daniel J.**  
University of Saskatchewan  
Saskatoon, SK  
Canada
- Madueke-Laveaux, Obianuju Sandra**  
University of Chicago  
Chicago, IL  
United States

**Maeder, Angela B.**

Chicago, IL  
United States

**Magee, Laura A.**

Redhill  
United Kingdom

**Magness, Ronald R.**

University of South Florida  
Tampa, FL  
United States

**Mahalingaiah, Shruthi**

Harvard Chan School of Public Health  
Boston, MA  
United States

**Mahendroo, Mala**

UTSW Medical Center  
Dallas, TX  
United States

**Maher, Jacqueline Y.**

Silver Spring, MD  
United States

**Mainigi, Monica**

Philadelphia, PA  
United States

**Mak, Winifred**

Dell Medical School, UT Austin  
Austin, TX  
United States

**Makino, Shintaro**

Juntendo University  
Chiba  
Japan

**Mamillapalli, Ramanaiah**

Cheshire, CT  
United States

**Manuck, Tracy**

UNC-Chapel Hill  
Chapel Hill, NC  
United States

**Marbaix, Etienne**

Universite Catholique de Louvain  
Bruxelles  
Belgium

**Marconi, Anna Maria**

Milano  
Italy

**Marsh, Erica E.**

University of Michigan  
Ann Arbor, MI  
United States

**Martens, Mark G.**

Reading, PA  
United States

**Martinez de Tejada, Begoña**

University Hospitals of Geneva  
Geneva  
Switzerland

**Maruyama, Tetsuo**

Keio University SOM  
Tokyo  
Japan

**Mas Perucho, Aymara**

Igenomix Foundation. INCLIVA Health  
Research Institute  
Paterna  
Spain

**Mata-Greenwood, Eugenia**

Loma Linda University  
Loma Linda, CA  
United States

**Matsubara, Keiichi**

Ehime University Graduate School of  
Medicine  
Toon, Ehime  
Japan

**Matthews, Stephen G.**

University of Toronto  
Toronto, ON  
Canada

**McAllister, Stacy**

Decatur, GA  
United States

**McBride, Carole A.**

University of Vermont  
Burlington, VT  
United States

**McElroy, Steven J.**

University of Iowa  
Iowa City, IA  
United States

**McGee, Elizabeth A.**

University of Vermont Medical Center  
Burlington, VT  
United States

**McGovern, Peter Gerard**

Rutgers-New Jersey Medical School  
Hasbrouck Heights, NJ  
United States

**McLean, Kelley C.**

University of Vermont  
Burlington, VT  
United States

**Mejia, Rachel**

University of Iowa  
Iowa City, IA  
United States

**Mendelson, Carole R.**

UT Southwestern  
Dallas, TX  
United States

**Menkhorst, Ellen M.**

The University of Melbourne  
Melbourne  
Australia

**Menon, Ramkumar**

The University of Texas Medical Branch  
Galveston, TX  
United States

**Merhi, Zaher**

New Rochelle, NY  
United States

**Merriam, Audrey A.**

Milford, CT  
United States

**Merrill, David C.**

Wausau, WI  
United States

**Mesiano, Sam A.**

Case Western Reserve University  
Cleveland, OH  
United States

**Metz, Christine N.**

The Feinstein Inst. for Med. Rsch.  
Manhasset, NY  
United States

**Metz, Torri D.**

University of Utah Health  
Salt Lake City, UT  
United States

**Meyer, Marjorie**

University of Vermont  
Burlington, VT  
United States

**Miller, Bradley T.**

Reproductive Medicine Associates of  
Michigan, PLC  
Troy, MI  
United States

**Miller, Eliza C.**

Columbia University  
New York, NY  
United States

- Miller, Richard K.**  
University of Rochester Medical Center  
Rochester, NY  
United States
- Minegishi, Takashi**  
Gunma University  
Maebashi Gunma  
Japan
- Mitchell, Murray D.**  
Queensland University of Technology  
Brisbane, QLD  
Australia
- Mohan, Manoj**  
Aster DM Healthcare, Qatar  
Doha  
Qatar
- Moley, Kelle H.**  
Seattle, WA  
United States
- Monga, Manju**  
Baylor College of Medicine  
Houston, TX  
United States
- Montgomery, Grant W.**  
The University of Queensland  
Brisbane, QLD  
Australia
- Moore, John J.**  
CWRU - MetroHealth  
Cleveland, OH  
United States
- Moore, Lorna G.**  
University of Colorado Denver  
Aurora, CO  
United States
- Moravek, Molly B.**  
Ann Arbor, MI  
United States
- Morelli, Sara Sinha**  
Rutgers-New Jersey Medical School  
Newark, NJ  
United States
- Morgan, Terry K.**  
Oregon HS University  
Portland, OR  
United States
- Morrison, Janna Leigh**  
University of South Australia  
Adelaide, SA  
Australia
- Mucowski, Sara**  
Dallas IVF  
Dallas, TX  
United States
- Muglia, Louis J.**  
Burroughs Wellcome Fund  
Raleigh, NC  
United States
- Murtha, Amy P.**  
UCSF Obstetrics, Gynecology &  
Reproductive Sciences  
San Francisco, CA  
United States
- Murthi, Padma**  
Glen Iris, VIC  
Australia
- Myatt, Leslie**  
Portland, OR  
United States
- Myers, Dean A.**  
University of Oklahoma HSC  
Oklahoma City, OK  
United States
- Myers, Jenny**  
University of Manchester  
Manchester  
United Kingdom
- Myers, Kristin**  
Columbia University  
New York, NY  
United States
- Mysorekar, Indira U.**  
Washington University SOM  
St. Louis, MO  
United States
- Nakajima, Steven T.**  
Stanford University  
Sunnyvale, CA  
United States
- Nallasamy, Shanmugasundaram**  
University of Vermont  
Burlington, VT  
United States
- Nathanielsz, Peter W.**  
University of Wyoming  
Laramie, WY  
United States
- Nayak, Nihar R.**  
Wayne State University School of  
Medicine  
Kansas City, MO  
United States
- Neal-Perry, Genevieve S.**  
University of North Carolina School of  
Medicine  
Chapel Hill, NC  
United States
- Nelson, D. Michael**  
St. Louis, MO  
United States
- Nelson, Linda R.**  
Scottsdale, AZ  
United States
- Newnham, John P.**  
University of Western Australia  
Perth  
Australia
- Nicholson, Wanda**  
University of North Carolina  
Durham, NC  
United States
- Nie, Guiying**  
RMIT University  
Melbourne, VIC  
Australia
- Nold, Christopher J.**  
Glastonbury, CT  
United States
- Nothnick, Warren B.**  
University of Kansas Medical Center  
Kansas City, KS  
United States
- Nuñez Calonge, Rocio**  
UR International Group  
Madrid  
Spain
- Ober, Carole**  
Chicago, IL  
United States
- Odunsi, Kunle O.**  
Roswell Park Cancer Institute  
Buffalo, NY  
United States
- Okamura, Kunihiro**  
Tohoku Kosai Hospital  
Sendai  
Japan
- Olson, David M.**  
University of Alberta  
Edmonton, AB  
Canada
- O'Neill, Kathleen**  
University of Pennsylvania  
Philadelphia, PA  
United States

- Ory, Steven J.**  
Florida International University  
Margate, FL  
United States
- Osteen, Kevin G.**  
Vanderbilt University Medical Center  
Nashville, TN  
United States
- Osuga, Yutaka**  
The University of Tokyo  
Tokyo  
Japan
- O'Tierney-Ginn, Perrie Faye**  
Tufts Medical Center  
Boston, MA  
United States
- Owen, David**  
UT Southwestern Medical Center  
Dallas, TX  
United States
- Padmanabhan, Vasantha**  
University of Michigan  
Ann Arbor, MI  
United States
- Pal, Lubna**  
Yale School of Medicine  
New Haven, CT  
United States
- Palatnik, Anna**  
Mequon, WI  
United States
- Palumbo, Angela**  
Centro de Asistencia A La  
Reproduccion Humana de Canarias  
La Laguna  
Spain
- Parast, Mana M.**  
University of California San Diego  
La Jolla, CA  
United States
- Parks, W. Tony**  
University of Toronto  
Toronto, ON  
Canada
- Parry, Samuel**  
University of Pennsylvania  
Philadelphia, PA  
United States
- Patel, Sravan Kumar**  
University of Pittsburgh  
Pittsburgh, PA  
United States
- Patterson, Amanda L.**  
University of Missouri  
Columbia, MO  
United States
- Paul, Soumen**  
University of Kansas Medical Center  
Kansas City, KS  
United States
- Paulson, Richard J.**  
University of Southern California  
Los Angeles, CA  
United States
- Pavone, Mary Ellen**  
Chicago, IL  
United States
- Pearce, William J.**  
Loma Linda University  
Loma Linda, CA  
United States
- Pellicer, Antonio**  
Fundacion IVI  
Valencia  
Spain
- Pennell, Craig E.**  
Kotara, NSW  
United States
- Pepe, Gerald J.**  
EVMS  
Norfolk, VA  
United States
- Petraglia, Felice**  
Siena  
Italy
- Phillippe, Mark**  
Massachusetts General Hospital  
Boston, MA  
United States
- Piper, Jeanna M.**  
Germantown, MD  
United States
- Pisarska, Margareta D.**  
Cedars Sinai Medical Center  
Los Angeles, CA  
United States
- Platt, Lawrence D.**  
Center for Fetal Medicine and Women's  
Ultrasound  
Los Angeles, CA  
United States
- Popovici, Roxana M.**  
Center for Gynecologic Endocrinology  
and Reproductive Medicine  
Munich  
Germany
- Poston, Lucilla**  
King's College London  
London  
United Kingdom
- Powell, Theresa**  
University of Colorado Anschutz  
Medical Campus  
Aurora, CO  
United States
- Powers, Robert W.**  
University of Pittsburgh  
Pittsburgh, PA  
United States
- Pressman, Eva K.**  
University of Rochester  
Rochester, NY  
United States
- Price, Thomas M.**  
Duke University Medical Center  
Durham, NC  
United States
- Prins, Jelmer**  
University Medical Center Groningen  
Groningen  
Netherlands
- Raha, Sandeep**  
McMaster University  
Hamilton, ON  
Canada
- Rajkovic, Aleksandar**  
University of California San Francisco  
San Francisco, CA  
United States
- Ramadoss, Jay**  
Texas A&M University  
College Station, TX  
United States
- Ramella-Roman, Jessica**  
Florida International University  
Miami, FL  
United States
- Randolph, John F.**  
Michigan Medicine  
Ann Arbor, MI  
United States
- Reddy, Uma**  
Yale University  
New Haven, CT  
United States

- Reed, Susan D.**  
University of Washington  
Seattle, WA  
United States
- Reese, Jeff**  
Vanderbilt University Medical Center  
Nashville, TN  
United States
- Reinheimer, Torsten M.**  
Reinheimer.Expert  
Copenhagen  
Denmark
- Rice, Laurel W.**  
University of Wisconsin-Madison  
Madison, WI  
United States
- Riddell, Meghan**  
University of Alberta  
Edmonton, AB  
Canada
- Rivera-Alsina, Manuel E.**  
El Pasol, TX  
United States
- Roberts, Claire T.**  
Flinders University  
Bedford Park, SA  
Australia
- Roberts, James M.**  
Pittsburgh, PA  
United States
- Roberts, Victoria HJ**  
Oregon National Primate Res. Ctr.  
Beaverton, OR  
United States
- Robertson, Sarah Anne**  
The University of Adelaide  
Adelaide, SA  
Australia
- Robinson, Randal D.**  
University of Texas HSC-San Antonio  
San Antonio, TX  
United States
- Rogers, Peter A.W.**  
University of Melbourne  
Parkville, VIC  
Australia
- Rosenn, Barak M.**  
West New York, NJ  
United States
- Ross, Michael G.**  
David Geffen School of Medicine and  
Fielding School of Public Health at  
UCLA  
Los Angeles, CA  
United States
- Rozance, Paul J.**  
University of Colorado School of  
Medicine  
Aurora, CO  
United States
- Rueda, Bo R.**  
MGH / Harvard Medical School  
Boston, MA  
United States
- Sadovsky, Yoel**  
Magee-Womens Research Inst.  
Pittsburgh, PA  
United States
- Sahin, Cagdas**  
Ege University, Medicine Faculty,  
Department of Ob&Gyn  
Izmir  
Turkey
- Salafia, Carolyn Margaret**  
New Rochelle, NY  
United States
- Salih, Sana M.**  
Farmington Hills, MI  
United States
- Santoro, Nanette F.**  
University of Colorado  
Aurora, CO  
United States
- Sapin, Vincent**  
Universite Clermont Auvergne  
Clermont-Ferrand  
France
- Sasa, Hidenori**  
National Defense Medical College  
Tokorozawa  
Japan
- Sauer, Mark V.**  
Rutgers Robert Wood Johnson Medical  
School  
New Brunswick, NJ  
United States
- Saunders, Philippa T.**  
University of Edinburgh  
Edinburgh  
United Kingdom
- Sayegh, Raja A.**  
Boston University SOM  
Boston, MA  
United States
- Schalekamp-Timmermans, Sarah**  
Rotterdam  
Netherlands
- Schiff, Isaac**  
Massachusetts General Hospital  
Boston, MA  
United States
- Schmella, Mandy J.**  
University of Pittsburgh School of  
Nursing  
Pittsburgh, PA  
United States
- Schoeberlein, Andreina**  
University of Bern & University  
Women's Hospital Bern  
Bern  
Switzerland
- Schubert, Frank P.**  
Zionsville, IN  
United States
- Schulz, Laura C.**  
University of Missouri  
Columbia, MO  
United States
- Schwartz, Peter E.**  
Yale SOM  
New Haven, CT  
United States
- Seferovic, Maxim D.**  
Baylor College of Medicine  
Houston, TX  
United States
- Segars, James H.**  
Johns Hopkins University School of  
Medicine  
Baltimore, MD  
United States
- Seifer, David B.**  
Milford, CT  
United States
- Seli, Emre**  
Yale School of Medicine  
New Haven, CT  
United States
- Selvanesan, Blesson C.**  
Baylor College of Medicine  
Houston, TX  
United States

**Session, Donna R.**

Vanderbilt University  
Franklin, TN  
United States

**Shah, Dinesh M.**

University of Wisconsin-Madison  
Madison, WI  
United States

**Shamshirsaz, Alireza A.**

Baylor College of Medicine  
Houston, TX  
United States

**Shao, Jianhua**

University of California San Diego  
La Jolla, CA  
United States

**Shibata, Eiji**

Univ. of Occupational and  
Environmental Hlth.  
Kitakyushu  
Japan

**Shifren, Jan L.**

Massachusetts General Hospital  
Boston, MA  
United States

**Shree, Raj**

University of Washington Medical  
Center  
Seattle, WA  
United States

**Siler-Khodr, Theresa M.**

Center for Investigation of Cell  
Regulation & Replication  
San Antonio, TX  
United States

**Silver, Richard K.**

NorthShore University HealthSystem  
Evanston, IL  
United States

**Silver, Robert M.**

University of Utah  
Salt Lake City, UT  
United States

**Simon, Melissa Andrea**

Chicago, IL  
United States

**Simpson, Joe Leigh**

Florida International University  
Miami, FL  
United States

**Singh, Natasha**

Cambridgeshire  
United Kingdom

**Sites, Cynthia K.**

University of Massachusetts--Baystate  
Springfield, MA  
United States

**Slater, Donna M.**

Calgary University  
Calgary, AB  
Canada

**Slayden, Ov Daniel**

Oregon Health & Sciences University  
Beaverton, OR  
United States

**Smith, Gordon C.S.**

University of Cambridge  
Cambridge  
United Kingdom

**Smith, Graeme N.**

Queen's University  
Kingston, ON  
Canada

**Smith, Roger**

Bar Beach, NSW  
United States

**Smith, Yolanda R.**

Michigan Medicine  
Ann Arbor, MI  
United States

**Snegovskikh, Victoria V.**

Warren Alpert Medical School of Brown  
University  
Providence, RI  
United States

**Soares, Michael J.**

University of Kansas Medical Center  
Kansas City, KS  
United States

**Socol, Michael L.**

Northwestern University Feinberg SOM  
Chicago, IL  
United States

**Sojka, Dorothy K.**

Loyola University Chicago  
Maywood, IL  
United States

**Song, Rui**

Loma Linda University  
Loma Linda, CA  
United States

**Sood, Anil K.**

UT MD Anderson Cancer Ctr.  
Houston, TX  
United States

**Spencer, Jessica B.**

Emory University SOM  
Atlanta, GA  
United States

**Spencer, Thomas E.**

University of Missouri  
Columbia, MO  
United States

**Stanic, Aleksandar K.**

University of Wisconsin-Madison  
Madison, WI  
United States

**Steegers, Eric A.P.**

Erasmus MC, University Medical  
Center  
Rotterdam  
Netherlands

**Stephenson, Mary D.**

University of Illinois at Chicago  
Chicago, IL  
United States

**Stewart, Elizabeth A.**

Rochester, MN  
United States

**Stock, Sarah J.E.**

University of Edinburgh  
Edinburgh  
United Kingdom

**Stonestreet, Barbara S.**

Women & Infants Hospital of RI  
Providence, RI  
United States

**Stratton, Pamela**

Rockville, MD  
United States

**Straughen, Jennifer K.**

Henry Ford Health System  
Detroit, MI  
United States

**Strauss, Jerome F.**

Virginia Commonwealth University  
Philadelphia, PA  
United States

**Su, Emily J.**

University of Colorado School of  
Medicine  
Aurora, CO  
United States

**Sugawara, Junichi**

Tohoku University Graduate School of  
Medicine  
Sendai  
Japan

**Sugimura, Motoi**  
Hamamatsu University SOM  
Hamamatsu  
Japan

**Surbek, Daniel**  
University of Bern  
Bern  
Switzerland

**Sykes, Lynne**  
Imperial College  
London  
United Kingdom

**Tal, Reshef**  
Yale School of Medicine  
New Haven, CT  
United States

**Tanaka, Mamoru**  
Keio University SOM  
Tokyo  
Japan

**Taylor, Hugh S.**  
Yale University SOM  
New Haven, CT  
United States

**Taylor, Robert N.**  
University at Buffalo, Obstetrics &  
Gynecology Research Network  
Buffalo, NY  
United States

**Teixeira, Jose**  
Michigan State University  
Grand Rapids, MI  
United States

**Thompson, Jennifer A.**  
University of Calgary  
Calgary, AB  
Canada

**Tribe, Rachel M.**  
King's College London  
London  
United Kingdom

**Tschugguel, Walter**  
Medical University of Vienna  
Vienna  
Austria

**Tsibris, John C. M.**  
University of South Florida  
Tampa, FL  
United States

**Tskitishvili, Ekaterine**  
Faculty of Medicine, University of Liege  
Liege  
Belgium

**Ural, Serdar**  
Hummelstown, PA  
United States

**Vadillo-Ortega, Felipe**  
Instituto Nacional de Medicina  
Genomica  
Mexico City  
Mexico

**Valent, Amy M.**  
Oregon Health & Science University  
Portland, OR  
United States

**Valenzuela, Guillermo J.**  
Valley Ob-Gyn  
Colton, CA  
United States

**Van Den Veyver, Igna**  
Baylor College of Medicine  
Houston, TX  
United States

**Van Rijn, Bas B.**  
Erasmus MC, Rotterdam  
Rotterdam  
Netherlands

**Van Voorhis, Bradley J.**  
University of Iowa Hospitals & Clinics  
Iowa City, IA  
United States

**Vарner, Michael W.**  
University of Utah HSC  
Salt Lake City, UT  
United States

**Vasicka, Ian M.**  
First Faculty of Medicine, Charles  
University in Prague  
Prague  
Czech Republic

**Velez Edwards, Digna R.**  
Vanderbilt University Medical Center  
Nashville, TN  
United States

**Vilella, Felipe**  
INCLIVA; Igenomix Foundation  
Paterna (Valencia)  
Spain

**Vollenhoven, Beverley J.**  
Monash University  
Clayton, VIC  
Australia

**Vora, Neeta L.**  
UNC Chapel Hill  
Chapel Hill, NC  
United States

**Walker, James**  
Leeds  
United Kingdom

**Walsh, Scott W.**  
Virginia Commonwealth University  
Richmond, VA  
United States

**Wang, Yuping**  
LSUHSC-S  
Shreveport, LA  
United States

**Ward, Kenneth**  
Juneau Biosciences  
Salt Lake City, UT  
United States

**Warren, Wendy B.**  
New Jersey Perinatal Associates, LLC.  
Livingston, NJ  
United States

**Wax, Joseph R.**  
MMC  
Portland, ME  
United States

**Wegienka, Ganesa**  
Henry Ford Health System  
Detroit, MI  
United States

**Weiner, Carl Philip**  
University of Kansas SOM  
Mission Hills, KS  
United States

**Werner, Erika F.**  
Brown University  
Providence, RI  
United States

**Wernimont, Sarah**  
University of Minnesota  
Minneapolis, MN 55455, MN  
United States

**Wesolowski, Stephanie R.**  
University of Colorado  
Aurora, CO  
United States

**Whirledge, Shannon D.**  
Yale School of Medicine  
New Haven, CT  
United States

**Wild, Robert A.**  
University of Oklahoma HSC  
Oklahoma City, OK  
United States

**Williams, Carmen J.**  
NIH/NIEHS  
Research Triangle Park, NC  
United States

**Williams, David**  
University College London Hospital  
London  
United Kingdom

**Wilson, Ronee E.**  
Tampa, FL  
United States

**Wing, Deborah A.**  
Korn Ferry  
Irvine, CA  
United States

**Winger, Quinton**  
Colorado State University  
Fort Collins, CO  
United States

**Winn, Hung N.**  
University of Missouri-Columbia SOM  
Columbia, MO  
United States

**Winn, Virginia D.**  
Stanford University School of Medicine  
Stanford, CA  
United States

**Wise, Lauren A.**  
Boston University School of Public  
Health  
Boston, MA  
United States

**Wood, Charles E.**  
University of Florida  
Gainesville, FL  
United States

**Wu, Sheng**  
Temple University School of Medicine  
Philadelphia, PA  
United States

**Xenakis, Elly**  
Univ. of Texas HSC at San Antonio  
San Antonio, TX  
United States

**Xiao, Daliao**  
Loma Linda University  
Loma Linda, CA  
United States

**Xue, Qing**  
First Hospital of Beijing University  
Beijing  
Peoples Republic of China

**Yallampalli, Chandrasekhar**  
Baylor College of Medicine  
Houston, TX  
United States

**Yamaleyeva, Liliya M.**  
Wake Forest University School of  
Medicine  
Winston-Salem, NC  
United States

**Yang, Huanghe**  
Duke University Medical Center  
Durham, NC  
United States

**Yang, Peixin**  
University of Maryland SOM  
Baltimore, MD  
United States

**Yang, Xiaohua**  
Case western reserve university  
Willoughby, OH  
United States

**Yee, Lynn M.**  
Northwestern University Feinberg  
School of Medicine  
Chicago, IL  
United States

**Yellon, Steven M.**  
Loma Linda University  
Loma Linda, CA  
United States

**Yoneyama, Yoshio**  
Tokyo Institute of Repro. Sci.  
Tokyo  
Japan

**Young, Steven L.**  
University of North Carolina School of  
Medicine  
Chapel Hill, NC  
United States

**Yu, Bo**  
Seattle, WA  
United States

**Yu, Jie**  
Amherst, NY  
United States

**Yu, Liang**  
Eastern Virginia Medical School  
Norfolk, VA  
United States

**Zakar, Tamas**  
University of Newcastle  
New Lambton Hgts, NSW  
United States

**Zamudio, Stacy**  
Hackensack University MC  
Hackensack, NJ  
United States

**Zhang, Kewei**  
Westmead Hospital, the University of  
Sydney, Australia  
Westmead, NSW  
Australia

**Zheng, Jing**  
University of Wisconsin-Madison  
Madison, WI  
United States

**Zhou, Chi**  
University of Arizona  
Tucson, AZ  
United States

**Zondervan, Krina T.**  
University of Oxford  
Oxford  
United Kingdom

## Associate Members

**Bird, Cynthia E.**  
University of Wisconsin Madison  
Madison, WI  
United States

**Kuhn, Katherine**  
University of Colorado  
Denver, CO  
United States

**Majidi Zolbin, Masoumeh**  
Karaj  
Iran

**McCarthy, Ronald T.**  
Washington University at St. Louis  
St. Louis, MO  
United States

**Nadeem, Lubna**  
Lunenfeld-Tanenbaum Research  
Institute  
Toronto, ON  
Canada

**Ogle, Amy**  
Private Practice Dietitian  
San Diego, CA  
United States

**Poojary, Keerthana Karunakar**  
Kasturba Medical College, Manipal  
Manipal, Karnataka  
India

**Scalise, Maria Lujan**

IFIBIO-Houssay (UBA-CONICET)  
Cuidad Autonoma de Buenos Aires  
Argentina

**Shynlova, Oksana**

Sinai Health System  
Toronto, ON  
Canada

**Spaans, Floor**

University of Alberta  
Edmonton, AB  
Canada

**Zhang, Jianhong**

Mount Sinai Hospital  
Toronto, ON  
Canada

**In Training Members****Alvarado Flores, Fernanda**

Tufts Medical Center  
Boston, MA  
United States

**Amabebe, Emmanuel**

University of Sheffield  
Sheffield  
United Kingdom

**Arrowsmith, Sarah**

University of Liverpool  
Liverpool  
United Kingdom

**Aye, Irving**

University of Cambridge  
Cambridge  
United Kingdom

**Bai, Jin**

University of California, Irvine  
Irvine, CA  
United States

**Barnett, Scott D.**

University of Nevada, Reno  
Reno, NV  
United States

**Branham, Ki'ara K.R.**

Columbia, MO  
United States

**Braun, Amy E.**

San Francisco, CA  
United States

**Burns, Gregory W.**

Michigan State University  
Grand Rapids, MI  
United States

**Chang, Eileen**

Denver, CO  
United States

**Chu, Xiaodan**

Shreveport, LA  
United States

**Coggin-Carr, David J.**

South Burlington, VT  
United States

**Colon-Caraballo, Mariano**

Dallas, TX  
United States

**Colson, Arthur**

Woluwé-Saint-Pierre  
Belgium

**DeAngelis, Anthony M.**

Beltsville, MD  
United States

**Dotts, Ariel J.**

Northwestern University  
Chicago, IL  
United States

**Garg, Deepika**

SANDY, UT  
United States

**Ghnenis, Adel B.**

University of Michigan  
Ann Arbor, MI  
United States

**Goad, Jyoti**

University of California, San Francisco  
San Francisco, CA  
United States

**Gordon, Catherine**

Brookline, MA  
United States

**Gumina, Diane L.**

Denver, CO  
United States

**Han, Leo**

Portland, OR  
United States

**Hebert, Jessica Faith**

Oregon Health & Science University  
Portland, OR  
United States

**Hula, Nataliia**

University of Alberta  
Edmonton, AB  
Canada

**Jain, Varsha**

University of Edinburgh  
Edinburgh

**James-Allan, Laura B.**

Los Angeles, CA  
United States

**Jimenez Ramirez, Norma D.**

Stanford, CA  
United States

**Khader, Nawrah**

University of Toronto  
Toronto, ON  
Canada

**Kinnear, Hadrian M.**

Ann Arbor, MI  
United States

**Lai, Pei F.**

Imperial College London  
London  
United Kingdom

**Leimert, Kelycia B.**

University of Alberta  
Edmonton, AB  
Canada

**Lewis, Emma L.**

Philadelphia, PA  
United States

**Li, Kayla**

University of Oxford  
Oldsmar, FL  
United States

**Liu, Jin**

Johns Hopkins University  
Baltimore, MD  
United States

**Liu, Yang**

Johns Hopkins University  
Baltimore, MD  
United States

**Lorenzatti Hiles, Guadalupe**

University of Michigan  
Ann Arbor, MI  
United States

**Louis-Jacques, Adetola F.**

University of South Florida Morsani  
College of Medicine  
Tampa, FL  
United States

**Marquardt, Ryan M.**

Michigan State University  
Grand Rapids, MI  
United States

- Marshall, Sarah A.**  
Monash University  
Oakleigh East  
Australia
- McDonald, Thomas**  
North Carolina State University  
Raleigh, NC  
United States
- Moldovan, Genna E.**  
Michigan State University  
Grand Rapids, MI  
United States
- Motomura, Kenichiro**  
Wayne State University  
Detroit, MI  
United States
- Mousa, Mira**  
University of Oxford  
Sharjah  
United Arab Emirates
- Mowla, Shahriar**  
Imperial College London  
London  
United Kingdom
- Narice, Brenda Fernanda**  
Sheffield  
United Kingdom
- Naule, Lydie**  
Brigham and Women's hospital,  
Harvard Medical School  
Boston, MA  
United States
- Neisani Samani, Elham**  
Garden City Hospital  
Garden City, MI  
United States
- Newman, Rachel A.**  
Long Beach, CA  
United States
- Nguyen-Ngo, Caitlyn**  
The University of Melbourne  
Melbourne, Victoria  
Australia
- O'Callaghan, Jessica L.**  
Queensland University of Technology  
Brisbane  
Australia
- Ok, Linda**  
INRS Centre Armand Frappier Sante  
Biotechnologie  
Laval, QC  
Canada
- Ona, Samsiya**  
Bronx, NY  
United States
- Pasha, Mazhar**  
University of Alberta  
Edmonton, AB  
Canada
- Paul, Emmanuel N.X**  
Michigan State University  
Grand Rapids, MI  
United States
- Peterse, Dirkje**  
Boston Children's Hospital/Harvard  
Medical School  
Boston, MA  
United States
- Prince, Lillian**  
Cuyahoga Falls, OH  
United States
- Puckett, Kenisha A.**  
Stanford University  
Stanford, CA  
United States
- Rahmioglu, Nilufer**  
University of Oxford  
Oxford  
United Kingdom
- Rengarajan, Aishwarya**  
University of Wisconsin Madison  
Madison, WI  
United States
- Roura, Jaime A.**  
Bethesda, MD  
United States
- Sacha, Caitlin Redd**  
Waban, MA  
United States
- Saez, Tamara**  
University of Alberta  
Edmonton, AB  
Canada
- Safrai, Myriam**  
Hadassah Hebrew Medical Center  
Jerusalem  
Israel
- Schwartz, Amanda R.**  
University of Michigan  
Ann Arbor, MI  
United States
- Seckin, Serin**  
Brooklyn, NY  
United States
- Selvaratnam, Roshan J.**  
Monash University  
Melbourne  
Australia
- Shangaris, Panicos**  
King's College London  
London  
United Kingdom
- Sheng, Wenji**  
LSUHSC-Shreveport  
Shreveport, LA  
United States
- Shukla, Vinay**  
University of Kansas Medical Center  
Kansas City, KS  
United States
- Skvarca, Lauren B.**  
UPMC  
Pittsburgh, PA  
United States
- Spelke, Mae Bridget**  
Lusaka  
Zambia
- Strug, Michael**  
Grand Rapids, MI  
United States
- Sun, Tianyanxin**  
Stanford University  
Palo Alto, CA  
United States
- Talia, Chiara**  
University of Aberdeen  
Aberdeen  
United Kingdom
- Tong, Mancy**  
Yale University  
New Haven, CT  
United States
- Tsolova, Aleksandra**  
Edinburgh  
United Kingdom
- Varberg, Kaela**  
KUMC - University of Kansas Medical  
Center  
Kansas City, KS  
United States
- Vazquez, Jessica**  
University of Wisconsin-Madison  
Madison, WI  
United States
- Verstraeten, Barbara S. E.**  
Edmonton, AB  
Canada

**Wald, Kaitlyn A.**

Seattle Reproductive Medicine  
Everett, WA  
United States

**Whitaker, Lucy HR**

Edinburgh  
United Kingdom

**Wilson, Rachel C.**

Oregon National Primate Research  
Center, Oregon Health and Sciences  
University  
Beaverton, OR  
United States

**Wilson, Rebecca**

Cincinnati Children's Hospital Medical  
Center  
Cincinnati, OH  
United States

**Wolf, Hope M.**

Virginia Commonwealth University  
Richmond, VA  
United States

**Wooldridge, Amy L.**

University of Alberta  
Edmonton, AB  
Canada

**Woolston, Esther M.**

University of Auckland  
Auckland  
New Zealand

**Yang, Yihua**

UC Irvine  
Irvine, CA  
United States

**Yin, Ophelia**

Los Angeles, CA  
United States

**Zarate, Miguel A.**

University of Colorado Denver  
Aurora, CO  
United States

**Zhao, Yingjie**

University of Wisconsin-Madison  
Madison, WI  
United States

**Zierden, Hannah**

Baltimore, MD  
United States

**Emeritus Members****(Sharpe) Timms, Kathy L.**

University of Missouri-Columbia  
Sunrise Beach, MO  
United States

**Abdul-Karim, Raja W.**

SUNY Upstate Medical University  
Syracuse, NY  
United States

**Amesse, Lawrence S.**

Florida Atlantic University  
Boynton Beach Florida, FL  
United States

**Ances, Isadore G.**

Robert Wood Johnson SOM  
Camden, NJ  
United States

**Barker, Kenneth L.**

SUNY Upstate Medical University-  
Syracuse  
Zanesville, OH  
United States

**Bhavnani, Bhagu R.**

University of Toronto  
Toronto, ON  
Canada

**Blackwell, Richard E.**

University of Alabama  
Birmingham, AL  
United States

**Blumenfeld, Zeev**

Technion- Israel Institute of Technology  
Haifa  
Israel

**Brace, Robert A.**

OHSU School of Medicine  
Portland, OR  
United States

**Brenner, Paul F.**

University of Southern California  
North Hollywood, CA  
United States

**Brenner, Robert M.**

ORPRC/ OHSU  
Beaverton, OR  
United States

**Brinkman, Charles R.**

UCLA School of Medicine  
Grand Junction, CO  
United States

**Bryant-Greenwood, Gillian D.**

Honolulu, HI  
United States

**Calder, Andrew Alexander**

Simpson Ctr. for Repro. Health  
Edinburgh, Scotland  
United Kingdom

**Castracane, V. Daniel**

Landenberg, PA  
United States

**Cederqvist, Lars L.**

New York, NY  
United States

**Challis, John R.G.**

West Vancouver, BC  
Canada

**Chang, R. Jeffrey**

University of California San Diego SOM  
La Jolla, CA  
United States

**Chatterton, Robert T.**

Chicago, IL  
United States

**Chwalisz, Kristof**

Retired  
Palm Harbor, FL  
United States

**Coddington, Charles C.**

Charlotte, NC  
United States

**Cohen, Wayne R.**

University of Arizona  
Tucson, AZ  
United States

**Coulam, Carolyn B.**

Reproductive Medicine Institute  
Evanston, IL  
United States

**Coustan, Donald R.**

Women & Infants Hospital of RI  
Providence, RI  
United States

**Creasy, Robert K.**

Corte Madera, CA  
United States

**Croy, Barbara Anne**

Queen's University  
Kingston, ON  
Canada

- Cruikshank, Dwight P.**  
Medical College of Wisconsin  
Waukesha, WI  
United States
- Curet, Luis B.**  
University of New Mexico  
Albuquerque, NM  
United States
- Davison, John M.**  
Newcastle University  
Newcastle Upon Tyne  
United Kingdom
- De Haan, Jelte**  
Research Institute Grow  
Slenaken  
Netherlands
- Delivoria-Papadopoulos, Maria**  
Drexel University College of Medicine  
Philadelphia, PA  
United States
- Devoe, Lawrence D.**  
Georgia Regents University School of  
Medicine  
Augusta, GA  
United States
- DiSaia, Philip J.**  
University of California-Irvine  
Orange, CA  
United States
- Dmowski, W. Paul**  
Reproductive Medicine Institute  
Oak Brook, IL  
United States
- Dubin, Norman H.**  
Union Memorial Hospital  
Baltimore, MD  
United States
- Fox, Harold E.**  
Johns Hopkins University  
Baltimore, MD  
United States
- Gant, Norman F.**  
UT Southwestern  
Dallas, TX  
United States
- Garfield, Robert E.**  
University of Arizona College of  
Medicine-Phoenix  
Phoenix, AZ  
United States
- Gibb, William**  
University of Ottawa  
Ottawa, ON  
Canada
- Gibbs, Ronald S.**  
University of Colorado Denver SOM  
Aurora, CO  
United States
- Gilstrap, Larry C.**  
ABOG  
Dallas, TX  
United States
- Gokina, Natalia I.**  
University of Vermont  
Burlington, VT  
United States
- Golichowski, Alan M.**  
St Joseph, MI  
United States
- Goodlin, Robert C.**  
Cameron Park, CA  
United States
- Hammond, Charles B.**  
Duke University Med. Ctr.  
Durham, NC  
United States
- Haseltine, Florence P.**  
Fort Worth, TX  
United States
- Helmerhorst, Frans M.**  
Leiden University Med. Ctr.  
Leiden  
Netherlands
- Huszar, Gabor B.**  
Yale University SOM  
New Haven, CT  
United States
- Jackson, Benjamin T.**  
Weston, MA  
United States
- Jewelewicz, Raphael**  
Columbia University  
Alpine, NJ  
United States
- Jimenez, Juan M.**  
Dallas, TX  
United States
- Kohorn, Ernest I.**  
Yale University  
Orange, CT  
United States
- Lala, Peeyush**  
Dept of Anatomy and Cell  
Biology, University of western Ontario  
London, ON  
Canada
- Langer, Oded**  
Knoxville, TN  
United States
- Lasley, Bill L.**  
Inverness, CA  
United States
- Leppert, Phyllis C.**  
Duke University SOM  
Durham, NC  
United States
- Lindheimer, Marshall D.**  
Chicago, IL  
United States
- Mabie, Bill C.**  
Greenville Hospital System  
Greenville, SC
- McDonough, Paul G.**  
Georgia Health Sciences University  
Augusta, GA  
United States
- Menon, K. M. J.**  
University of Michigan  
Ann Arbor, MI  
United States
- Miodovnik, Menachem**  
Arlington, VA  
United States
- Moawad, Atef**  
Burr Ridge, IL  
United States
- Monroe, Scott E.**  
Atherton, CA  
United States
- Morrison, John C.**  
University of Mississippi Med. Ctr.  
Jackson, MS  
United States
- Mueller-Heubach, Eberhard**  
Wake Forest University School of  
Medicine  
Clemmons, NC  
United States
- Naftolin, Frederick**  
New York University SOM  
New York, NY  
United States
- Nagamani, Manubai**  
Houston, TX  
United States

**Niebyl, Jennifer R.**

University of Iowa Hospitals & Clinics  
Iowa City, IA  
United States

**Novy, Miles J.**

OHSU  
Portland, OR  
United States

**Oh, William**

Women and Infants' Hospital  
Providence, RI  
United States

**Osol, George J.**

University of Vermont College of  
Medicine  
Burlington, VT  
United States

**Peeters, Louis L.**

Utrecht  
Netherlands

**Rao, C.V.**

Florida International Univ.  
Miami, FL  
United States

**Rebar, Robert W.**

Birmingham, AL  
United States

**Redman, Christopher W.**

University of Oxford  
Oxford  
United Kingdom

**Reed, Kathryn L.**

University of Arizona  
Tucson, AZ  
United States

**Repke, John T.**

Penn State University College of  
Medicine  
Hershey, PA  
United States

**Riddick, Daniel H.**

Glade Hill, VA  
United States

**Rose, James C.**

Wake Forest University SOM  
Winston-Salem, NC  
United States

**Rosenfeld, Charles Richard**

UTSW Medical Center at Dallas  
Dallas, TX  
United States

**Rotmensch, Jacob**

Rush University Medical Center  
Chicago, IL  
United States

**Roux, Jacques F.**

Wilson, WY  
United States

**Sabbagha, Rudy E.**

Northwestern University  
Chicago, IL  
United States

**Salamonsen, Lois A.**

Hudson Institute of Medical Research  
Clayton, VIC  
Australia

**Sanborn, Barbara M.**

Houston, TX  
United States

**Schneider, Henning**

University of Berne  
Kehrsatz  
Switzerland

**Schroder, Hobe J.**

Loma Linda University  
Loma Linda, CA  
United States

**Schruefer, John J.**

Rockville, MD  
United States

**Schulman, Joseph D.**

Genetics & IVF Institute  
Fairfax, VA  
United States

**Sciarra, John J.**

Wilmette, IL  
United States

**Seeds, John W.**

Virginia Commonwealth University  
Richmond, VA  
United States

**Sibley, Colin P.**

University of Manchester  
Oxford Rd, Manchester  
United Kingdom

**Sokol, Robert J.**

West Bloomfield, MI  
United States

**Sorokin, Yoram**

Wayne State University  
Detroit, MI  
United States

**Strickler, Ronald C.**

Grosse Pointe Shores, MI  
United States

**Subramanian, Marappa G.**

Wayne State University  
Detroit, MI  
United States

**Talledo, O. Eduardo**

Augusta, GA  
United States

**Teramo, Kari A.**

Helsinki University Hospital  
Helsinki  
Finland

**Tredway, Donald R.**

Tredway Consulting, LLC  
Broken Arrow, OK  
United States

**Trudinger, Brian J.**

University of Sydney at Westmead  
Hospital  
Beecroft, NSW  
United States

**Tyson, John E.**

C.A.R.E. Health Resources  
Clifford, ON  
Canada

**Van Assche, Frans Andre**

Univ Leuven  
Leuven  
Belgium

**Wallach, Edward E.**

Johns Hopkins at Greenspring Station  
Lutherville, MD  
United States

**Wallenburg, Henk C.S.**

Erasmus University Rotterdam  
Rhoon  
Netherlands

**Weiss, Gerson**

New Jersey Med Sch-Rutgers  
New York, NY  
United States

**Wentz, Anne Colston**

Bozeman, MT  
United States

**Witkin, Steven S.**

Weill Cornell Medicine  
New York, NY  
United States

**Wynn, Ralph M.**

New York, NY  
United States

**Yeh, Sze-Ya**

Arcadia, CA  
United States

**Young, Bruce K.**

New York University SOM  
New York, NY  
United States

**Young, Roger C.**

PreTel, Inc  
Portland, OR  
United States

**Honorary Members****Benirschke, Kurt**

University of Calif.-San Diego  
San Diego, CA  
United States

**Carsten, Mary E.**

UCLA Medical Center  
Los Angeles, CA  
United States

**Cohen, Jean**

Paris  
France

**Rudolph, Abraham M.**

UCSF  
San Francisco, CA  
United States

**Seppala, Markku T.**

University of Helsinki  
Helsinki  
Finland

**Zarate, Arturo**

National University of Mexico, Instituto  
Mexicano Seguro Social  
Mexico City  
Mexico

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555 East Wells Street  
Suite 1100  
Milwaukee, WI 53202

Leah Miller  
Jamie Brouws  
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Ciera Arias

+1 (414) 918-9888  
+1 (414) 918-9888  
+1 (414) 918-9888  
+1 (414) 918-9888

lmiller@sri-online.org  
jbrouws@sri-online.org  
mderby@sri-online.org  
carias@sri-online.org

Fax: +1 (414) 276-3349  
Web Site: [www.sri-online.org](http://www.sri-online.org)