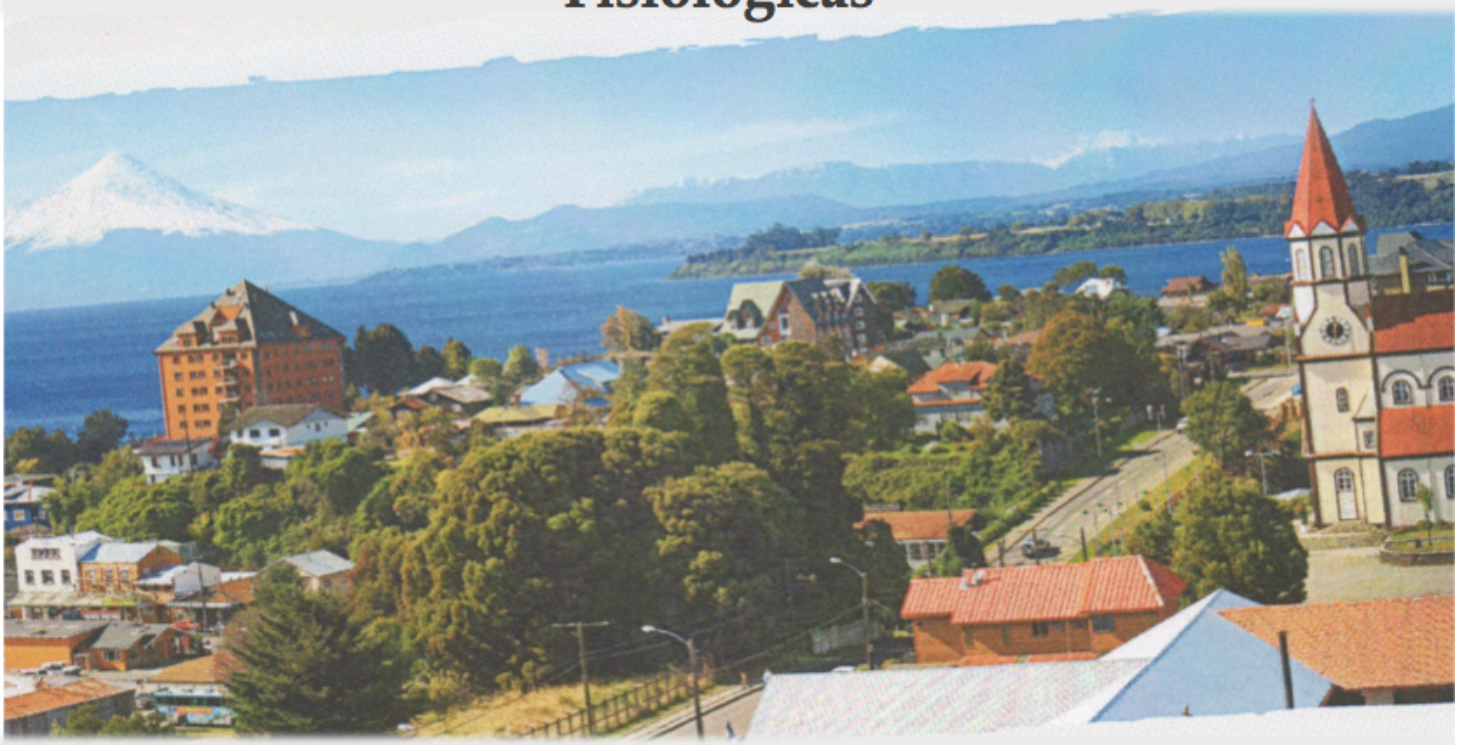
SLIMP - SCHCF 2017

Latin American Society for Maternal Fetal Interaction and Placenta

Sociedad Chilena de Ciencias Fisiológicas



PROGRAM

11 - 13 April, 2017
Hotel Enjoy Puerto Varas
Chile

SLIMP / SCHCF
Joint Meeting 2017

Latin American Society for Maternal Fetal Interaction and Placenta (SLIMP)

Sociedad Chilena de Ciencias Fisiológicas (SCHCF)

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Latin American Society for Maternal Fetal Interaction and Placenta (SLIMP)

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Hotel Enjoy Puerto Varas
11 - 13 April, 2017

Organizing Committee

Luis Sobrevía
Andrea Leiva
Dolores Busso
Marcelo Farías
Bredford Kerr

Scientific Committee

Alejandro Tapia
Alicia Jawerbaum
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Carlos Escudero
Silvia Daher
Ulrike Kemmerling

Acknowledgements

- Placenta Associations of the Americas (PAA)
- International Federation of Placenta Associations (IFPA)
- University Medical Center Groningen (UMCG), University of Groningen, The Netherlands
- Lawson Health Research Institute (Lawson), Canada
- Sociedad Chilena de Ciencias Fisiológicas (SCHCF)
- ELSEVIER
- Comisión Nacional para Investigación en Ciencia y Tecnología (CONICYT), Chile
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- Turismo Chile, Turismo de Reuniones, SERNATUR, Ministry of Economy, Development and Tourism, Government of Chile

Scientific program *in brief*

Tuesday April 11th

Rooms A, B, C
14:00
Opening

14:10
Lawson Plenary Lecture 1: DJ Hill (Canada)

Room A
15:00 – 16:30
Symposium 1. Placental Dysfunction and Pregnancy Outcome: AM Franchi (Argentina), P Casanello (Chile), C Pérez Leiros (Argentina)

Room C
15:00 – 16:30
Symposium 2. Metabolic and Endocrine Modulators of Pregnancy: B de Vrijer (Canada), C Varone (Argentina), E Arany (Canada)

Room D
16:30 – 17:30
Poster Communications I (*and Coffee*)

Room A
17:30 – 19:00
Symposium 3. SCHCF – Skeletal Muscle Function: Focus on Physiological Signaling for Autophagy, Mitochondrial Ca^{2+} Control and GLUT4 Trafficking: D Valladares (Chile), A Contreras-Ferrat (Chile), C Osorio-Fuentealba (Chile)

Room C
17:30 – 19:00
Symposium 4. Key Players at Implantation: A Tapia-Pizarro (Chile), MªL Ribeiro (Argentina), E Cebral (Argentina)

Rooms A, B, C
19:00 – 19:30
SLIMP 2015 Prize Award Lecture: N Portilho (Brazil)

19:30 – 20:30
SCHCF – UMCG Plenary Lecture 2: JL Hillebrands (The Netherlands)

20: 30
Welcome reception (Hotel Enjoy Puerto Varas)

Wednesday April 12th

Rooms A, B, C
08:30 – 09:30
Oral Communications I: M.F. Camisay (Argentina), N. Blanco (Chile), H.G.S. Oliveira (Brazil), Ch. Castillo (Chile), A. Sotelo (Argentina), N Santander (Chile).

Room A
09:30 – 11:00
Symposium 5. Role of Thyroid Hormones in Pregnancy: S Bueno (Chile), C Riedel (Chile), R Moreno-Reyes (Belgium)

Room B
09:30 – 11:00
Symposium 6. Early Life Programming: B Kerr (Chile), C Torres-Farfan (Chile), A Chicco (Argentina)

Room C
09:30 – 11:00
Symposium 7. Angiogenesis and Vascular Remodelling: C Escudero (Chile), V Palma (Chile), JL Hillebrands (The Netherlands)

Room D
11:00 - 12:00
Poster Communications II (*and Coffee*)

Rooms A, B, C
12:00 – 13:00
SCHCF – Plenary Lecture 3: S Zamudio (USA)

Room A
14:30 -16:00
Symposium 8. SCHCF – Young Scientist: I Ruminot (Chile), G Peña-Münzenmayer (Chile), JA Gutiérrez (Chile), F Sepúlveda (Chile)

Room B
14:30 -16:00

Symposium 9. Frontiers in Maternal Foetal Research I: M Farina (Argentina), G Cruz (Chile), C Sóñora (Uruguay), E Grasso (Argentina), R Higa (Argentina)

Room C
14:30 -16:00
Symposium 10. Frontiers in Maternal Foetal Research II: F Pardo (Chile), F Sacerdoti (Argentina), E Capobianco (Argentina), P Delgado-Olguin (Canada)

Rooms A, B, C
16:00 – 17:00
Plenary Lecture 4: G Rice (Australia)

Room D
17:00 – 18:00
Poster Communications III (*and Coffee*)

Rooms A, B, C
18:00 – 18:30
Forum Placenta Associations of the Americas (PAA) Research Opportunities: N Ilsley (USA), S Zamudio (USA), I Caniggia (Canada)

18:30 – 19:30
Plenary Lecture 5: I Caniggia (Canada)

Room A
19:30 – 20:15
SLIMP members meeting

Room B
19:30 – 20:15
SCHCF members meeting

Thursday, April 13th

Rooms A, B, C

08:30 – 09:30
Oral Communications II: N. Szpilbarg (Argentina), D. Fornes (Argentina), J. Araos (Chile), I.A. Neves (Brazil), T. Sáez (The Netherlands), J.B. Moreli (Brazil)

09:30 – 10:30
Plenary Lecture 6: N Illsley (USA)

10:30 – 11:00
Coffee

Room A
11:00 – 12:30
SCHCF – Symposium 11. Vascular Modulators: Role of Adenosine Receptors and Thyroid Hormones: AI Rodriguez (Chile), E Guzmán-Gutiérrez (Chile), C Escudero (Chile)

Room B
11:00 – 12:30
Symposium 12. Infection, Toxins, and Pregnancy: C Ibarra (Argentina), E Bevilacqua (Brazil), U Kemmerling (Chile)

Room C
11:00 – 12:30
Symposium 13. Pathogenesis of Preeclampsia: M Faas (The Netherlands), A Damiano (Argentina), M Post (Canada)

Rooms A, B, C
12:30 – 13:30
Plenary Lecture 7: A Jawerbaum (Argentina)

13:30 – 14:00
Closing Ceremony and Awards

Scientific program *in detail*

Tuesday April 11th

Rooms A, B, C
14:00 – 15:00 Opening L Sobrevia (Chairman)

Lawson Plenary Lecture 1 (Chair: L Sobrevia)

David J Hill (Lawson Health Research Institute, Canada)

Metabolic adaptations in the mother for an optimal pregnancy outcome. What goes wrong in gestational diabetes?

15:00 – 16:30 Symposia 1 and 2

Room A

Symposium 1. Placental Dysfunction and Pregnancy Outcome (Chair: AMª Franchi)

Ana María Franchi (Universidad de Buenos Aires, Argentina)

New strategies to prevent preterm birth

Paola Casanello (Pontificia Universidad Católica de Chile, Chile)

Intrauterine growth trajectories modify the epigenetic programming of vascular-related genes in human umbilical artery endothelium and cord adiponectin levels

Claudia Pérez Leiros (Universidad de Buenos Aires, Argentina)

Trophoblast cells regulate immune cell functional profile through VIP-mediated pathways

Room C

Symposium 2. Metabolic and Endocrine Modulators of Pregnancy (Chair: E Arany)

Barbra de Vrijer (Western University, Canada)

Insight Inside: Imaging fetal adipose tissue development with 3D water-fat MRI

Cecilia Varone (Universidad de Buenos Aires, Argentina)

Role of leptin in the molecular physiology of the placenta

Edith Arany (Western University, Canada)

Maternal dietary composition programs offspring pancreatic development reducing or increasing the risks of glucose impairment in adulthood

Room D

16:30 – 17:30 Poster Communications I (*and Coffee*)

17:30 – 19:00 Symposia 3 and 4

Room A

Symposium 3. SCHCF – Skeletal Muscle Function: Focus on Physiological Signaling for Autophagy, Mitochondrial Ca^{2+} Control and GLUT4 Trafficking (Chair: P Llanos)

Denisse Valladares (Universidad Finis Terrae, Chile)

IP3 receptor modulates autophagy, mitochondrial dynamics and mitophagy in skeletal muscle: New insights muscular dystrophy treatment

Ariel Contreras-Ferrat (Universidad Finis Terrae, Chile)

Excitation-metabolism coupling in skeletal muscle: Ca^{2+} handling and mitochondrial function

Cesar Osorio-Fuentealba (Universidad Metropolitana de Ciencias de la Educación, Chile)

Cell physiology of skeletal muscle: mechanisms of GLUT4 traffic and cytoskeleton implications

Room C

Symposium 4. Key Players at Implantation (Chair: A Tapia)

Alejandro Tapia-Pizarro (Universidad de Chile, Chile)

Hyperglycosylated chorionic gonadotropin as a modulating factor for embryo implantation and trophoblast invasion

Mª Laura Ribeiro (Universidad de Buenos Aires, Argentina)

Role of lipid mediators at the maternal-foetal interphase

Elisa Cebral (Universidad de Buenos Aires, Argentina)

In vivo and in vitro models for implantation studies in maternal alcohol ingestion. Role of matrix metalloproteinases and cell adhesion molecules

Rooms A, B, C

19:00 – 19:30 SLIMP 2015 Prize Award Lecture (Chair: A Jawerbaum)

Nathália Portilho (Oswaldo Cruz Institute (Fiocruz), Brazil)

Characterization and distribution profile of hematopoietic cells in mouse placenta

19:30 – 20:30 SCHCF – UMCG Plenary Lecture 2 (Chair: L Sobrevia**)**

Jan-Luuk Hillebrands (University of Groningen. The Netherlands)

Angiogenesis and αKlhoto

20: 30 Welcome reception (Hotel Enjoy Puerto Varas)

Wednesday April 12th

Rooms A, B, C

08:30 – 09:30 Oral Communications I (Chair: E Capobianco, F Pardo)

OC1 M.F. Camisay (University of Buenos Aires, Argentina)
FK506 binding protein 52 modulated AP-1 functions in human trophoblast cells

OC2 N. Blanco (Universidad Andrés Bello & Pontificia Universidad Católica de Chile, Chile)
The blood brain barrier of the offspring gestated in hypothyroxinemia has higher permeability to macromolecules and to the transmigration of immune cells to the central nervous system

OC3 H.G.S. Oliveira (Federal University of Alagoas, Brazil)
Versican expression and roles in hydatidiform moles

OC4 Ch. Castillo (Universidad de Chile, Chile)
Trypanosoma cruzi exosomes increases susceptibility to parasite infection in human placental chorionic villi explants

OC5 A. Sotelo (Universidad de Buenos Aires, Argentina)
Angiotensin II induces decidualisation markers and chemoatractants in human endometrial stromal cells and regulates trophoblast migration

OC6 N. Santander (Pontificia Universidad Católica de Chile, Santiago, Chile)
RNA-Seq analysis reveals candidate genes that may explain neural tube defects in mouse embryos lacking SR-BI

09:30 – 11:00 Symposia 5, 6, and 7

Room A

Symposium 5. Role of Thyroid Hormones in Pregnancy (Chair: S Bueno)

Susan Bueno (Pontificia Universidad Católica de Chile, Chile)
The impact of thyroid hormone deficiency during pregnancy in infectious diseases

Claudia Riedel (Universidad Andrés Bello, Chile)
The physiological consequences of maternal thyroid hormone deficiencies in their offspring

Rodrigo Moreno-Reyes (Université Libre de Bruxelles, Belgium)
Micronutrient deficiencies and the thyroid in pregnancy

Room B

Symposium 6. Early Life Programming (Chair: Adriana Chicco)

Bredford Kerr (Centro de Estudios Científicos (CECs), Chile)

Exposure to an experience-dependent plasticity paradigm during pregnancy and lactation modulates offspring energy homeostasis

Claudia Torres-Farfan (Universidad Austral de Chile, Chile)

Gestational chronodisruption impairs circadian physiology in rat male offspring, increasing the risk of chronic disease

Adriana Chicco (Universidad del Litoral, Argentina)

Can weaning dietary manipulation counteract the effect of poor nutrition during pregnancy and lactation?

Room C

Symposium 7. Angiogenesis and Vascular Remodelling (Chair: Carlos Escudero)

Carlos Escudero (Universidad del Bío-Bío, Chile)

Is angiogenesis altered in the offspring of mothers with preeclampsia? Potential role of A2A adenosine receptor-nitric oxide-vascular endothelial growth factor pathway

Verónica Palma (Universidad de Chile, Chile)

Netrin-1 acts as a non-canonical angiogenic factor produced by human Wharton's Jelly Mesenchymal Stem Cells

Jan-Luuk Hillebrands (University of Groningen, The Netherlands)

αKlotho in vascular (patho)physiology

Room D

11:00 - 12:00 Poster Communications II (*and Coffee*)

Rooms A, B, C

12:00 – 13:00 SCHCF – Plenary Lecture 3 (Chair: AMª Franchi)

Stacy Zamudio (Hackensack University Medical Center (HUMC), USA)

Placental metabolic reprogramming induces adaptive maternal/foetal physiological responses to placental hypoxia

13:00 – 14:30 Lunch break

14:30 – 16:00 Symposia 8, 9, and 10

Room A

Symposium 8. SCHCF – Young Scientist (Chair: B Kerr)

Iván Ruminot (Centro de Estudios Científicos (CECs), Chile)

Tight coupling of astrocyte energy metabolism to synaptic activity revealed in brain tissue with genetically encoded FRET nanosensors

Gaspar Peña-Münzenmayer (Universidad Austral de Chile, Chile)

Participation of Ae4 anion exchanger in Cl^--dependent saliva secretion

Jaime A Gutiérrez (Universidad San Sebastián, Chile)

Preeclampsia, an opportunity to learn about placental development: Role of RECK?

Fernando Sepúlveda (Universidad de Concepción, Chile)

Endocannabinoid signaling in glia-neuron communication and the use of phytocannabinoids as a biomedical tool for the Chilean population

Room B

Symposium 9. Frontiers in Maternal Foetal Research I (Chair: A Leiva)

Mariana Farina (Universidad de Buenos Aires, Argentina)

Participation of the endocannabinoid system in the pathophysiology of human placenta

Gonzalo Cruz (Universidad de Valparaíso, Chile)

Reproductive and metabolic alterations in the progeny of obese rats: role of sympathetic nervous system

Cecilia Sóñora (Universidad de la República, Uruguay)

β2GPI-specific antibodies induce pro-inflammatory mediators and tissue transglutaminase differential variant expression on trophoblast cells and monocytes-macrophages

Esteban Grasso (Universidad de Buenos Aires, Argentina)

Relevance of the blastocyst conditioned media on immunotolerance: focus on the control of the inflammatory response

Romina Higa (Universidad de Buenos Aires, Argentina)

Maternal treatments to prevent diabetic embryopathy

Room C

Symposium 10. Frontiers in Maternal Foetal Research II (Chair: A Borbely)

Fabián Pardo (Universidad de Valparaíso, Chile)

Excessive gestational weight gain reduces the response to vasoactive molecules in human foetoplacental microvessels

Flavia Sacerdoti (Universidad de Buenos Aires, Argentina)

Shiga toxin during pregnancy and breastfeeding: Can human milk protect children from typical HUS?

Evangelina Capobianco (Universidad de Buenos Aires, Argentina)

Placental mTOR signaling in metabolic diseases

Paul Delgado-Olguin (The Hospital for Sick Children & University of Toronto, Canada)

EHMT2/G9a controls maturation of the placental vasculature

Rooms A, B, C

16:00 – 17:00 Plenary Lecture 4 (Chair: M Farías)

Gregory Rice (University of Queensland, Australia)

Developing screening modalities for improving pregnancy outcomes - ex nihilo nihil fit

Room D
17:00 – 18:00 **Poster Communications III** (*and Coffee*)

Rooms A, B, C
18:00 – 18:30 **Forum Placenta Associations of the Americas (PAA) Research Opportunities** (Moderator: L Sobrevia)
Nicholas Ilsley (USA)
Stacy Zamudio (USA)
Isabella Caniggia (Canada)

18:30 – 19:30 **Plenary Lecture 5** (Chair: L Sobrevia)
Isabella Caniggia (Mount Sinai Hospital & University of Toronto, Canada)
Oxygen sensing: Novel epigenetic mechanisms

19:30 – 20:15 **SLIMP and SCHCF Members Meeting**

Room A
SLIMP members meeting

Room B
SCHCF members meeting

21:00 *Congress dinner*

Thursday, April 13th

Rooms A, B, C
08:30 – 09:30 **Oral Communications II** (Chairs: G Cruz, P Delgado)
OC7 N. Szpilbarg (Universidad de Buenos Aires, Argentina)
Evidence for oxygen-mediated regulation of aqp4 expression in human placenta
OC8 D. Fornes (University of Buenos Aires, Argentina)
microRNA-130 and microRNA-122 alteration are related to lipid metabolic impairments in the foetal liver of rats with gestational diabetes mellitus
OC9 J. Araos (Pontificia Universidad Católica de Chiile, Chile)
NHE1 modulates intracellular pH and cell proliferation in human ovarian cancer
OC10 I.A. Neves (University of Sao Paulo, Brazil)
Effects of recreational use of marijuana during pregnancy: placental morphofunctional changes in mice

OC11 T. Sáez (University of Groningen, The Netherlands & Pontificia Universidad Católica de Chile, Chile)
Feto-placental endothelial exosomes modulate high glucose-induced endothelial dysfunction in human umbilical vein endothelial cells

OC12 J.B. Moreli (Federal University of São Paulo, Brazil)
The role of endogenous annexin A1 (AnxA1) in pregnancy

09:30 – 10:30 Plenary Lecture 6 (Chair: E Bevilacqua)

Nick Illsley (Hackensack University Medical Center (HUMC), USA)
Epithelial-mesenchymal transition: the basic mechanism behind trophoblast invasion

10:30 – 11:00 Coffee

11:00 – 12:30 Symposia 11, 12, and 13

Room A

Symposium 11. Vascular Modulators: Role of Adenosine Receptors and Thyroid Hormones (Chairs: M González, C Escudero)

Andrés I Rodriguez (Universidad del Bío-Bío, Chile)
Role of adenosine A2A receptor in an in vivo model of melanoma angiogenesis and growth

Enrique Guzmán-Gutiérrez (Universidad San Sebastián, Chile)
Role of thyroid hormones metabolism on endothelial and trophoblast cells in gestational diabetes mellitus

Carlos Escudero (Universidad del Bío-Bío, Chile)
Reduced pro-angiogenic capacity in male mice lacking adenosine A2A receptor

Room B

Symposium 12. Infection, Toxins, and Pregnancy (Chair: Estela Bevilacqua)

Cristina Ibarra (Universidad de Buenos Aires, Argentina)
Virulence factors from Shiga toxin producing E coli could be one of the causes of foetal morbimortality

Estela Bevilacqua (Universidade de São Paulo, Brazil)
ZIKA virus infection in human placental cells

Ulrike Kemmerling (Universidad de Chile, Chile)
The epithelial turnover of the trophoblast constitutes a local placental innate immune response against Trypanosoma cruzi

Room C

Symposium 13. Pathogenesis of Preeclampsia (Chairs: F Sacerdoti, N Martínez)

Marijke Faas (University of Groningen, The Netherlands)

Immune cells in the placental bed in pregnancy and preeclampsia

Alicia Damiano (Universidad de Buenos Aires, Argentina)

New insights into the pathogenesis of preeclampsia: the role of placental aquaporins

Michael Post (Hospital for Sick Children & University of Toronto, Canada)

Lipid-endoglin interactions in preeclampsia

Rooms A, B, C

12:30 – 13:30 Plenary Lecture 7 (Chair: D Busso)

Alicia Jawerbaum (Universidad de Buenos Aires, Argentina)

Postnatal consequences of impaired development in maternal diabetes

13:30 – 14:00 Closing Ceremony and Awards

Plenary lectures (PL)

PL1
Metabolic adaptations in the mother for an optimal pregnancy outcome. What goes wrong in gestational diabetes?

D.J. Hill. *Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario N6A 4V2, Canada.*

The development of gestational diabetes (GDM) has been associated with a failure to adapt to the insulin resistance of pregnancy, with abnormal changes in placental function, the maternal-fetal immune interface, and in maternal β-cell mass during pregnancy. A number of clinical trials aimed at reducing the risk of GDM in at-risk women based on lifestyle changes alone have had minimal impact. We have focused on understanding the mechanisms controlling the adaptive changes in pancreatic β-cell mass that must occur in the mother to sustain euglycemia during pregnancy. The increase in β-cell number derives from both proliferation within islets, and from the maturation of immature β-cells that lack glucose transporter 2 (GLUT2) ($Ins^{+}Glut2^{LO}$ cells) and are predominantly located within small, extra-islet β-cell aggregates (BCA). Pancreata were collected from pregnant C57Bl/6 mice at gestational days (GD) 6 to 18, and postpartum D 7 and compared to control, non-pregnant animals. Beta cell mass was three times greater at GD 18 compared to non-pregnant mice. Total β-cell proliferation peaked at GD12, occurring both within islets (>6 β-cells) and in BCA (1-5 β-cells). The proportion of $Ins^{+}Glut2^{LO}$ cells also increased significantly during pregnancy and this preceding the increase in β-cell mass and proliferation, peaking at GD9 for both the islet and BCA compartments. An increase in the proportion of $Ins^{+}Glut2^{LO}$ cells that were proliferative ($Ki67^{+}$ positive) persisted until GD 15 within BCA, and the overall number of BCA was increased significantly at GD9. These results indicate that $Ins^{+}Glut2^{LO}$ β-cell progenitors likely contribute to β-cell expansion during pregnancy through an increased rate of proliferation and differentiation into new, functional β-cells. In order to model the onset of GDM pregnant mice were fed low protein (8%, LP) or control (20%, C) diets during gestation and lactation, and offspring weaned onto C diet. At 6-10 weeks age female offspring (F1) of LP or C-fed mothers were mated and given C diet throughout pregnancy. F1 females previously exposed to LP diet had impaired glucose tolerance compared to controls on GD 12 and PPD 7. In control mice β-cell mass increased three-fold during pregnancy (0.5±0.2 mg to 1.4±0.4 mg on GD 18, $p<0.001$). However, LP-exposed F1 females had a lower β-cell mass throughout gestation (GD 18, 1.0±0.3 mg, $p<0.05$ vs C), and the percent proliferating β-cells was significantly reduced in both islets and BCA. In LP-exposed F1 mice the relative number of $Ins^{+}Glut2^{LO}$ β-cells was significantly lower than in controls throughout gestation. Results suggest that the adaptive increase in β-cell mass seen during pregnancy was impaired in mothers previously exposed to LP diet, with poorer glucose tolerance

during and following gestation. This was associated with a decreased gestational proliferation of β-cells, and the number of putative progenitors in BCA. Hence, prior environmental programming in early life can precipitate GDM.

PL2
Angiogenesis and αKlhoto

J.L. Hillebrands. *University Medical Center Groningen (UMCG), University of Groningen, The Netherlands*

(*Abstract not available*)

PL3
Placental metabolic reprogramming induces adaptive maternal/foetal physiological responses to placental hypoxia

S. Zamudio, A. Al-Khan, N.P. Illsley. *Center for Abnormal Placentation, Department of Obstetrics and Gynecology, Hackensack University Medical Center, Hackensack, NJ, USA.*

Relative placental hypoxia is widely considered etiological in a variety of pregnancy pathologies. We describe placental metabolic reprogramming (MRP), an evolutionarily conserved cellular response to lowered oxygen availability. MR has been identified in cancer, where it is considered pathological, but also in exercising muscle, in hypertensive fibroblasts and ischemic angiogenic cells. MR is characterized by reversible down-regulation of mitochondrial oxygen consumption (hypometabolism) and increased aerobic glucose utilization. In pregnancy MRP appears to increase the gradient for O_2 transfer from mother to placenta to foetus, and is accompanied by altered maternal glucose homeostasis. We hypothesized that these alterations would show a gradient of response based on disease severity. Using Doppler ultrasound we quantified maternal and fetal blood flows and fetal O_2 and glucose delivery and consumption. Subjects comprised 68 control, 25 late-onset preeclamptics (PE), 13 early onset PE and 9 idiopathic intrauterine growth restriction (IUGR). Each pathology was associated with varying degrees of increase in maternal arterial-venous glucose extraction, lowered fetal glucose concentrations and O_2 and glucose consumption. MRP was most pronounced in early onset PE and least evident in late onset PE. Our investigations of MRP highlight how maternal physiology is impacted by placental function. It suggests that placental mitochondrial changes in PE and IUGR may be adaptive as opposed to pathological, the more common interpretation. Moreover, the MR mechanism of increased aerobic glucose consumption, resulting in lowered fetal glucose levels and consumption, likely contributes to fetal programming, which alters risk of chronic disease later in life.

PL4
Developing screening modalities for improving pregnancy outcomes - *ex nihilo nihil fit*

G.E. Rice. *Centre for Clinical Research, University of Queensland, Brisbane, Australia.*

The foundations of health throughout life are laid down during fetal development. Events that compromise fetal development have profound effects not only on the immediate outcomes of pregnancy but also on the life-long disease risk susceptibility of the offspring. In most cases, however, poor pregnancy outcome is not anticipated or diagnosed early enough to significantly change health outcomes. The early identification of women at risk of developing complications of pregnancy, therefore, is the first step in reducing the prevalence and severity of such complications. Currently available tests are either not of sufficient accuracy for first trimester, population-based screening; lack sufficient data to define clinical utility and justify implementation into standard practice; or no effective treatment is available. To deliver end-user requirements (*i.e*. improved pregnancy outcomes): better performing biomarkers and/or panels of biomarkers are required; the effectiveness of intervention strategies needs to be evidenced; and processes established to facilitate translation into practice. This presentation will highlight recent advances in the identification of early pregnancy biomarkers; clinical intervention strategies; and the translation of new clinical knowledge into improved health outcomes.

PL5
Oxygen sensing: Novel epigenetic mechanisms

I. Caniggia[1,2,3], M. Post[4], S, Alahari[1,2]. *[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital; Departments of [2]Physiology & [3]Ob/Gyn, University of Toronto, Toronto, Ontario, Canada. [4]Program in Physiology & Experimental Medicine, Peter Gilgan Centre for Research and Learning, Hospital for Sick Children, Toronto, Ontario, Canada.*

Persistent hypoxia due to impaired oxygen sensing is involved in the pathogenesis preeclampsia leading to excess HIF1A. The von Hippel Lindau tumour suppressor (VHL) protein is a key executor of the cellular hypoxic response and a crucial component of HIF1A degradation. However, its expression and regulation in preeclampsia remains to be established.
Objective: To examine the epigenetic mechanism(s) regulating the gene expression of *VHL* in the human placenta in physiological and pathological conditions.
Results: We show that the expression of VHL is downregulated in placentae from early-onset, but not late-onset (E-PE) preeclamptic pregnancies. In particular, we found that *VHL* gene expression is controlled by Jumonji domain containing protein

6 (JMJD6), a Fe(II) and oxygen-dependent demethylase that targets histones 3 (H3R2me2) and 4 (H4R3me2) at specific arginine residues, and that JMJD6 function/activity is altered in E-PE due to changes in oxygen and iron availability. Furthermore, we demonstrate that methylation of *VHL* DNA is also altered in preeclamptic placentae, resulting in its transcriptional repression.
Conclusion: We propose that epigenetic events involving methylation and demethylation act in concert to decrease *VHL* gene levels that, in turn, influence on impaired HIF1A homeostasis observed in preeclampsia.
Funding: CIHR.

PL 6
Epithelial-mesenchymal transition: the basic mechanism behind trophoblast invasion

N.P. Illsley, S. DaSilva-Arnold, A. Al-Khan, A. Natenzon, S. Zamudio.
Center for Abnormal Placentation, Department of Obstetrics and Gynecology, Hackensack University Medical Center, Hackensack, NJ, USA.

Many components of human trophoblast differentiation and invasion have been described. However there has little systematic investigation of the underlying mechanism. We hypothesized that the evolutionarily conserved process called the epithelial-mesenchymal transition (EMT) and known in gastrulation, wound healing, and cancer metastasis, was the mechanism by which cytotrophoblast (CTB) differentiated into the invasive extravillous trophoblast (EVT). Analysing gene expression markers of EMT we showed that this process was responsible for the conversion of first trimester CTB to EVT, although the mechanism differs from previously-defined, developmental EMT. In the third trimester, while EVT also displayed an EMT profile, the process was more limited, consistent with the less invasive phenotype of these cells. By contrast EVT obtained from abnormally invasive placenta (AIP; placenta accreta) demonstrated a profile closer to first trimester EVT, a record of the over-invasive trophoblast that characterize this pathology. EVT isolated from severe early-onset preeclamptic (PE) placenta showed the opposite; EMT markers suggest that these cells have a phenotype closer to the originating CTB, which matches the evidence showing unusually shallow or absent invasion in PE. Together, these data show that the primary molecular process in trophoblast differentiation, converting the anchored, polarized CTB to migratory, invasive EVT, is an EMT. It is active early in gestation but constrained by the third trimester. Abnormal invasion, as exemplified by AIP or PE, is associated with significant alterations in the EMT, consistent with over- and under-invasion respectively.

PL7
Postnatal consequences of impaired development in maternal diabetes

A. Jawerbaum. *Laboratory of Reproduction and Metabolism. CEFYBO-CONICET. School of Medicine, University of Buenos Aires, Argentina.*

Intrauterine programming of metabolic and cardiovascular diseases occurs in pregnancies complicated by diabetes. To understand the mechanisms of induction of these alterations animal models of diabetes and pregnancy are required. In our laboratory we have found that the offspring of mild diabetic rats have increased markers of a pro-oxidant/pro-inflammatory state in their hearts from the neonatal stage, alterations evident in a sex-dependent manner. Besides, the offspring of mild diabetic rats have increased circulating lipid levels from day 21 of age although increased circulating glucose concentrations from the fifth month of age. Interestingly, if normoglycaemic three month-old offspring of pregestational diabetic rats are mated with control males, the pregnant rats develop gestational diabetes (GDM). This *in utero*-programmed GDM model shows increased pro-inflammatory and pro-oxidant markers in the fetal liver and placenta. Besides, maternal treatments with mitochondrial antioxidants during the F0 pregnancy of mild diabetic rats prevent the increase in pro-oxidant/pro-inflammatory markers in the heart of the offspring as well as in the fetal liver and the placenta of the pregnant offspring that develop GDM. Moreover, diets enriched in unsaturated fatty acids capable of activating PPAR nuclear receptors, administered to mild diabetic rats during pregnancy (F0 generation), reduce the pro-oxidant/pro-inflammatory state in the heart of the weanling offspring, prevent aberrant lipid accumulation in the heart of the adult offspring, and prevent fetal overweight and increased pro-oxidant/pro-inflammatory markers in the placenta from the female offspring that develop GDM. These results point to oxidative stress as a relevant mechanism involved in intrauterine programming in maternal diabetes and highlight the ability of treatments with antioxidant capacity to ameliorate the impact of adverse intrauterine programming.

SLIMP Award 2015 Lecture

Characterization and distribution profile of hematopoietic cells in mouse placenta

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Previous studies identified hematopoietic potential of mid-gestational human and mouse placentas. However, placenta contribution to hematopoietic ontogeny is not clear. **Objectives:** (a) Characterize hematopoietic cells in mouse placenta over midgestation, identifying their topography and niche (b) Establish the origin of

hematopoietic cells and whether progeny from these placental cell lineages contribute to postnatal hematopoiesis. **Methods:** We used histology, immunohistochemistry (IHC) and whole-mount fluorescence to visualize hematopoietic cells *in situ*. Further experiments include qRT-PCR after Laser Capture Microdissection (LCM) and renal subcapsular grafts, in which ectoplacental cones from mice with GFP background were implanted underneath kidney capsule of receptor mice. **Results:** Two niches were present in the labyrinth of mouse placentas at 10.5 and 11.5 gestational days. Placenta seems to produce hematopoietic stem/progenitor cells and a second wave of erythrocytes in the labyrinthine region close to the junctional zone. Some trophoblast giant cells and decidual cells may provide an erythopoiesis-promoting niche. A close relationship was apparent between placental hematopoietic and endothelial cell origins, suggesting the existence of a bipotent precursor or a hemogenic endothelium. IHC suggested close association between trophoblast and hematopoietic precursor cells. Preliminary data co-localized GFP+ cells with endothelial (CD31) or hematopoietic marker (Runx1) in the renal subcapsular grafts. These results highlight that at least some hematopoietic cells in placenta have unique placental precursor cells that are present prior to allantoic fusion. Further analyses of renal subcapsular grafts and gene expression profile after LCM are being undertaken. FIOCRUZ, CAPES, CNPq, Faperj, NSERC and Canada Research Chairs Program.

Symposia (S)

Symposium 1 (S1)
Placental Dysfunction and Pregnancy Outcome

S1-1
New strategies to prevent preterm birth

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Preterm delivery (PD) is the leading cause of neonatal mortality and contributes to delayed physical and cognitive development in children. At present, there is no efficient therapy to prevent preterm labour. Our group developed a murine model of preterm labour, induced by injections of bacterial lipopolysaccharide (LPS) that induces a 100% incidence of PD. In this model we assayed new therapeutic tools to prevent preterm labour and to increase offspring survival. Environmental enrichment (EE) strategy has been found to be beneficial in animal models of several

neurodegenerative diseases and reverses some consequences of prenatal stress. In general, EE appears to be associated with an overall improvement in the psychological and physical wellbeing of animals. So we assayed the possibility that the EE could prevent the consequences of inflammation induced by bacterial components on pregnancy and labour. In this sense mice were maintained either under a standard condition or upon an enriched environment. Surprisingly we observed that EE prevented preterm delivery in 35% of cases. We also tested two pharmacological treatments to prevent PD. Melatonin, the main product of the pineal gland, contributes to gestation maintenance by stimulating progesterone production and maintaining uterine quiescence. We observed that maternal administration of melatonin prevented LPS-induced PD in 50% of the cases and increased offspring survival. Resveratrol is a polyphenol abundantly found in grapes and red wine exhibiting beneficial health effects owing to its antioxidant and anti- inflammatory properties. We investigated the putative preventive effect of resveratrol on PD as well as the mechanism involved in such protection.

S1-2

Intrauterine growth trajectories modify the epigenetic programming of vascular-related genes in human umbilical artery endothelium and cord adiponectin levels

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The association of low and excessive foetal weight with cardiovascular risk are in the central interest of DOHaD. We have previously showed that umbilical artery endothelium (HUAEC) from intrauterine growth restricted (IUGR) foetuses show deficient endothelium-dependent relaxation, high *NOS3* expression and low NOS activity. The correlation of foetal adiponectin levels and body size are under debate. **Objective:** Due to the key role of endothelium in the umbilical cord vascular function we studied markers of endothelial epigenetic heterogeneity in HUAEC from IUGR, control and large for gestational age (LGA) foetuses, and adiponectin cord levels. **Methods:** In HUAEC from IUGR, control and LGA fetuses the expression of several vascular-related genes were determined. The methylation (pyrosecuencing) at the promoter of these genes as well as histone modifications (H3 & H4) was determined by ChIP. Knockdown of DNMT1 was performed to study methylation-dependent changes. Adiponectin concentrate on was determined by ELISA. **Results:** In IUGR-derived HUAEC there is an increase in the basal expression of eNOS. *NOS3* showed significant decrease in methylation at its promoter region in IUGR and LGA along with open chromatin hallmarks (H3K9 Ac, H4K12 Ac). DNMT1-knockdown

modified the expression of eNOS in IUGR and LGA compared to AGA, and this was associated to changes in methylation in CpG -352 in the NOS3 gene promoter. Foetal plasma adiponectin levels were increased in LGA compared to AGA foetuses and it was correlated with maternal weight and BMI at first visit, fetal birth weight and height. **Conclusion**: In summary both extreme phenotypes (IUGR and LGA) show epigenetic programming in some key endothelial genes and differ in adiponectin concentration, which is a determinant of birth size, independent of maternal nutritional status and other anthropometric foetoplacental variables.
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S1-3

Trophoblast cells regulate immune cell functional profile through VIP-mediated pathways

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Deep placentation disorders such as preeclampsia are associated with loss of immune homeostasis. Maternal leukocytes are recruited to the maternal-placental interface from the beginning of pregnancy and both normal placentation and the success of pregnancy strongly depend on an appropriate communication established with trophoblast cells. From an immunological standpoint, normal placentation is associated with the maintenance of immune homeostasis through redundant loops of cell-to-cell interaction as well as the local release of mediators to sustain an anti-inflammatory microenvironment. Among those factors we have proposed the vasoactive intestinal peptide (VIP) and its high affinity receptors VPAC to have a central role. **Objective**: To explore the relevance of trophoblast cell VIP at the early maternal-placental interface as well as its impact on trophoblast function and interaction with immune cells. **Methods**: Trophoblast derived cell lines Swan 71 and HTR8 were transfected with a VIP siRNA to knock down its expression or with an irrelevant siRNA as a control. Blood monocytes or neutrophils from healthy donors were co-cultured with trophoblast cells or their conditioned media and immune and trophoblast cell functional profiles were assessed. **Results**: VIP deficient trophoblast cells exhibited an impaired migration and failed to promote an M2 macrophage profile, as well as to deactivate neutrophils primed with pro-inflammatory stimuli. **Conclusions**: Results support that the loss of immune homeostasis at the maternal-placental interface involves an impaired trophoblast migration and invasion associated with a defective activation of VIP/VPAC system that fails to modulate both trophoblast function and trophoblast-immune interaction.

Symposium 2 (S2)
Metabolic and Endocrine Modulators of Pregnancy

S2-1
Role of leptin in the molecular physiology of the placenta

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Leptin is a key hormone in placental physiology. It regulates trophoblast proliferation, inhibits apoptosis, stimulates protein synthesis, and regulates foetal growth and development. Previous results demonstrated that estradiol (E_2) regulates leptin expression. **Objectives:** We analysed the effect of specific protein 1 (SP1) and cAMP signalling pathway in the induction of leptin expression by E_2 in human placental cells. We also study the mechanisms that mediate the antiapoptotic effect of leptin in placenta and a possible role of leptinon cell migration and invasion. **Methods**: BeWo and Swan cells, cultured under standard conditions, and human placental explants were used. Western blot, qRT-PCR and transfection assays were carried out. All procedures counted with the approval from the Ethical review committee of the Hospital Nacional Alejandro Posadas. **Results**: We observed that SP1 and cAMP-protein kinase A pathway increased leptin promoter activity. SP1 effect is oestrogen receptor alpha (ER ✔) dependent. The inhibitors H89 and SQ22536, and HDAC protein diminished E_2 induction of leptin. We have demonstrated also that p53, is downregulated in the presence of leptin under serum deprivation or hypoxia involving mitogen-activated protein kinases (MAPK) and phosphatidylinositol-3-kinase (PI3K) signalling pathways. Leptin also augments the level of Mdm2 protein, a regulator of p53 half-life. On the other handleptin reduced E-cadherin and induced β1 integrin expression. Moreover wound healing assay and invasion assay demonstrated that leptin promotes cell migration and invasion. **Conclusions**: All these findings suggest that leptin expression is tightly regulated and improve the comprehension of the mechanisms whereby E_2 regulates leptin expression and leptin function during pregnancy.

S2-2
Maternal dietary composition programs offspring pancreatic development reducing or increasing the risks of glucose impairment in adulthood

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Maternal health due to Type 1 (T1D) and Type 2 (T2D) diabetes and diets lacking proteins or in excess of fat and fructose during gestation impacts fetal development. The chronic hyperglycaemia and lipotoxicity of diabetes leads to oxidative stress affecting fetal-placental unit increasing prenatal morbidity and mortality despite the advances in glycaemic control. Macroscopic alterations in the process of closure of the neural tube and cardiac defects are still present at birth. Programming molecular and cellular modifications in utero may have serious consequences in adulthood. Previously, we have shown that protein restriction during pregnancy increases glucose intolerance in 4 months rats. More so, the addition of safflower oil or olive oil to the diets of T1D mother's reduced the inflammatory fetal-placental environment rats by the activation of a family of peroxisome proliferator activated nuclear receptors (PPARs) regulating metabolic and anti-inflammatory pathways during development and reducing fatty acid oxidation in fetal diabetic hearts. **Objective**: The objective of this study was to examine the molecular mechanisms involved on the development and function of the endocrine pancreas postnatally when olive oil was supplemented to the diets of T1D mothers or a westernized diet rich in fats and fructose was given to an intrauterine restriction model with uterine ligation. **Methods:** Morphometric and gene expression of PPARs and target genes involved in the differentiation of β-cells were analysed. **Results:** the addition of olive oil to dams rescued the offspring from insulin resistance meanwhile the westernized diet increased this predisposition at 4 months. **Conclusions**: Individual customized dietary intervention is needed during pregnancy to reduce the burden of metabolic disease.

S2-3

Insight Inside: Imaging fetal adipose tissue development with 3D water-fat MRI

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Infants born small (SGA) or large for gestational age (LGA) and after pregnancies complicated by maternal diabetes have increased risk of mortality and morbidity promoting future obesity and metabolic disease. In SGA, this is likely the result of a nutritional mismatch before and after birth, while excess storage and increased availability of nutrients appears to be the pathophysiology in LGA offspring; resulting in promotion of adipose tissue development in both situations. **Objective:** We sought to investigate abnormal fetal fat development indicative of future metabolic health risk *in vivo* using a novel MRI technique. **Methods:** 3D water-fat MRI, a technique sensitive to the lipid content of tissues, was used to assess the transition from water-filled pre-adipocytes into mature fat-filled adipocytes in fetal subcutaneous adipose tissue (FSAT). 3D water-fat MRI provides a fat fraction (FF = fat/(water + fat)) that quantifies the proportion of MRI signal received from lipid. **Results:** In pregnancies with gestational ages between 28 and 38 weeks, we found no correlation between FF

or lipid volume and maternal pre-pregnancy BMI or EFW percentile, but observed a significant positive correlation between FF and lipid volume and gestational age. We describe the distribution and maturation of FSAT in normal, obese and diabetic pregnancies. **Conclusion:** 3D water-fat MRI can be used to non-invasively study fetal adipose tissue development in mid- to late gestation, a crucial developmental stage during which adipocytes rapidly fill with lipid. This technique can improve our understanding of fetal metabolic health in pregnancies complicated with diabetes, obesity and abnormal fetal growth.

Symposium 3 (S3)

SCHCF – Skeletal Muscle Function: Focus on Physiological Signaling for Autophagy, Mitochondrial Ca^{2+} Control and GLUT4 Trafficking

S3-1

IP3 receptor modulates autophagy, mitochondrial dynamics and mitophagy in skeletal muscle: New insights muscular dystrophy treatment

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Duchenne Muscular Dystrophy (DMD) is a recessive X-linked genetic disease, caused by mutations of the dystrophin gene. Several reports describe that IP_3 receptor (IP_3R) is essential for muscle function and maintenance of cellular bioenergetics. This is due to the participation of this receptor in calcium transfer from the ER to the mitochondria. Changes in IP_3R function can compromise mitochondrial function to finally alter autophagy. Until now there is no detailed study of the IP_3R/calcium/autophagy axis in DMD. **Objective**: Our aim was to investigate the participation of IP_3R in the regulation of mitochondria calcium and basal autophagy/mitophagy in fibers from mdx model. **Methods:** Mice were either electroporated with an shRNA for IP_3R1 or treated with the purinergic receptor inhibitor, suramine. Afterwards we analyzed the expression of several autophagy/mitophagy proteins and mitochondrial calcium in isolated muscle fibers. Finally, mice performed strength tests to evaluate the beneficial effects of treatment. **Results:** We found differences in basal expression levels of autophagy/mitophagy proteins, like LC3II, p62 and Parkin, between mdx and wt fibers. When IP_3R knockdown were performed, we observed changes in the expression of almost all of the proteins analyzed. Similar results were observed in mice treated with suramine. Finally, mdx mice increased their strength performance after suramine treatment and also presented a reduction in the number of central nuclei and extracellular matrix that correlated with lower levels of serum CK. **Conclusion:** Inhibition of the IP_3 pathway

should be tested as a new therapeutic target for the muscle weakness observed in DMD patients.
Funding: FONDECYT 3140491(DV), 3140532(FW), 1151293(EJ), FONDAP 15130011(SL)

S3-2
Excitation-metabolism coupling in skeletal muscle: Ca^{2+} handling and mitochondrial function

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Elucidating the mechanisms that link fiber contraction with the necessary increase in ATP synthesis is important to understand skeletal muscle function. Mitochondria exhibit a particular architecture in muscle. A large fraction of mitochondria resides in close proximity to myofibrils flanking the z line, where ATP production is essential for the shortening of sarcomere and contraction. Subsarcolemmal mitochondria have a different distribution, contain different components of the metabolic protein complexes and appear to have different properties in the regulation of muscle metabolism. The combined role of these two mitochondria populations is completely unknown. **Methods:** We studied mitochondrial Ca^{2+} transients with molecular tools, shown specific subcellular distribution, following depolarization or electrical stimulation of single skeletal muscle fibers, and their relation with metabolic output and membrane potential changes in mitochondria. **Results:** Surface depolarization of adult fibers increased both cytoplasmic and mitochondrial Ca^{2+} levels. Depolarization-dependent mitochondrial Ca^{2+} uptake required functional IP3R and RyR1 channels. Moreover, inhibition of either one of these channels decreased basal O_2 consumption rate but only RyR1 inhibition decreased ATP-linked O_2 consumption. Depolarization induced Ca^{2+} signals in sub-sarcolemmal mitochondria were accompanied by a reduction in mitochondria membrane potential but Ca^{2+} signals propagate towards intermyofibrillar mitochondria, where mitochondrial membrane potential increases. These results are compatible with Ca^{2+}- dependent propagation of mitochondrial membrane potential from the surface towards the center of the fiber. **Conclusion:** Signaling in the form of mitochondrial membrane potential propagation appears to be a new and central mechanism for fast metabolic regulation of skeletal muscle by surface depolarizing stimuli.
Funding: Proyecto Fondecyt 11130340.

S3-3
Cell physiology of skeletal muscle: mechanisms of GLUT4 traffic and cytoskeleton implications

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Skeletal muscle glucose uptake during exercise involves the active recruitment of GLUT4 to the muscle cell surface. Key to this process is Rac-dependent reorganization of actin beneath the plasma membrane both in response to insulin and to contraction, but the intracellular mechanisms that mediate these effects are not well understood. Using L6 rat skeletal myoblasts stably expressing myc-tagged GLUT4, we found that exogenous ATP increased GLUT4 translocation to the cell surface and glucose uptake, both process requires actin filament remodeling. Members of the Rho GTPases family, like Rac1 and Cdc42 are responsible for the cortical actin polymerization evoked by exogenous ATP, ATP-dependent Rac1 and Cdc42 activation was also inhibited by the phosphatidylinositol 3-kinase (PI3K) inhibitor LY-294002, suggesting that both Rac1 and Cdc42 are effectors downsteam of PI3K. ATP-dependent GLUT4 translocation was inhibited by Arp2/3 inhibitor (CK-869) or by siRNA-mediated silencing of Arp3 subunit of the Arp2/3 complex. ATP also led to dephosphorylation of the actin-severing protein cofilin on Ser3. Cofilin knockdown via siRNA partially inhibited GLUT4 translocation. We propose that ATP-dependent actin remodeling mediated by Rac1 and Cdc42 effectors downstream of PI3K, modulates both Arp2/3 and cofilin in a coordinate a dynamic cycle of actin branching and severing at the cell cortex, essential for ATP-mediated GLUT4 translocation in muscle cells.
Funding: Proyecto Fondecyt 11130424, Proyecto anillo ACT1411

Symposium 4 (S4)
Key Players at Implantation

S4-1
Hyperglycosylated chorionic gonadotropin as a modulating factor for embryo implantation and trophoblast invasion

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Hyperglycosylated human chorionic gonadotropin H (hCG-H) is a variant for hCG with much larger oligosaccharides which has shown to have unique biological functions on maternal and embryonic tissues. hCG-H could be detected in conditioned medium from developing human embryos *in vitro* after hatching, suggesting it may play a paracrine role in the endometrial epithelium during the implantation process.

Later on, hCG-H accounts for up to 90% of total circulating hCG during the 2nd to 3rd week of pregnancy when trophoblast invasion is high and falls to negligible levels (<5%) by the end of the first trimester. Functionally, hCG-H seems to promote trophoblast invasion during early placentation and has potential roles in regulating immune cell function and angiogenesis within the endometrium at the beginning of pregnancy. However, the effects that it may exert in the endometrial cells and its role in modulating the endometrial receptivity are unknown. We have studied the signal transduction pathways activated by hCG-H in endometrial epithelial (EEC) and stromal cells (ESC) and its potential crosstalk with the transforming growth factor beta 1 (TGF-beta1). In addition, we have found that hCG-H induces unique gene expression regulation responses in EEC relevant for endometrial receptivity. Also, we have studied the effects of hCG-H on the secretion of extracellular remodelling elements from ESC and decidualized ESC; and how this modulation regulates the invasive potential of HTR8/SVneo cells. Understanding the functional roles of hCG-H may contribute to our knowledge on the aetiology of conditions such as recurrent pregnancy loss and preeclampsia.

S4-2
Role of lipid mediators at the maternal-foetal interphase

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We study the role of lipid mediators at the maternal-fetal interface. Lipids are a very heterogeneous group of substances that play important role in diverse biologic functions. In particular, we have paid special attention to endocannabinoids, prostaglandins, and lysophosphatidic acid, and their actions on the uterine function and first trimester trophoblast behaviour. We observed that these lipids crosstalk in the uterine tissue at the sites of embryo implantation, regulating the expression of molecular markers of vascularization (interleukin-10) and decidualization (insulin-like growth factor binding protein 1 (IGFBP-1) and prolactin), and thus acting as proimplantatory factors. Endocannabinoids level close to and at implantation sites seem to modulate nitric oxide synthase activity, via cannabinoid receptors and the presence of the blastocyst. Also, the disruption of endogenous lysophosphatidic acid signalling by blocking lysophosphatidic acid 3 receptor (LPA3) modified the development of uterine vessels with consequences in the formation of the decidua and placenta, and in the growth of the embryos. Lysophosphatidic acid enhances vascularization and migration of first trimester trophoblast cells through LPA3 and increasing the expression of cyclooxygenase-2 protein and prostaglandin 2 generation. Interestingly, lysophosphatidic acid-stimulated trophoblast exerts paracrine actions over endothelial cells increasing their migration. Recently, we observed that oestradiol and progesterone, the master hormones that orchestrate the

events that take place during implantation, regulate trophoblast vascularization through lysophosphatidic acid and LPA3 receptor. All together, our results show that lipid mediators could regulate the implantation process by modulating crucial events at the maternal and the fetal components of the maternal-foetal interphase.

S4-3

In vivo and *in vitro* models for implantation studies in maternal alcohol ingestion. Role of matrix metalloproteinases and cell adhesion molecules

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During the implantation, complex events of trophoblast invasion and penetration take place into maternal uterus to conduct normal embryonic development at post-implantation stages. In preimplantation, cavitation, expansion and hatching of the blastocyst as well as the correct expression and assembly of cell adhesion molecules, are requirements for the beginning of the implantation. During the implantation window, the trophoblast differentiation and invasiveness becomes relevant to lead the complete penetration of the embryo into the maternal tissue (day 8 of gestation). Throughout implantation, the matrix metalloproteases (MMPs), the key regulatory mediators in the placental cells, are involved in regulating trophoblast invasion to maintain a normal utero-placental homeostasis and to determine the proper embryo development and fetal survival at term. Dysregulation of these mechanisms may lead to early embryo loss. *In vitro* implantation as well as the ectoplacental cone explants and culture are useful to study the implantation loss, the inadequate trophoblast invasiveness and the maternal-fetal interaction defects, addressing the quantity and quality of adaptive and/or altered processes at periimplantation stages. We previously reported complications of pregnancy and embryo loss due to perigestational alcohol consumption up to early pregnancy. Alcohol 10 % exposure before gestation and up to day 4 of gestation induced disassembly and altered expression of E-cadherin and ZO-1 leading to abnormal cavitation and expansion of the blastocysts. At day 5 of gestation, the reduced number of hatched-implantative embryos resulted in delayed embryo development at advanced implantation stages (24 and 48 h of *in vitro* culture). During *in vitro* implantation, the trophoblast invasiveness was dysregulated, which can lead to deficient expansion of the ectoplacental cone (EC) *in vivo* (day 8 of gestation). In parallel with the reduced expansion of EC at 24 and 48 h of *in vitro* EC-culture, the *in vivo* studies revealed diminished EC-MMP-9 gene and protein expression. Instead, the increase of MMP-2 expression in the proliferative zone of the EC was detected with diminish E-cadherin in trophoblast cells and maternal-fetal interface. Perigestational alcohol consumption produces imbalances of MMP-2 and MMP-9 expression in trophoblast cells during peri-implantation stages probably

conducting to the embryo resorption seen at early organogenesis (day 10 of gestation) and/or abnormal placenta at term.

Symposium 5 (S5)
Role of Thyroid Hormones in Pregnancy

S5-1
The impact of thyroid hormone deficiency during pregnancy in infectious diseases

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A deficit of maternal thyroid hormones during gestation has detrimental consequences in the central nervous system of the offspring, impairing motor abilities, learning, and memory. However, there are no studies showing if maternal hypothyroidism during gestation impacts the response of the offspring to infections.
Objectives: To evaluated whether adult mice gestated in hypothyroid mothers have an altered response to pneumococcal pneumonia. **Methods**: The drug methimazole was used to induce hypothyroidism in pregnant mice, and then the adult progeny was infected with *Streptococcus pneumoniae* D39, an invasive strain able to cause pneumonia, sepsis, and meningitis. Survival rate, clinical score, bacterial load in lungs, blood and brain, immune cell presence in lungs and BAL, cytokine mRNA in lungs, spleen and brain, cytokine protein levels in blood, lung tissue damage, and lung vascular permeability was evaluated. **Results**: As compared to mice gestated under normal conditions, female mice gestated in hypothyroid mothers had increased survival rate, lower clinical score, and lower bacterial load in blood and brain. Further, female mice gestated under maternal hypothyroidism had higher amounts of innate cells inside the lungs, and reduced production of cytokines characteristic of sepsis in spleen and blood at 48 h after infection. Interestingly, these mice also have their vascular permeability basally increased in the lungs. **Conclusions**: These results suggest that the innate immune response and the permeability of barriers could be

affected in the female offspring from hypothyroid mothers, increasing the resistance to bacterial infections.

S5-2
The physiological consequences of maternal thyroid hormone deficiencies in their offspring

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Hypothyroidism and hypothyroxinemia are thyroid hormone deficiencies conditions highly frequent worldwide. The most important causes of these conditions are iodide deficiency diet and thyroid autoimmune diseases. Both conditions during gestation are responsible for irreversible consequences in the offspring. It has been shown that maternal hypothyrodism or maternal hypothyroxinemia induce an imprinting on the fetus central nervous system impairing cognitive functions. We have shown that the consequences of maternal hypothyroidism and maternal hypothyroxinemia during gestation spread out to the immune system of the offspring by increasing the immune response and inflammation in autoimmune diseases. We have studied the immune response of mice gestated in hypothyroidism or hypothyroxinemia once in the adulthood they were induced with experimental autoimmune disease (EAE). EAE is an experimental model to study multiple sclerosis. Our results strongly support that the maternal thyroid hormones can cause an imprinting to the offspring immune system and can be determinant in the outcome of autoimmune diseases. These results support the importance to study the impact of thyroid hormones deficiencies in human aiming to improve the quality of life of the future generations. Funding: FONDECYT 1161525, Instituto Milenio P09/016-F, Núcleo UNAB DI-471-15/N.

S5-3
Micronutrient deficiencies and the thyroid in pregnancy

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Many environmental and nutritional determinants have an impact on the epidemiology and severity of thyroid diseases. Among them deficiencies in iodine, selenium and iron are well documented. In some regions combined deficiencies of these elements interact to impair thyroid function. We reviewed these interactions. Severe iodine deficiency causes endemic goiter and impaired cognitive function. Mild iodine deficiency (MID), prevalent in many European countries, increases the

frequency of thyroid nodular disease. In addition, a potential effect on the cognitive function of children exposed to MID during pregnancy has been suggested. Selenium deficiency is restricted to a few regions in the world. The enzymes activating or deactivating thyroid hormones are selenoproteins, consequently selenium deficiency decreases their activity. In iodine and selenium deficient regions both deficiencies interact to affect thyroid metabolism. Iron deficiency is common worldwide including in industrialized countries. In women with iron deficiency anemia thyroxine and triiothyronine concentrations are reduced. Iron deficiency anemia reduces the activity of thyroid peroxidase, a Fe-dependent enzyme, involved in the iodination of thyroglobulin. In pregnant women iron deficiency has been shown to predict lower thyroxine and higher thyroid stimulating hormone. In conclusion, severe iodine deficiency during pregnancy may result in maternal and fetal hypothyroidism and impaired cognitive function in children. However, the deleterious effect of MID on fetal thyroid and child cognitive function is not yet fully established. Another area for research concerns the effects of the very frequent combination of iron deficiency and MID on thyroid function during pregnancy.

Symposium 6 (S6)
Early life programming

S6-1
Gestational chronodisruption impairs circadian physiology in rat male offspring, increasing the risk of chronic disease

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Chronic exposure to light at night, as in shift work, alters biological clocks (chronodisruption), impacting negatively pregnancy outcome in humans. Actually, the interaction of maternal and foetal circadian systems could be a key factor determining a fitting health in adults. **Objectives:** We investigated whether chronic photoperiod shifts (CPS) during pregnancy modify maternal circadian rhythms and impair circadian physiology in their adult offspring, increasing health risks. Thus, pregnant rats were exposed to normal photoperiod (12 h-light/12 h-dark) or to CSP until 85% gestation. The effects of gestational CPS were evaluated on the mother and adult offspring. **Methods:** In the mother at 18 days of gestation we measured rhythms of heart-rate, body temperature, and activity through gestation, and daily rhythms of plasma variables: melatonin, corticosterone, aldosterone, and markers of renal function. In adult offspring we measured rhythms of clock gene expression in the

suprachiasmatic nucleus (SCN), locomotor activity, body temperature, heart rate, blood pressure, plasma variables, glucose tolerance, and corticosterone response to adrenocorticotropic hormone (ACTH). **Results:** CPS altered all maternal circadian rhythms; lengthened gestation and increased newborn weight. The adult CPS offspring presented normal rhythms of clock gene expression in the SCN, locomotor activity and body temperature. However, the daily rhythm of plasma melatonin was absent, and corticosterone, aldosterone, renal markers, blood pressure, and heart-rate rhythms were altered. Moreover, CPS offspring presented decreased glucose tolerance and abnormal corticosterone response to ACTH. **Conclusion:** Gestational chronodisruption induced long-term effects on the offspring circadian system, where a normal SCN coexists with altered endocrine, cardiovascular and metabolic function.

S6-2

Can weaning dietary manipulation counteract the effect of poor nutrition during pregnancy and lactation?

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The interaction between fetal programming and postnatal environment suggests that weaning diet could amplify or attenuate programmed outcomes under the 'two hit hypothesis'. **Objectives:** I) To examine if the offspring from sucrose-rich diet-dams (SRD-dams) weaned on a control diet develop normal glucose and lipid homeostasis in adulthood; II) To investigate whether dietary n-3 long-chain polyunsaturated fatty acids (n-3 PUFAs) at weaning result in an amelioration of dyslipidaemia, adiposity, liver steatosis, hypertension, and glucose homeostasis induced by SRD from the onset of pregnancy up to adulthood. **Methods:** At weaning and until 150 days of life offspring from SRD-dams were fed: A) control diet (CD), B) SRD+fish oil (rich in 20:5n-3 and 20:6n-3 fatty acids) or SRD+chia seed (rich in 18:3n-3 fatty acids) replacing corn oil (rich in 18:2n-6 fatty acids) in the SRD. **Results**: I) Offspring from SRD-dams weaned on a CD showed: increased adiposity, dyslipidaemia, hepatic steatosis, and altered glucose homeostasis, supporting the hypothesis that early life exposure to SRD lead to an unfavorable profile in adulthood regardless of whether offspring consumed SRD after weaning. II) The presence of n-3PUFAs from both sources of fat in the SRD at weaning was able to prevent the major lipid disorders in adult offspring associated with accretions of n-3 PUFA in liver homogenate. Moreover the presence of 18:3n-3 at weaning improved hypertension and glucose homeostasis. **Conclusion**: These data confirm that dietary manipulation at weaning with both sources of n-3 PUFAs provide a viable option for prevent/mitigate adverse outcomes induced by SRD from *in utero* to adulthood.

S6-3

Exposure to an experience-dependent plasticity paradigm during pregnancy and lactation modulates offspring energy homeostasis

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Environmental factors have a major role on metabolic programming. Environmental enrichment is a widely used paradigm to induce experience-dependent plasticity and it is capable of changing the expression of energy homeostasis-related genes when it is administrated at post weaning period. However, little is known about the effect of this paradigm when it is administrated during early development. **Objective:** To determine the physiological and molecular consequences on energy homeostasis phenotype in the offspring of mice exposed to an experience-dependent plasticity paradigm during pregnancy and lactation. **Methods:** We established C57/B6 mice breeding pairs exposed to either an enriched or control conditions until weaning. After that, male offspring from both conditions were housed in standard cages and later evaluated. **Results:** Our results show that the offspring of mice exposed to an experience-dependent plasticity paradigm had an increased body weight, enhanced locomotor activity, improved glucose tolerance and insulin sensitivity, and a transient decrease in food intake. To determine the molecular changes underlying this physiological phenotype, we evaluated the expression of genes associated to energy homeostasis and its promoter methylation. We found changes the expression of genes associated to a modification of its promoter methylation patterns. **Conclusions:** Our findings show that the exposure to an experience-dependent plasticity paradigm during pregnancy and lactation modulates offspring metabolic phenotype through epigenetic modifications.
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Symposium 7 (S7)
Angiogenesis and Vascular Remodelling

S7-1

Is angiogenesis altered in the offspring of mothers with preeclampsia? Potential role of A_{2A} adenosine receptor-nitric oxide-vascular endothelial growth factor pathway

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Indirect evidences suggest reduced angiogenesis in offspring born to preeclampsia. Adenosine, via nitric oxide (NO) and vascular endothelial growth factor (VEGF) may regulate angiogenesis. **Objectives**: To investigate whether A_{2A} adenosine receptor (A_{2A}AR)/NO/VEGF expression is impaired in offspring from pre-eclampsia. **Methods**: In human, cultures of umbilical vein endothelial cells (HUVEC) and placental microvascular endothelial cells (hPMEC) from normal pregnancies and women with preeclampsia were used for estimation of mRNA levels of VEGF, cell proliferation and angiogenesis *in vitro* in presence or absence of siRNA for A_{2A}AR. Also, preeclampsia-like syndrome was generated in pregnant mice (C56BJ) exposed to the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 50 mg/kg). *In vivo* blood perfusion using Laser Doppler was analysed at day 7 postnatal in offspring (F1), exposed or not to preeclampsia-like syndrome. **Results**: In human, A_{2A}AR stimulation increases cell proliferation and VEGF in HUVEC and hPMEC derived from normal pregnancy. This last effect was blocked by the A_{2A}AR antagonist, ZM-241385 (10^{-5} M), or by L-NAME. Contrary to HUVEC from preeclampsia, S-nitroso-N-acetyl-penicillamine oxide (SNAP, 10^{-9} M), a NO donor, partially recovered VEGF expression, cell proliferation and *in vitro* angiogenesis capacity, in cells from normal pregnancies exposed to siRNA for A_{2A}. In mice, peripheral blood perfusion was reduced in F_1 from preeclampsia compared to respective littermate in control group. **Conclusions**: Functional presence of A_{2A}AR/NO/VEGF pathway for regulating proangiogenic behaviour was demonstrated in foetoplacental endothelium from normal pregnancy. But, in cells from preeclampsia, impaired cell response to NO-donor, was observed. Whether this alteration might be linked with reduced peripheral angiogenesis in F1 from preeclampsia is still unclear.
Funding: FONDECYT 1140586, FONDEQUIP EQM140104, DIUBB GI153109/EF and GI 152920/EF.

S7-2

Netrin-1 acts as a non-canonical angiogenic factor produced by human Wharton's Jelly Mesenchymal Stem Cells

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Recently classified as non–canonical angiogenic molecules, Netrins promote or inhibit angiogenesis depending on the physiological context. These chemotropic ligands act through their immunoglobulin-like transmembrane receptors, which

belong to the DCC/NEO1 and UNC families. Stromal tissue of the umbilical cord, named WJ-MSC, is known to produce classic angiogenic factors; however, the putative role of stromal Netrins to attract/repel growing blood vessels in the foetal-placental unit has not been explored yet. **Objective:** We investigated Netrins function in relation to their putative receptors.**Methods**: Human umbilical vein endothelial cells (HUVEC) were used, and complemented with *in vivo* experiments using the classical Chicken Chorioallantoic Membrane (CAM) assay. **Results:** WJ-MSCs conditioned medium promotes angiogenesis *in vitro* and *in vivo,* and this effect is partially mediated by Netrin-1 signalling, but independent of DCC and/or G-protein-coupled adenosine A_{2A} and A_{2B}-receptors. **Conclusions:** Our findings reveal that stromal production of Netrin-1 is a critical component of the vascular regulatory machinery. Netrin-1, through an unknown receptor, presumably acts as a trophic mediator that contributes to angiogenic homeostasis in the microenvironment of the foetoplacental unit.
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S7-3
αKlotho in vascular (patho)physiology

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Cardiovascular disease, including vascular calcification, is prevalent in patients with chronic kidney disease (CKD). CKD is a state of α-Klotho deficiency. α-Klotho is highly expressed in the kidney and exerts a role as obligate co-factor for the bone-derived phosphaturic hormone Fibroblast Growth Factor-23. When CKD progresses, renal α-Klotho expression decreases and which may be accompanied by reduced circulating α-Klotho levels. Circulating α-Klotho is mainly derived from the kidney and is shed in blood and urine by ADAM10 and ADAM17 cleavage from the cell membrane. Data obtained in pre-clinical models revealed that the vascular phenotype of α-Klotho deficiency features endothelial dysfunction, medial calcification, arterial stiffening, and hypertension, with characteristics similar to aged human arteries. α-Klotho gene expression or protein supplementation can prevent and rescue these α-Klotho-deficient phenotypes. Precise mechanisms underlying the vasculoprotective effects of α-Klotho are yet to be revealed, and may include indirect effects (*e.g.* α-Klotho-dependent phosphaturia) but also direct effects on vascular cells. Endogenous vascular Klotho expression is a controversial subject and, currently, no compelling evidence exists that supports the existence of vascular membrane-bound Klotho expression, as expressed in kidney. Based on current available data it has become clear Klotho has many vasculoprotective effects and may constitute a promising therapeutic target.

Symposium 8 (S8)
SCHCF – Young Scientist

S8-1
Tight coupling of astrocyte energy metabolism to synaptic activity revealed in brain tissue with genetically encoded FRET nanosensors

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The potassium ion, K^+, a neuronal signal that is released during excitatory synaptic activity, produces acute activation of glucose consumption in cultured astrocytes, a phenomenon mediated by the sodium bicarbonate cotransporter NBCe1 (*SLC4A4*). We have explored here the relevance of this mechanism in brain tissue by imaging the effect of neuronal activity on CA1 astrocytic pH, glucose and pyruvate dynamics using BCECF and FRET nanosensors. Electrical stimulation of Schaffer collaterals produced fast activation of glucose consumption with a parallel increase in intracellular pyruvate. These responses were blocked by TTX and were absent in tissue slices prepared from NBCe1-KO mice. Direct depolarization of astrocytes with elevated extracellular K^+ or Ba^{2+} mimicked the metabolic effects of electrical stimulation. We conclude that the glycolytic pathway of astrocytes *in situ* is acutely sensitive to neuronal activity, and that extracellular K^+ and the NBCe1 cotransporter are involved in metabolic crosstalk between neurons and astrocytes. Glycolytic activation of astrocytes in response to neuronal K^+ helps to provide an adequate supply of lactate, a metabolite that is released by astrocytes and which acts as neuronal fuel and an intercellular signal.

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S8-2
Participation of Ae4 anion exchanger in Cl^--dependent saliva secretion

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Transcellular Cl^- movement across acinar cells is the rate-limiting step for salivary gland fluid secretion. Recently, we demonstrated that Ae4 (Slc4a9) anion exchangers are expressed in mouse submandibular acinar cells where they contribute to Cl^--dependent fluid secretion. *Ae4* null mice show reduced HCO_3^--dependent Cl^- uptake,

in keeping with Cl^-/HCO_3^- exchanger activity. However, the functional properties of Ae4 remain controversial. It has been proposed that Ae4 mediates Cl^-/HCO_3^- exchange or Na^+-HCO_3^- cotransport. We studied the biophysical proprieties of Ae4 to better understand how it promotes saliva secretion. We find that native Ae4 activity in mouse salivary gland acinar cells supports Na^+-dependent Cl^-/HCO_3^- exchange that is comparable with that obtained upon heterologous expression of Ae4 in CHO-K1 cells. Additionally, whole cell recordings and ion concentration measurements demonstrate that Na^+ is transported by Ae4 in the same direction as HCO_3^- (and opposite to that of Cl^-) and that ion transport is not associated with changes in membrane potential. We also find that Ae4 can mediate Na^+-HCO_3^- cotransport–like activity under Cl^--free conditions. However, whole cell recordings show that this apparent Na^+-HCO_3^- cotransport activity is in fact electroneutral HCO_3^-/ Na^+-HCO_3^- exchange. Although the Ae4 anion exchanger is thought to regulate intracellular Cl^- concentration in exocrine gland acinar cells, our thermodynamic calculations predict that the intracellular Na^+ concentrations required for Ae4-mediated Cl^- influx differ markedly from those reported for acinar secretory cells at rest or under sustained stimulation. Given that K^+ ions reach intracellular concentrations of 140–150 mM (essentially the same as extracellular [Na^+]), we hypothesize that Ae4 could mediate K^+-dependent Cl^-/HCO_3^- exchange. Indeed, we find that Ae4 mediates Cl^-/HCO_3^- exchange activity in the presence of K^+ as well as Cs^+, Li^+ and Rb^+. In summary, our results strongly suggest that Ae4 is an electroneutral Cl^-/nonselective cation–HCO_3^- exchanger. We postulate that the physiological role of Ae4 in secretory cells is to promote Cl^- influx in exchange for K^+(Na^+) and HCO_3^- ions.
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S8-3

Preeclampsia, an opportunity to learn about placental development: Role of RECK?

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The human placenta is one of the most important organs in the body. It is essential for the foetus development and mother health during pregnancy, but also their lifelong health. Nevertheless, it is the least understood and studied of all human organs. Problems with the placenta can lead to serious consequences, such as preeclampsia,

which is the leading cause of maternal morbidity and mortality. Despite the exact pathophysiology of preeclampsia remains elusive, the placenta seems to be the inception problem, triggering maternal hypertension. In normal pregnancies, foetal trophoblast invade the maternal decidua to reach and modify the spiral arteries where differentiate to an endothelial-like phenotype, which is required to increase the blood flow to the placenta. However, in preeclampsia, the invasion and differentiation capacities of this trophoblast are affected, emerging as a proposed cause of this syndrome. RECK is a plasma membrane protein that inhibits different metalloproteinases, acting as a key regulator of cellular migration, invasion, and angiogenesis. Our results show that RECK is overexpressed in trophoblast as well as in umbilical vein endothelial cells from preeclampsia compared to normal pregnancies. Moreover, RECK expression reduces migration, invasion, and differentiation to the endothelial-like phenotype of first trimester human trophoblast cell line HTR8/SVneo. We propose RECK as a potential common regulator of both invasion and spiral arteries remodeling, thus playing a role in the pathogenesis of preeclampsia. These findings open a line of research related to increase our knowledge about placenta development as well as to give new clues on the aetiology of preeclampsia, allowing to early diagnose, to prevent, and to develop therapeutic strategies for the treatment of preeclampsia.
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S8-4

Endocannabinoid signaling in glia-neuron communication and the use of phytocannabinoids as a biomedical tool for the Chilean population

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The endocannabinoid system is probably one of the most important signaling system in mammals. Food ingestion and energy balance, and metabolism are all partially controlled by endocannabinoid signaling in specialized neurons located at the arcuate nucleus (AN) of the hypothalamus. These neurons sense moment-to-moment changes in physiological inputs and modulate their activity in response to circulating molecules as glucose and other. Tanycytes are the major glial cells present in the basal hypothalamus and are widely present in the third ventricle (3v). These cells also have extensive basal processes that form close contact with AN neurons, in particular orexigenic and anorexigenic neurons. Our work has consisted of the study of

expression machinery of synthesis and degradation of 2-AG, the main endocannabinoid in the hypothalamus. Our data indicate that tanycytes present all the molecular machinery to produce 2-AG in vitro and in vivo, but the participation of 2-AG from tanycytes in the communication tanycyte-AN neurons and its effect on food intake need to be studied in more detail. In order to develop innovative and effective biomedical tools we are currently very interested in the use of molecules capable of modulating the endocannabinoid signaling. In this sense, phytocannabinoids as CBD, the main non-psychoactive phytocannabinoid present in Cannabis Sativa strains, could be a very interest opportunity to find novel non-psychoactive anti-obesity drugs. Some effects of CBD in humans will be discussed in this seminar, as well as the biomedical use of CBD and other phytocannabinoids in important cellular processes relevant to human physiology.

Symposium 9 (S9)
Frontiers in Maternal Foetal Research I

S9-1
Participation of the endocannabinoid system in the pathophysiology of human placenta

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Cannabis use during pregnancy has been associated with an increase risk of adverse birth outcomes suggesting that the endogenous ligands for cannabinoid receptors could have a significant influence on pregnancy complications. Endocannabinoids (ECs) are an important family of lipid-signalling molecules that act as endogenous ligands of cannabinoid receptors (CB1 and CB2). We previously reported an aberrant endocannabinoid system in placentas from preeclampsia. Preeclampsia is a multisystem syndrome that represents a major factor for maternal and perinatal morbid-mortality, and affects 7–10% of pregnancies. Lipids are the principal components of biological membranes and play essential roles in cellular function regulating important processes such as proliferation, apoptosis and cell migration. In the placenta, apoptosis is a homeostatic mechanism that participates in

syncytialization. Increasing evidence demonstrates the relevance of ECs and hypoxia as modulators of trophoblast cell turnover. Hypoxia inducible factor 1 (HIF-1) regulates the expression of a large number of genes including those involved in cell cycle arrest and apoptosis. HIF-1α was detected in trophoblast of human placentas and its expression is exacerbated in pathological conditions. We found that HIF-1α stabilization decreased fatty acid amide hydrolase FAAH mRNA and protein levels, critical regulator in the metabolic control of ECs levels in normal and pathological pregnancy. Our results suggest that changes in ECs levels might be contribute to the upregulation of apoptotic parameters by a mechanism that involves activation of CB1 receptor. Thus, we proposed that abnormal expression of endocannabinoide system might contribute to deregulate important pathway connected to the placentation process.

S9-2

Reproductive and metabolic alterations in the progeny of obese rats: role of sympathetic nervous system

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Maternal obesity is related to non-alcoholic fatty liver disease and polycystic ovary in the progeny. Both alterations are associated with conditions in which an increased sympathetic activity has been observed (i.e. obesity, obstructive sleep apnea, stress). **Objectives**: We pretend to demonstrate that there is an increase in sympathetic nerves projecting both to the ovary and the liver in the progeny of rats with obesity induced by high fat diet consumption. **Methods**: Virgin Sprague Dawley female rats were feed with a high fat diet (60%Kcal/fat, Researchdiets, USA) for 1 month prior to pregnancy, during pregnancy and nursing. The female offspring was feed with control diet from weaning to postnatal day 60. At this day, serum, liver and ovaries were collected and stored at -80°C. Norepinephrine was measured by ELISA in liver and HPLC-EC in the ovary, tyrosine hydroxylase was analysed by Western blot and adrenergic receptors by qRT-PCR. Histologic analysis with hematoxylin-eosin was also performed. **Results**: We found an increase in norepinephrine concentration both in ovary and liver. The ß2 adrenergic receptor expression was increased in the ovary. In addition, we found a decrease in tyrosine hydroxylase in liver. These changes were associated to both ovarian cysts and fatty liver, respectively. **Conclusions**: These results suggest that an increase in sympathetic nervous system regulation is a feature of both non-alcoholic fatty liver disease and polycystic ovary in offspring of obese

rats. We propose that the increase in sympathetic activity has a pathogenic role in the developmental programming of both conditions.

S9-3

β2GPI-specific antibodies induce pro-inflammatory mediators and tissue transglutaminase differential variant expression on trophoblast cells and monocytes-macrophages

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Antiphospholipid syndrome (APS) is characterized by significant pregnancy morbidity. Beta-2 glycoprotein I (β2GPI) is constitutively expressed by trophoblast and anti-β2GPI antibodies are involved in obstetric disorders associated with APS inducing an inflammatory response in trophoblast. Transglutaminase-2 (TG2) crosslinking activity regulates nuclear factor-κB signalling and its inflammatory effects. TG2 truncated variants encoded by alternatively spliced mRNA are involved in enzyme regulation. **Objectives**: We analysed effects of anti-β2GPI antibodies on proinflammatory cytokine production and TG2 isoforms expression in trophoblast cells and monocytes/macrophages. **Methods:** Serum samples were obtained from women with APS and fertile non-pregnant women. Trophoblast Swan-71 and phorbol 12-myristate 13-acetate treated THP-1 cell lines were used to evaluate anti-β2GPI antibodies effect on cytokine production by ELISA and TG2 variants expression by qPCR. **Results:** Sera with anti-β2GPI antibodies increased IL-6 production and induced TG2 expression in trophoblast (dose dependent effect). In differentiated THP-1 cells exposed to anti-β2GPI antibodies displayed significant and dose dependent increase in interleukin 1 (IL-1) and IL-6 production and induces changes in expression of TG2 truncated isoforms. **Conclusions:** Results support β2GPI-specific antibodies effects on inflammation induction at maternal-foetal interface and suggest that modulation of TG2 variants expression plays a role in this process. Further work is currently underway to decipher molecular mechanisms underlying this regulation.

S9-4

Relevance of the blastocyst conditioned media on immunotolerance: focus on the control of the inflammatory response

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Implantation is associated with a sterile inflammatory response that should be later controlled to a tolerogenic microenvironment by maternal and blastocyst-derived factors. At decidualization, cells undergo reticular stress and unfolded protein response, allowing them to expand their endoplasmic reticulum and change their secretome increasing immunomodulators' production. **Objective:** We investigated whether human Blastocyst Conditioned Media (BCM) could control the initial inflammatory response during the implantation period. **Methods:** Human endometrial stromal cell line (HESC) were decidualized or not with medroxiprogesterone + dbcAMP. Then, HESC cells were treated with human BCM for 24 h. Gene and protein expression was evaluated by PCR or FACS, respectively. An *in vitro* implantation model based on co-culture of blastocyst-like spheroids (BLS) from Swan-71 trophoblast cells line over decidualized-HESC cells and a transwell migration system for Tregs recruitment were used to evaluate decidua functionality. **Results:** We observed ($P<0.05$, n = 5, folds relative to negative control) increased expression of interleukin-8 (CXCL8) (1.21 ± 0.06), stromal derived factor 1 (CXCL12) (1.43 ± 0.18), and interleukin-1β intracellular protein production (IL-1β) (4.72 ± 1.21) after the decidualization. Since IL-1β can act as a 'double-edge' mediator in early pregnancy, we evaluated BCM effect on IL-1β. BCM reduced IL-1β in decidualized cells. This was paralleled by decreased mRNA expression of the reticular stress sensors PKR-like endoplasmic reticulum kinase (0.26 ± 0.03) and activating-transcription factor 6 (0.48 ± 0.04). Using the *in vitro* implantation model we observed that BCM obtained from developmentally impaired blastocysts decreased the invasion index of BLS on decidualized cells and Tregs recruitment. **Conclusion:** BCM might contribute to the crosstalk with decidualized cells controlling the inflammatory response and allowing blastocyst invasion accordingly with blastocyst quality.

S9-5
Maternal treatments to prevent diabetic embryopathy

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Maternal diabetes induces alterations in embryo development and increases resorption and malformation rates. The pro-oxidant/pro-inflammatory intrauterine environment is an important factor involved in the induction of diabetic embryopathy. We focus on the study of maternal treatments able to protect and prevent impaired embryo development. The antioxidant idebenone, a structural analogue of coenzyme Q10 with potent effects as antioxidant, ameliorates the altered parameters related to a pro-oxidant/pro-inflammatory environment, improves mitochondrial function and biogenesis, and prevents apoptosis in embryos from mild diabetic rats. Also related to its antioxidant and anti-inflammatory properties, unsaturated fatty acids that activate the nuclear receptors PPARs can be dietary administered to mothers during

pregnancy. PPARδ is the only PPAR isoform expressed in the embryo during early organogenesis. PPARδ expression, the levels of other components of the PPAR system, and PPARδ-regulated genes such as matrix metalloproteinases (MMPs, involved in tissue remodelling) are altered in embryos from diabetic rats. Olive oil (oleic acid-enriched, PPAR activator) supplementation in the maternal diet modulates the expression of components of the PPAR system and the activity of MMPs. Also folic acid and safflower oil (linoleic acid-enriched, PPAR activator) supplementation prevent embryo malformations in diabetic rats, acting through the modulation of reactive oxygen species, prostacyclin (PPARδ endogenous activator) and nitric oxide (embryo morphogen) production and the regulation of MMPs and their endogenous inhibitors. Our results show the capacity of maternal treatments with antioxidants and/or PPAR agonists to improve embryo development and prevent embryo resorption and malformations in diabetes and experimental models of pregnancy.

Symposium 10 (S10)
Frontiers in Maternal Foetal Research II

S10-1
Excessive gestational weight gain reduces the response to vasoactive molecules in human fetoplacental microvessels

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Excessive gestational weight gain (eGWG) occurs when women increase their weight beyond the recommended range. eGWG is related with reduced insulin nitric oxide (NO)-mediated dilation of human umbilical vein rings; meanwhile, obesity is associated with alterations in the vascular response to endothelin-1 (ET-1, vasoconstrictor). **Objective**: To determine whether eGWG associates with reduced foetoplacental microvascular response to vasoactive molecules. **Methods**: Anthropometric parameters were recorded, and placental microvascular veins rings from the third chorionic branch were obtained from women with normal (NW) or

obese (OB) pregestational body mass index coursing with eGWG or adequate GWG (aGWG). Vascular reactivity to adenosine (10^{-6} to 10^{-3} M, 5 min), insulin (10^{-10} to 10^{-6} M, 5 min) and ET-1 (10^{-14} to 10^{-6} M, 5 min) was evaluated in KCl-preconstricted (32.5 mM) vein rings using wire myography in the absence or presence of 100 µM N^G-nitro-L-arginine methyl ester (NOS inhibitor). **Results:** eGWG occurs in 45.7% of OB and 18.5% of NW women. Mothers with pregestational OB delivered larger ($P<0.05$, n = 295-879) newborn (2.7 ± 0.3 ponderal index (PI)) than NW (2.6 ± 0.3 PI). In NW, the eGWG reduces the response NO-dependent vasodilators molecules (87 ± 5% for insulin, 29 ± 3% for adenosine). In NW, the eGWG increases the maximal response (1.3 ± 0.5 fold) and the effective half-maximal concentration (EC_{50}) (23 ± 10-fold) of ET-1. In OB, insulin and adenosine were unable to alter vascular reactivity in both GWG conditions. In OB, the eGWG increases EC_{50} (7.6 ± 3-fold) in response to ET-1. **Conclusion:** Pregestational obesity and eGWG reduce the response to vasoactive molecules in human foetoplacental microvasculature.
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S10-2
Shiga toxin during pregnancy and breastfeeding: Can human milk protect children from typical HUS?

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Shiga toxin type 2 (Stx2) is a virulence factor of Shiga toxin producing *E. coli* (STEC) and is responsible for Haemolytic Uremic Syndrome development. We have previously demonstrated that an active immunization against Stx2 protects rats from early pregnancy loss mediated by the toxin and also protect pups through breastfeeding. We propose human milk can protect the breastfeed newborn from STEC infections through immune and/or non-immune components. The aim of this work was to evaluate if antibodies from human milk can recognize and inhibit Stx2 and other virulence factor of STEC. For that purpose human milks from healthy donors were collected at the Hospital Alejandro Posadas (Buenos Aires, Argentina). Milks were conserved at -20°C until used. Samples were defatted by centrifugation and the liquid phase was used. In order to analyse the presence of specific antibodies against STEC O157:H7 (n = 24) and the STEC virulence factor EspB (n = 8), ELISA and Western blot assays were performed, respectively. In order to analyse if human milk can neutralize Stx2, neutralization assays on Vero cell were performed. The results indicate that 50 % of analysed human milks had immunoglobulins against O157:H7 *E. coli*, although no specific anti EspB antibodies were detected in the analysed samples. Moreover human milks showed no capacity to neutralize *in vitro* Stx2. Our data indicate that human milk can recognize STEC O157:H7 but does not neutralize Stx nor identify EspB. Further analysis will be performed to evaluate if

other non-immune components of human milk (lipids or cells) can mediate protection against Stx2 through breastfeeding.

S10-3
Placental mTOR signalling in metabolic diseases

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The placenta is a metabolic active tissue that not only regulates the foetal supply of nutrients and oxygen and secretion of hormones into the maternal and foetal circulation, but also can adapt morphologically and functionally to optimize substrate supply, and thus support foetal growth under adverse intrauterine conditions. Mechanistic target of rapamycin (mTOR) play a central role in placental nutrient sensing and foetal growth. mTOR exists as two different protein complexes, mTOR Complex 1 and 2 (mTORC1 and mTORC2). These two mTOR complexes have distinct physiological functions and are regulated differently. The dysregulation in the mTORC signalling pathways has been related to the development of multiple human pathologies such as cancer, type 2 diabetes mellitus and obesity. Children whose mothers had diabetes during pregnancy are at increased risk of becoming obese and developing diabetes at young ages. Besides, many of these female offspring already have diabetes or abnormal glucose tolerance by the time they reach their reproduction age, prolonging the cycle of diabetes. There is some evidence that epigenetic and phenotypic traits induced by early life environment can be passed from one generation to the next. We have recently found that maternal diets enriched in n6/n3 polyunsaturated fatty acids (PUFAs) in the F0 generation can regulate intrauterine programming of metabolic and placental alterations in their offspring (F1 generation) that develops gestational diabetes mellitus. The regulation of foetal weight and placental mTORC pathways in the next generation highlights the potential use of diets enriched in n6/n3 PUFAs to prevent transgenerational adverse effects of maternal diabetes.

S10-4
EHMT2/G9a controls maturation of the placental vasculature

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G9a is an epigenetic regulator controlling embryogenesis; however, its function in the cellular processes and molecular pathways controlling placental vascular maturation and placental disease are unknown. **Objective:** To uncover the function of G9a in

maturation of the placental vasculature, and its involvement in placental disease.**Methods:** Extension and structure of the placental vasculature, and proliferation of endothelial cells and trophoblasts were quantified in endothelial-specific *G9a* mutant mice. Genomewide expression analysis and a genetic rescue uncovered the main G9a target pathway. Expression of human *G9A* and its main targets was analyzed in placentae from pregnancies affected by intrauterine growth restriction (IUGR). **Results:** Decreased proliferation of endothelial cells (35 ± 9%) ($P<0.01$, n = 4), concomitant with increased proliferation of trophoblasts (2.5 ± 0.4 fold) ($P<0.01$, n = 4) prevented vascular expansion in maturing *G9a* mutant placentae. In addition, genes encoding proteins in the Notch pathway were downregulated in *G9a* mutant placental endothelial cells. Moreover, constitutive activation of the Notch pathway in *G9a* mutant endothelial cells restored expansion of the placental vasculature. Furthermore, G9A and proteins in the Notch pathway were decreased in placentae from human pregnancies affected by IUGR. **Conclusion:** G9a-mediated activation of the Notch pathway in endothelial cells is required for placental vascular maturation. We are testing whether pharmacologic activation of the Notch pathway promotes placental vascular maturation.

Symposium 11 (S11)
SCHCF – Vascular Modulators: Role of Adenosine Receptors and Thyroid Hormones

S11-1
Role of adenosine A_{2A} receptor in an *in vivo* model of melanoma angiogenesis and growth

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Melanoma is the least common but the most deadly skin cancer, with a duplicated incidence rate in the last 30 years. Once the metastatic phase develops, it's almost always fatal. Different therapeutic approaches have been evaluated, including chemotherapy and biologic therapies. The aggressive nature of melanomas is related to several abnormalities in growth factors, cytokines, and their receptors expression, which impact on angiogenesis processes and tumor growth. Extracellular adenosine

is an immunomodulatory biomolecule produced by ATP hydrolysis. The purine nucleoside acts as a local vascular modulator stimulating angiogenesis. Adenosine exerts its effects by enrolling a G-protein coupled receptors family referred to as Adenosine Receptors (ARs). Some contradictory evidence shows adenosine pathway, is involved in in tumor pathological angiogenesis. To investigate exact the role of specific adenosine subtype receptor, Adenosine type 2 receptor (A_2AR) in melanoma-induced angiogenesis, we will perform mice melanoma autocraft transplant of a tumor cell line (B16F10) into a host C56BJ mouse (control and A_2AR silenced). For the next 16 days we will evaluate tumor morphology and growth; and also tissue angiogenesis and blood flow. Our result shows that tumor size, and tumor latency is increased in A_2AR KO mice versus wild type, and also A_2AR KO mice present a higher blood flow pattern in the area near the tumor. In addition, we will evaluate melanoma development by a mathematical model, based upon ordinary differential equations (ODE), employing Trust-Region-Reflective Algorithm, with a nonlinear optimization method specially designed to solve parameters estimation problems by nonlinear least squares criterion using Matlab © algorithm. We concluded that an A_2AR absence in mice stimulates melanoma development by experimental data and validated by a mathematical model.
Funding: Internal Proyect UBB, DIUBB 166709 3/R, Universidad del Bío-Bío, Chile

S11-2
Role of thyroid hormones metabolism on endothelial and trophoblast cells in gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is characterizes by abnormal maternal D-glucose metabolism, and associates with reduced maternal circulating free tyroxine (fT4). Deiodinase 3 (DIO3) is a thyroid hormones inactivator enzyme that catalyzes deiodination of thyroxine (T4) into reverse triiodothyronine (rT3) and triiodothyronine (T3) into 3,3'-diiodothyronine (T2). Moreover, placenta is involved in regulate thyroid hormones transport from the mother to fetus. It has been described that normal placenta expresses thyroid hormones transporters (THT) that include monocarboxylates transporters 8 and 10 (MCT8 and MCT10), L-amino acid transporters 1 and 2 (LAT1 and LAT2) and organic anion transporter polypeptides system A1 and A2 (OATPA1 and OATPA2). It is known that LAT2 is related with GDM, because increases L-arginine transport in this disease. Now, GDM effect on thyroid hormones metabolism protein expression is not reported. Objective: To evaluate whether thyroid hormones metabolism protein expression in endothelial and trophoblast cells are altered in GDM. Methods: Placentas were obtained from Hospital Guillermo Grantt Benavente of Concepción, Chile. Thyroid hormones metabolism

protein expression were evaluated in Placentas from normal and GD pregnancies by immunohistochemistry (IHC). HUVEC and HTR8-Svneo were exposed (6 hours) to D-glucose (11-25 mM). Total mRNA was extracted with Trizol reagent and used for real time PCR to estimate the relative abundance of Dio3 and 28S mRNA using the 2–ΔΔCt method. Moreover, protein extract were separated by electrophoresis and evaluated by westernblot. Results: Dio3 and LAT2 is localized in endothelial and syncytiotrophoblast cells, and this protein increased (2.1 ± 0.2 & 2.3 ± 0.2 folds, respectively) in GD pregnancies in both type cells. Dio3 and Lat2 mRNA level was higher (1.8 ± 0.2 & 4.2 ± 0.4 folds, respectively) in placenta. HUVEC and HTR8-Svneo exposed to D-glucose from 11 to 25 mM (effective half-maximal concentration (EC50) 15 ± 1 mM) increased Dio3 and Lat2 mRNA expression (2.8 ± 0.2 & 3.2 ± 0.3 folds, respectively). Conclusions: GDM increases Dio3 and Lat2 expression in endothelial and trophoblast cell possibly by high levels of D-glucose similarly as placenta from GDM.
Funding: Dirección de Investigación, Universidad San Sebastián (DIUSS), Chile.

S11-3
Reduced pro-angiogenic capacity in male mice lacking adenosine A2A receptor

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Since adenosine via activation of adenosine A_{2A} receptor increase pro-angiogenic activity in progenitor endothelial and differentiated endothelial cells, the main focus of this article was to investigate whether the adenosine receptor-mediated pro-angiogenic behavior of endothelial cells, observed *in vitro* and *in vivo,* was sex-dependent. Using primary cultures of mice pulmonary endothelial cells (mPEC), isolated from female and male wild type (WT) or adenosine deficient mice (A_{2A}KO), we analyzed mRNA levels for A_1, A_{2B} and A_3 adenosine receptors using quantitative PCR. Proliferation, migration, and *in vitro* angiogenic capacities were analyzed in the absence or presence of the nonselective adenosine receptor agonists (NECA) or the selective agonist for A_{2A} receptor, CGS-21680, in mPEC cells from WT and A_{2A}KO mice. For the *in vivo* analyses, WT and A_{2A}KO mice were utilized to monitor wound-healing capacity, histology, and blood vessel counting in the dermis. Laser Doppler

analyzed tissue perfusion. Low mRNA level for A_3 were observed in mPEC from A_{2A}KO, especially in mPEC cells from male A_{2A}KO mice. Only female mPEC from A_{2A}KO mice showed lower sensitivity to NECA than mPEC from WT mice. Low migration capacity was observed basally in mPEC from A_{2A}KO males. NECA and GCS-21680 treatments increased cell migration in mPEC from female WT mice, but not in A_{2A}KO mice. Both, NECA and GCS-21680, increased *in vitro* tube formation capacity in female and male WT mice, whereas NECA increased tube formation capacity only in female A_{2A}KO mice. Male A_{2A}KO showed a dorsal wound larger than their respective littermate in the WT group at 10 days of wound healing. Male A_{2A}KO mice had a thinner dermis, which associated with reduced number of blood vessels compared to male WT or female A_{2A}KO mice. Lastly, tissue perfusion was reduced only in male A_{2A}KO compared to male WT. In summary, *in vitro* and *in vivo* results suggest that, contrary to female mice, male A_{2A}KO mice exhibited a reduced pro-angiogenic capacity which is associated with less extracellular matrix that could explain, at least in part, their impaired wound healing capacity.
Funding: FONDECYT 1140586 (Chile). Group of Research and Innovation in Vascular Health (GRIVAS-Health).

Symposium 12 (S12)
Infection, Toxins, and Pregnancy

S12-1
Virulence factors from Shiga toxin producing *E coli* could be one of the causes of foetal morbimortality

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Infection associated with Shiga toxin producing *E coli* (STEC) became relevant in public health since it was considered one of the most important emergent food-borne pathogens. Shiga toxin (Stx) is the main virulence factor of STEC and is responsible for systemic complications including Haemolytic Uremic Syndrome (HUS) characterized by haemolytic anemia, thrombocytopenia and acute renal failure. HUS is mostly seen in young children including neonates although the outbreak in 2011 in central Europe caused by Stx2-producing STEC affected more adults than children, and women were overrepresented. To our knowledge, there are no reports of Stx2 effects during human pregnancy. We had reported that rats treated with Stx2 induced foetomaternal damage and abortion. Our results indicate that Stx2 reaches the uteroplacental unit where Gb3 is present and triggers damage in decidual tissue, hypoxia, intrauterine growth restriction and an increase of local TNF-α levels. Stx2 altered the maternal microvasculature and reduce blood flow and oxygen delivery to

the fetus. Poor oxygen supply accompanied with damages in the uteroplacental tissue and inflammation could be responsible for the early pregnancy loss. Immunization with the B subunit of Stx2 totally protects rats from early pregnancy loss induced by Stx2 and confers anti-Stx2 immunity to the offspring. Although there are no reports of Stx2-mediated fetal damage or fetal death in humans, we speculate that humoral immunity against Stx2 in pregnant women could prevent possible Stx2 damage to the fetus, as consequence of STEC infections during pregnancy. Additionally, it could benefit neonates by conferring anti-Stx2 antibodies passively transferred by lactation.

S12-2
ZIKA virus infection in human placental cells

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Zika virus (ZIKV), the mosquito-borne flavivirus that has currently been associated with severe congenital malformations, has also been considered the focus of an ongoing pandemic and public health emergency. Initially limited to sporadic cases in Africa and Asia, since 2015 this infection has spread throughout Brazil and other regions of the Americas. Infection can cause varying symptoms ranging from a subclinical disease to severe conditions such as microcephaly in neonates born to infected mothers and Guillain-Barre syndrome in adults. In this study, we examine in placental tissues the entry of ZIKV and its contribution to the induction of antiviral responses. We show that trophoblast cells are permissive to the entry of ZIKV isolated from a Brazilian patient during the first 6 hours of infection. After 48 hours, viral particles were rarely found, which favors the idea that trophoblast may not be an ideal site for viral replication. The ZIKV permissiveness of the syncytiotrophoblast was confirmed by immunohistochemistry and RT-qPCR. ZIKV induced the expression of specific proteins but did not change cell death rates, which may be associated with restrictions in viral replication. Our preliminary results herein, increase the knowledge on ZIKV the biology, particularly giving the need to develop strategies to contain viral infection during gestation.
Funding: Fapesp and CNPq.

S12-3
The epithelial turnover of the trophoblast constitutes a local placental innate immune response against *Trypanosoma cruzi*

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Congenital Chagas disease, caused by *Trypanosoma cruzi*, is partially responsible for the progressive globalization of Chagas disease despite of its low transmission rate. The probability of congenital transmission depends on complex interactions between the parasite, the maternal and fetus/newborn immune responses and placental factors, being the latter the least studied one. During congenital transmission, the parasite must cross the placental barrier where the trophoblast, a continuous renewing epithelium, is the first tissue to have contact with the parasite. Importantly, the epithelial turnover is considered part of the innate immune system since pathogens, prior to cell invasion, must attach to the surface of cells. The trophoblast turnover involves cellular processes such as proliferation, differentiation and apoptotic cell death, all of which are induced by the parasite. Here, we analyse the current evidence about the trophoblast epithelial turnover as a local placental innate immune response.
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Symposium 13 (S13)
Pathogenesis of Preeclampsia

S13-1
Immune cells in the placental bed in pregnancy and preeclampsia

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Normal placentation is associated with the presence of immune cells, mainly NK cells and macrophages. Especially at the implantation site and in the placental bed many NK cells and macrophages are present. These cells are necessary for normal implantation and placentation and especially spiral artery remodelling. The main function of these cells is a (immune) regulatory function: the cells regulate amongst others trophoblast invasion and spiral artery remodelling. The phenotype of both uNK cells and macrophages is specific for the placental bed: in contrast to the main population of peripheral NK cells, uterine NK (uNK) cells express high levels of CD56 and mainly lack CD16 expression. Although a small population of $CD56^{bright}CD16^{-}$ NK cells is detectable in peripheral blood, these are usually agranular, while uNK cells are highly granulated. Also the macrophages in the placental bed have a unique phenotype: the macrophages in the placental bed can be classified as M2-like macrophages, with functions as immunomodulation and tissue remodelling. The pertinent role of these immune cells for normal pregnancy can be demonstrated by looking at these cells in pregnancy complications such as preeclampsia. Altered numbers of uNK cells and/or macrophages have been found in preeclampsia and may be involved in the defective spiral artery remodelling seen in

preeclampsia. Since it is difficult to study the role of immune cells in the placental bed in the pathophysiology of preeclampsia in humans, placental bed immune cells in animal models will also be discussed.

S13-2

New insights into the pathogenesis of preeclampsia: the role of placental aquaporins

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Although the etiology of preeclampsia remains uncertain, it is well-known that the placenta plays a central role in the pathophysiology of this syndrome. Abnormal syncytiotrophoblast differentiation and altered expression of a variety of trophoblast transporters were associated with preeclampsia. Although differentiation into syncytium results in a decrease of caveolin-1 and a marked reduction of caveolas, in placentas from women with preeclampsia we found no expression of caveolin-1 which correlated to changes in the membrane lipid composition of trophoblast. Caveolin-1/caveolas domains orchestrate different cellular events such as migration. In addition, we previously reported that the expression and function of aquaporins (AQPs) are also altered in the placenta from preeclampsia. However, preeclampsia is not known to be associated with an altered foeto-maternal water flux. Recently, AQPs were proposed to have cellular unexpected roles. In this context, we found that placental AQPs may be involved in the apoptosis of the trophoblast and their dysregulation may be associated to an increase of the apoptotic events in preeclampsia. However, our findings are related to term placentas when this disorder is well established. Therefore, we evaluated the roles of AQPs and caveolin-1/caveolas during the early stages of placental development. Our results showed that inhibition of AQPs and the lipid raft disruption significantly attenuates migration of trophoblast cells. In all cases, metalloproteinases expression and function was not modified and invasion process was unaltered. Thus, we proposed that abnormal expression of these proteins might produce failures in placentation, resulting in an increase of trophoblast apoptosis, which finally triggers the clinical manifestations of preeclampsia.

S13-3

Lipid-endoglin interactions in preeclampsia

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Preeclampsia has increased circulating levels of a short form of the auxillary TGF-beta (TGFB) receptor endoglin (sENG). However, its processing, release and functionality in preeclampsia remains poorly understood. **Objective**: To elucidate whether ENG is attracted to syncytial sphingomyelin-enriched apical membrane subdomains that facilitate ENG cleavage and subsequent shedding of sENG into the maternal circulation in preeclampsia. **Results**: We show that ENG interacts with specific sphingomyelin(SM)s which promote its clustering with particular metalloproteinases (MMP) in SM-enriched lipid rafts of the apical syncytial membranes from preeclamptic placenta where ENG is cleaved by MMP into sENG. The SM-enriched lipid rafts also contain TGFB receptors (TGFBR1 and TGFBR2), but not soluble fms-like tyrosine kinase 1 (sFLT1), another protein secreted in excess in the circulation of women with preeclampsia. The truncated ENG is then released into the maternal circulation via PLAP/CD63-positive and SM-enriched exosomes together with TGFBR1 and 2. **Conclusion**: Hypoxia-induced SMs facilitate ENG cleavage in the apical syncytial membrane via MMP and subsequent exosomal release of sENG with TGFBR1 and 2 into the maternal circulation. This TGFB receptor complex could block the vascular effects of TGFB in the circulation of preeclamptic women.

Oral Communications I (OC)

OC1 (and PC12)
FK506 binding protein 52 modulated AP-1 functions in human trophoblast cells

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FK506 binding protein 52 (FKBP52) is a cochaperone that influences steroid receptors function and has peptidylprolyl-isomerase (PPIase) activity. Reduced FKBP52 protein has been detected in placentas from preeclampsia (PE), also it has been suggested that c-fos is implicated in regulating invasive mechanism of trophoblast in PE as well as in placentas from normal gestations. **Objective**: The aim of this work was to investigate the effect of FKBP52 on the transcription factor activator protein 1 (AP-1) in trophoblast cells. **Methods**: BeWo were used as *in vitro*

choriocarcinoma model. Cells were transfected with wild type FKBPs or their putative PPIase mutants, and then, stimulated by miristic-acetated phorbol ester 12-myristate 13-acetate (PMA). AP-1 signalling was evaluated by luciferase assays and Western blot, analysing endogenous c-fos expression and monitoring phophorylated extracellular signal-regulated kinases 1 and 2 (pERK1/2)/total ERK ratio along time (90 min). Interleukin 6 (IL-6) secretion was measured by ELISA, and matrix metalloproteinase 2 (MMP-2) proteolytic activity was determined by zymography. **Results**: After 30 min of PMA treatment ERK1/2 acquired its maximum phosphorylation level. In the presence of FKBP52 total ERK1/2 was unaltered, but pERK1/2 / total ERK1/2 remained increased along time ($P<0.01$, n = 6), and increased c-fos protein level (1.9 ± 0.2 fold). Interestingly FK506 binding protein 51 (FKBP51), structurally similar but functional opposite to FKBP52, did not show significant differences in ERK1/2 phosphorylation neither c-fos endogenous protein abundance. FKBP52 stimulated AP-1 transcriptional activity on a concentration dependent manner (range: 2.9 - 47 fold of increment for 0.5-1.5 μg wtFKBP52 plasmid transfected, $P<0.01$, n = 5). Besides, in order to analyse the effects of these regulatory events, we studied IL-6 secretion and MMP-2 proteolytic activity. We observed an increased on IL-6 medium concentration (2.3 ± 0.3 fold) and MMP-2 enzymatic activity (2.5 ± 0.1 fold), abrogated by the PPIase mutants of FKBP52. **Conclusions**: We demonstrated that FKBP52 enhances ERK1/2 phosphorylation, increases c-fos protein abundance and AP-1 transcriptional activity as well as the expression or activity of its target genes. We concluded that FKBP52 could be considering as a positive new regulator of AP-1 in trophoblast cells.
Funding: UBACyT and CONICET.

OC2 (and PC38)

The blood brain barrier of the offspring gestated in hypothyroxinemia has higher permeability to macromolecules and to the transmigration of immune cells to the central nervous system

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Maternal hypothyroxinemia is a highly frequent condition characterized by low levels of T_4 with normal levels of T_3 and thyrotropin. The offspring gestated in hypothyroxinemia suffers an early onset and more severe symptoms of experimental autoimmune encephalomyelitis (EAE). The offspring gestated in hypothyroxinemia that has EAE showed an increase infiltration of T $CD4^+$ and T $CD8^+$ cells in the central nervous system (CNS). **Objective:** The aim of this work is to evaluate whether the

blood brain barrier (BBB) of the offspring gestated in hypothyroxinemia is more permeable to macromolecules and to the transmigration of immune cells to the CNS. **Methods:** BBB permeability was analysed by the extravasation of Evans blue in the CNS by absorbance and the presence of immune cells in the CNS parenchyma by flow cytometry. **Results**: The analysis of our results showed that the offspring gestated in hypothyroxinemia has higher BBB permeability to macromolecules; however, the presence of T $CD4^+$ and $CD8^+$ in the CNS remained normal. **Conclusion:** Our results support the idea that the BBB of the progeny gestated in hypothyroxinemia has higher permeability to macromolecules.
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OC3 (and PC41)
Versican expression and roles in hydatidiform moles

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Proteoglycans such as biglycan, decorin, and versican have interesting functions on proliferation, survival, migration, and differentiation in a variety of healthy tissues and pathologies, including gestational diseases. Amid them, hydatidiform moles have increasing worldwide incidence and are characterized by abnormal trophoblast proliferation and differentiation, increased survival and chorionic gonadotropin production. **Objectives**: It was aimed to determine decorin, biglycan, and versican expression in molar pregnancies and if they influentiate trophoblast differentiation. **Methods:** Partial, complete, and invasive moles expression were accessed by immunohistochemistry for decorin, biglycan, and versican. The choriocarcinoma-derived BeWo cell line was employed for versican mRNA silencing and their differentiation into syncytiotrophoblast was verified. Versican mRNA silencing was confirmed by RT-PCR and flow cytometry. **Results**: Decorin and biglycan were strongly stained in syncytiotrophoblast and intermediate trophoblast in all analysed samples. Differently, versican was more expressed in all hydatidiform moles in comparison to first trimester villi, only staining in syncytiotrophoblast. BeWo only expressed versican when undergoing syncytialization. Versican mRNA silencing resulted in lower syncytialization and slightly increase in cell death. **Conclusions**: Versican may have a role in syncytialization and syncytiotrophoblast survival, important features from hydatidiform moles and as such it might be an interesting therapeutic target.

OC4 (and PC36)

***Trypanosoma cruzi* exosomes increases susceptibility to parasite infection in human placental chorionic villi explants**

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Congenital transmission of Chagas' disease, caused by the protozoan *Trypanosoma cruzi* (*T. cruzi*), is one of the major public health concerns in Latin America where more than one million women in fertile age are infected. The parasite crosses the placental barrier using its virulence factors such as exosomes. Parasite derived exosomes play an important role in inter-cellular communication by carrying complex cargoes, including mRNA, miRNA and others. **Objective**: We studied whether *T. cruzi*-derived exosomes modulate parasite infection and tissue damage during *ex vivo* infection of human placental chorionic villi explants (HPCVE). **Methods:** HPCVE obtained by informed consent from healthy donors were pre-treated with *T. cruzi* exosomes for 2 h (5 μg/ml) and later infected further 24 h with *T. cruzi* trypomastigotes (10^5 per ml, Y Strain). Parasite load was determined by qPCR, tissue damage was studied by routine histopathological analysis and histochemistry for glycosylated molecules (PAS stain) and collagen (Picro Sirius Red). **Results:** HPCVE pre-treated with exosomes and infected with the parasite show a significant increase in parasite DNA load compared to control, not infected, or infected with the parasite alone (149 ± 23%, $P \leq 0.05$, n = 3). Additionally, increased tissue damage is observed in HPCVE pre-treated with exosomes and infected with the parasite compared to samples incubated only with *T. cruzi*. Interestingly, exosomes by themselves induce histopathological and histochemical damage in HPCVE. **Conclusion:** *T. cruzi* exosomes, as virulence factors, enhance the infection of the parasite in *ex vivo* infected HPCVE.

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OC5 (and PC19)
Angiotensin II induces decidualisation markers and chemoatractants in human endometrial stromal cells and regulates trophoblast migration

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We previously demonstrated that angiotensin II (Ang II) modulates gene expression, proliferation, and metalloprotease activity in rat endometrial stromal cells (ESC).

Decidual prolactin and heparin binding-epidermal growth factor (HB-EGF) induced in rat ESC by progesterone-treated trophoblast conditioned medium, decreased in ESC pretreated with losartan. **Objective:** To analyse a) whether Ang II induces markers of uterine receptivity in human ESC (T-HESC), b) the effect of Ang II treated-T-HESC conditioned medium on the migration of trophoblast HTR8/SVneo cells, c) the involvement of angiotensin receptors. **Methods:** T-HESC were exposed or not to Ang II (125, 250, 500 ng/ml) for 24, 48, 72, or 96 h. Cells were pretreated (1 h) with INCA-6 (5 μM), as well as with losartan 1 μM or PD123319 (1 μM) to assess the involvement of calcineurin/nuclear factor of activated T cells (CN/NFAT) signalling pathway, and Ang II receptors, respectively. Insulin growth factor binding protein 1 (IGFBP-1) and HB-EGF -mRNA and protein expression was analysed by semiquantitative RT-PCR and Western blot. Interleukin (IL)-1β and IL-8 were analysed in cell culture supernatants by ELISA. HTR8/SVneo trophoblast cells were exposed or not to conditioned medium from T-HESC, which were cultured for 48 h in the presence or absence of Ang II, and their migration on scratch was then registered. **Results:** Ang II induced the expression of IGFBP-1 respect to control (7.81 ± 2.91 and 7.38 ± 1.52 fold, mRNA and protein, respectively), HB-EGF (1.52 ± 0.12 and 2.86 ± 0.35 fold, mRNA and protein, respectively) and IL-8 (59.29 ± 11.86 vs 23.39 ± 5.45 pg/ml), but not IL-1β in T-HESC. INCA-6 inhibited IGFBP-1 and HB-EGF induction. INCA-6 and losartan, but not PD123319 inhibited the induction of IL-8. Conditioned medium from T-HESC, which was stimulated with Ang II, increased the migration on scratch of HTR8/SVneo. Increased migration was not observed when trophoblast cells were incubated with conditioned medium from T-HESC, which were pretreated with losartan before the Ang II stimuli. **Conclusions:** Results suggest that Ang II acting at angiotensin receptor-1 induces NFAT-dependent gene expression related with receptivity in human ESC, which could favour the migration of trophoblast cells.

OC6 (and PC21)

RNA-Seq analysis reveals candidate genes that may explain neural tube defects in mouse embryos lacking SR-BI

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Scavenger Receptor Class B Type I (SR-BI) mediates bidirectional lipid uptake between HDL and cells. During murine early embryogenesis, SR-BI is expressed in trophoblast giant cells. SR-BI$^{-/-}$ embryos from SR-BI$^{+/-}$ intercrosses exhibit cephalic neural tube defects (NTD) and are severely vitamin E-deficient. Maternal supplementation with vitamin E prevents NTD in these embryos. **Objectives**: To find

genes differentially expressed in E9.5 SR-BI$^{-/-}$ embryos with NTD vs. normal SR-BI$^{-/-}$ or wild-type embryos, and to analyse the effect of maternal vitamin E supplementation on the expression of those genes. **Methods**: We determined the transcriptomic signature associated with NTD in SR-BI$^{-/-}$ mouse embryos by RNAseq. We next analysed the expression of a selection of genes related the antioxidant response or with a known participation in neural tube closure using qPCR in wild-type and NTD or non-NTD SR-BI$^{-/-}$ embryos from chow fed or vitamin E-supplemented dams. **Results**: We observed differential expression of a large set of genes in NTD SR-BI$^{-/-}$ embryos (797 genes vs. wild-type embryos), mainly related to developmental (e.g., Wnt signalling) and metabolic pathways (e.g., FXR signalling). Genes related to the antioxidant response showed similar expression in wild-type and SR-BI$^{-/-}$ embryos. Among different transcription factors associated with neural tube closure, three genes (*Pax3*, *Alx1*, and *Alx3*) showed reduced expression levels (0.67 ± 0.20, 0.30 ± 0.21, and 0.43 ± 0.17, respectively, $P<0.05$) in NTD SR-BI$^{-/-}$ embryos, which were normalized by vitamin E maternal supplementation. **Conclusions**: The gene expression analysis suggests a causal relationship between reduced expression of several transcription factors due to vitamin E deficiency and NTD in SR-BI$^{-/-}$ embryos.

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Oral Communications II (OC)

OC7 (and PC17)
Evidence for oxygen-mediated regulation of aqp4 expression in human placenta

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Preeclampsia is a multisystem syndrome unique to human pregnancy. Aberrations in the remodelling of the spiral arteries lead to fluctuations in the oxygen tension within the placenta. The resulting over-expression of hypoxia inducible factor 1α (HIF-1α) contributes to the dysregulation of numerous genes, which perturbs normal placental functions. The expression of a variety of syncytiotrophoblast transporters is abnormal in placentas from women with preeclampsia. **Objective:** To study the expression of Aquaporin-4 (AQP4) in placentas from women with preeclampsia and the effects of

changes in oxygen tension on AQP4 expression in placental villous tissue. **Methods:** Placental tissue from full-term normal pregnancy and preeclampsia were obtained. Normal placental tissue was cultured under different oxygen conditions (20 or 2% O_2). Some explants were treated with 250 μM $CoCl_2$ (a hypoxia mimicking agent that inhibits HIF-1α). Tissue viability was assessed by the MTT. AQP4 protein expression was analysed by Western blot and immunohistochemistry. A theoretical analysis of the promoter region of the AQP4 gene was carried out using the MatInspector tool from Genomatix. **Results**: In placentas from preeclampsia AQP4 was weakly detectable. In explants from normal placenta cultured in hypoxia (2% O_2) AQP4 protein expression increased (1.7 ± 0.3 fold) (P<0.05, n = 8) but it was significantly decreased (48 ± 7%) (P<0.01, n = 8) following reoxygenation. The *in-silico* analysis showed three putative binding sites for HIF-1α in AQP4 promoter. Incubation of explants with $CoCl_2$ increased AQP4 protein level (1.8 ± 0.16 fold) (P<0.01, n = 8). **Conclusions:** These data suggest that AQP4 expression is abnormal in placentas from women with preeclampsia, possibly because of fluctuations in oxygen tension within the placenta. We propose that oxygen may regulate the expression of placental AQP4 probably through a HIF-1α dependent pathway.

OC8 (and PC35)
microRNA-130 and microRNA-122 alteration are related to lipid metabolic impairments in the foetal liver of rats with gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a prevalent disease that increases the risk of adverse foetal outcomes. MicroRNA-130 (miR-130) targets the nuclear receptor PPARγ, a master regulator of lipid metabolism. MicroRNA-122 (miR-122), the most abundant microRNA in the liver regulates hepatocyte differentiation and metabolism. Fatty acid synthase (FAS) is a target gene of both PPARγ and miR-122. **Objectives**: Aiming to find putative lipid liver anomalies we analysed miR-130, miR-122, PPARγ, FAS expression, and triglyceride content in the foetal liver in a novel model of GDM in the rat. **Methods**: GDM was spontaneously induced by intrauterine programming in the offspring (F1) of diabetic rats (F0 diabetic rats were obtained through neonatal streptozotocin administration (90 mg/Kg), glycaemia values (180-230 mg/dL) significantly higher from controls values (80-100 mg/dL)). In control and GDM rats, livers of male foetuses were explanted on day 21 of gestation, miR-130, miR-122, and FAS analysed by qPCR, PPARγ by Western blot, and triglyceride content by thin-layer chromatography. **Results**: Foetal livers from GDM rats showed reduced miR-130 (52%, P<0.05, n = 8) when compared to controls, an alteration possibly related to the observed increase in PPARγ level (65%, P<0.05, n = 8). Triglyceride content and the expression of the PPARγ target gene FAS were increased (53% and 61%,

$P<0.05$ n = 8, respectively) in the foetal livers of GDM rats, while miR-122, a negative regulator of FAS expression, was reduced (57%, $P<0.05$, n = 8). **Conclusions**: Reductions of both miR-130 and miR-122 are likely to be involved in the synthesis and accumulation of lipids in the liver of male foetuses from GDM rats.

OC9 (and PC53)

NHE1 modulates intracellular pH and cell proliferation in human ovarian cancer

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Ovarian cancer is a gynaecological disease with high mortality rate. Cancer cells produce large amounts of protons (H^+). The Na^+/H^+ exchanger 1 (NHE1) is one of the main H^+ transport systems and participates in intracellular pH (pHi) control and cell proliferation, but its role in human ovarian cancer has not been described yet. **Objectives**: Our aim was to determine whether NHE1 plays a role in the pHi and proliferation in human ovarian cancer cells. **Methods:** NHE1 and Ki67 (proliferation marker) expression was assessed in ovarian non-tumour cell line (HOSE), tumour cell line (A2780), primary cultures of human ascites cancer cells (haOC), and in serial sections of ovary biopsies obtained from Hospital Clínico UC-CHRISTUS at the Pontificia Universidad Católica de Chile (Santiago de Chile). NHE1 activity in the absence or presence of zoniporide (100 nmol/L, 6 minutes, NHE1 inhibitor) was estimated by measuring pHi recovery rate (*dpHi/dt*) by the acid-pulse technique using 2,7-bicarboxyethyl-5,6-carboxyfluorescein (BCECF-AM) probe in a fluorimeter. Cell proliferation was evaluated by [H^3]-thymidine incorporation. **Results**: NHE1 is the main contributor to *dpHi/dt* in HOSE (66 ± 11% of total *dpHi/dt*) and A2780 (98 ± 2% of total *dpHi/dt*) cell lines, and haOC (76 ± 8% of total *dpHi/dt*). Zoniporide reduced [H^3]-thymidine incorporation in HOSE, A2780 and haOC cells. NHE1 was positively correlated with Ki67 in human ovarian tumour. **Conclusion**: These findings suggest that NHE1 plays a pro-proliferative role and regulates intracellular pHi in human ovarian cancer cells.

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OC10 (and PC11)

Effects of recreational use of marijuana during pregnancy: placental morphofunctional changes in mice

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Marijuana is one of the most commonly self-cited drug used during pregnancy. There are few studies concerning its gestational toxicity and results are contradicting. Experimental studies use non-realistic exposure routes and dose. **Objectives**: To better understand the impacts of recreational use of marijuana during pregnancy we used a mouse model of realistic exposure that mimic human's use under the aspects of dose and route of exposure to assess placental and fetal effects. **Methods**: We exposed pregnant BALB/c mice (n = 12) daily (nose-only) to either marijuana smoke [0.2 g of marijuana-0.3% Δ^{-9}Tetrahydrocanabinol] (MA group) or filtered air (FA group) during 5 min from 5.5-17.5 day post-conception (dpc). On 18.5 dpc pregnancy was terminated, foetus and placenta macroscopically examined, weighted and fixed. Stereology was used to estimate volumes (V), surface areas (S) and thickness (T) of placental structure and O_2 diffusive conductance (O_2DC) was calculated by $O_2DC = (K \bullet (S_{MBS} + S_{FC}))/2 \bullet T$, where MBS is maternal blood space, FC is foetal capillaries, and K is Krogh's diffusion coefficient). **Results**: No gross abnormalities in placenta or foetus were seen. Placentas from MA group presented greater total volumes (P<0.004) due to increased V of labyrinth (L) and decidua (D) compartments. Besides this compensatory growth of the placenta, foetuses from MA group were smaller (CR length P<0.01, 8% reduction in weight) and lighter (P<0.01, 8% reduction in weight). Analysis of fractional contribution of each placental compartment (chorionic plate, L, junctional zone, and D) and of L components (trophoblast, FC and MBS) did not show any difference. However, total surface area of MBS and FC were increased (34% and 66%, respectively, P<0.05) and the thickness of the interhemal membrane (IHM) is reduced (33%, P = 0.03) in cannabis group; together these changes improved the O_2DC, suggesting that these placentas are 44% more efficient (P = 0.04). **Conclusions**: Low dose exposure to marijuana smoke during pregnancy affects placental structure and impairs foetal growth. Increases in placental specific compartments suggest compensatory mechanisms that will potentially fails to attend foetal demand.
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OC11 (and PC73)

Feto-placental endothelial exosomes modulate high glucose-induced endothelial dysfunction in human umbilical vein endothelial cells

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Gestational diabetes is characterized by feto-placental endothelial dysfunction. This is associated with increased L-arginine/NO pathway in HUVEC, which could be induced by high-glucose. Exosomes can modulate vascular functions. **Objetives**: to evaluate the role of exosomes in the high glucose-induced endothelial dysfunction. **Methods**: HUVEC from normal pregnancies were exposed to high-glucose (HG, 25 mM) and normo-glucose (NG, 5 mM) in exosomes-free medium for 24 hours. Exosomes were isolated by ultracentrifugation, analyzed by Nanosight-3.0 and electron microscopy. HUVEC-NG (n=7) were exposed to 1 or 5 μg/cm^2 of exosomes-HG during 12 hours, and vice-versa. Wound healing assay was performed. VEGF (vascular endothelial growth factor), hCAT-1 (human cationic amino acid transporter type 1) and P~ser^{1177}-eNOS (phosphorylated-ser^{1177} endothelial nitric oxide synthase) expression were measured by western blot and qPCR. **Results**: HG increased the exosome concentration (1,640e+009 vs 1,100e+009) as compared with NG, while exosome size was lower in HG (121,2±3,6 vs 152,6±14,2). HG increased cell migration as compared with HUVEC-NG (0.78 vs 1). Exosomes-HG increased the migration in HUVEC-NG (0.82 vs 1) and, the opposite effect was observed in HUVEC-HG exposed to exosomes-NG (0.9 vs 1). HG increased the VEGF, hCAT-1 and P~ser^{1177}-eNOS expression vs HUVEC-NG (1.53 vs 1; 1.5 vs 1; 1.6 vs 1, respectively), while exosomes-HG increased P~ser^{1177}-eNOS expression in HUVEC-NG (1 vs 1.56), and vice-versa (1.6 vs 1.15). No changes were observed in hCAT-1 and VEGF expression. **Conclusions**: Our results suggest that feto-placental endothelial exosomes could be important in endothelial function and could play a role in the changes induced by high-glucose.
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OC12 (and PC28)
The role of endogenous annexin A1 (AnxA1) in pregnancy

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Annexin A1 (AnxA1) is a glucocorticoid-induced anti-inflammatory protein secreted by phagocytes in the innate response. AnxA1 also controls the secretion of steroids hormones and is found in the testis, ovaries, placenta, and seminal fluid. **Objective**: This work was performed to investigate the role of AnxA1 on pregnancy. **Methods**: In this study, male and female BALB/c mice wild type (WT, n = 10) and AnxA1 knockout (KO, n = 10) were used. In females during or at term of pregnancy parameters related to gestation success were evaluated. In male, spermatozoids characteristics and Y and X chromosome proportion were evaluated. **Results**: The coupled AnxA1 KO mice delivered higher number (5.0 in WT and 8.0 in KO) of puppies from a little, with upper percentage of female (female/male = 55/45 in WT and 28.3/71.7 in KO). This profile seems to be not dependent on male characteristics, as sperm of KO mice did not present functional alterations and equal proportions of Y and X chromosomes than WT mice. Furthermore, mismatched male WT mice with female KO had higher female puppies from a litter, which was not observed on male KO mice mated with female WT. Indeed, female of KO mice presented, arrested of oestrous cycle at proestrous phase, increased sites of implantation, reduced pre and post implantation losses, exacerbated inflammatory reaction in the uterine fluid during implantation phase, and enhanced plasma progesterone in the beginning of pregnancy. **Conclusions**: Together, the results highlight, for the first time, that AnxA1 pathway may be a target to be controlled during the early phase of the pregnancy.
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Poster Communications (PC)

PC1

Human chorionic gonadotropin block Smad2/3-mediated signalling of transforming growth factor β in human endometrial stromal cells, regulating the secretion of extracellular matrix remodelling elements and facilitating HTR8-SVneo invasion *in vitro*

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Transforming growth factor β **(**TGF-β) is expressed in the endometrium during the window of implantation however inhibits trophoblast invasion *in vitro*. On the other

hand, human chorionic gonadotropin **(**hCG) secreted by trophoblast cells has an opposite effect, facilitating the invasion. **Objective:** to determine the possible modulation of hCG on TGF-β1 signalling in endometrial stromal cells (ESCs) and its effect on the secretion of extracellular matrix remodelling elements and extravillous trophoblast invasion *in vitro*. **Methods**: ESCs were stimulated with TGF-β1 (10 ng/mL) and/or hCG (10 IU/mL) to evaluate TGF-β1-induced Smad2/3 phosphorylation (Western blot), mRNA level of the TGF-β1-induced gene SMAD 7 (qPCR), and secretion of metaloproteinase-2 (MMP-2) (gelatin zymography). The invasive potential of HTR8/SVneo cells was evaluated *in vitro* using Boyden chambers in the presence of the ESCs conditioned media. **Results:** Smad2/3 phosphorylation increased (2.4 ± 0.5 fold, $P<0.05$, n = 3) in ESCs stimulated with TGF-β1. This activation was blocked in the presence of hCG. Moreover, the mRNA level for SMAD 7 increased (1.7 ± 0.3 fold) in ESCs treated with TGF-β1, whereas no effect was observed upon co-stimulation with hCG. The secretion of MMP-2 decreased (20 ± 10%) with TGF-β and increased (2.0 ± 0.4 fold) with hCG in the presence or absence of TGF-β. HTR8/SVneo cell invasion decreased with TGF-β1 (30 ± 15%), but increased (1.4 ± 0.5 fold) in the presence of hCG. **Conclusion**: These results suggest a modulating effect of hCG on TGF-β in ESCs, facilitating the invasion of extravillous trophoblast cells, which may have direct implications in the process of embryo implantation and placentation.

PC2

Infection by *T. cruzi* up-regulates the catabolic pathway of tryptophan in human placental villi

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Tryptophan catabolic pathway participates in immunologic tolerance of the foeto-maternal relationship. Indoleamine 2,3-dioxygenase (IDO) which contributes to immune regulation by catalyzing the essential amino acid tryptophan along the kynurenine (Kyn) pathway, is highly expressed in the placenta. In turn, Kyn is a natural ligand for Aryl hydrocarbon receptor (AhR). This system has been described to participate in the infection of Chagas disease, but the interaction of placental tissue with *Trypanosoma cruzi* (*T. cruzi*), the causal agent of congenital Chagas transmission, is not yet studied. **Objective**: To evaluate the effect of *T. cruzi* infection on the catabolism of tryptophan in a human placenta villous model. **Methods:** Chorionic villi explants were co-cultured with (infected, n = 3) or without 10^5 trypomastigotes (Tulahuen strain; control, n = 3) for 24 h. PCR for *T. cruzi* DNA was employed to analyse infected explants. Spectrophotometric assays were used to

measure IDO enzymatic activity and Kyn production. Western blot (ß-actin was internal reference) and immunohistochemistry were used to explore protein. **Results**: PCR positive to *T. cruzi* DNA were observed in infected explants. Infected chorionic villi explants show higher IDO specific activity (6.05 ± 0.38 vs. 2.46 ± 0.26 nmoles/min/mg protein $P<0.001$), IDO protein expression (2.04 ± 0.16 vs. 1.83 ± 0.12 fold, relative to ß -actin, $P<0.05$), Kyn production (11.54 ± 1.43 vs. 6.75 ± 0.6 µM Kyn/mg protein, $P<0.05$), and AhR protein abundance (68.41 ± 1.44 vs. 61.09 ± 2.07 fold, relative to ß -actin, $P<0.05$). **Conclusion:** *T. cruzi* modifies the catabolic tryptophan pathway in the chorionic villi. This pathway could participate in the process of infection of placental tissue in the congenital transmission of Chagas.
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PC3
Cafeteria diet alters rat uterine morphology and foeto-placental development

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Maternal diet may be associated with foetal and placental growth restriction. **Objectives:** We evaluated the cafeteria diet effects on: 1) adult rat uterine morphology, 2) reproductive performance, 3) foetoplacental growth on gestational day 21 (GD21), 4) weight of pups at birth, and 4) oestrogen receptor α (ERα) mRNA expression in placentas. **Methods:** Female Wistar rats were fed after weaning with a standard rodent chow diet (control (C)) or cafeteria diet (CAF) with highly palatable energy dense foods. Some animals were sacrificed on diestrus, 20 weeks after treatment. The uterus were included in paraffin for histological and immunohistochemical determination of ERα, vimentin and Ki67 (proliferation marker). Then, some animals were mated to evaluate the reproductive performance and to determine foeto-placental weight and ERα mRNA expression on GD21-placentas by QPCR analysis. **Results:** CAF group showed increased uterine glandular area expressed as volume density (V_v) (C 4.78 ± 0.76 vs CAF 8.27 ± 1.87, $P<0.05$, n = 7) with a higher vimentin expression in the periglandular stroma (C 0.276 ± 0.036 vs CAF 0.404 ± 0.044). CAF group showed higher ERα and Ki67 expression in: luminal epithelium (ERα C 2.2 arbitrary units (AU) ± 0.3 vs CAF 4.7 AU ± 0.5, Ki67 C 40.6% ± 5.1 vs CAF 68.1% ± 2.2); and glandular epithelium (ERα C 3.6 AU ± 0.2 vs CAF 8.7AU ± 1.0, Ki67 C 63.5% ± 5.1 vs CAF 76.1% ± 1.4). CAF diet did not alter reproductive performance and foetal weight; however, a decrease of GD21-placental weight (C 0.507g ± 0.140 vs CAF 0.440g ± 0.08), and pup weights at birth (C 5.683g ± 0.199 vs CAF 4.732g ± 0.343) were detected. Furthermore, the ERα

mRNA relative expression was lower (C 2.750 ± 1.033 vs CAF 1.516 ± 1.017) on CAF-placentas. **Conclusion:** CAF diet affected the uterine morphology and altered placental development with a lower weight of pups at birth.

PC4

Treatment with cinaciguat improved the vasoactive properties of lamb small pulmonary arteries

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Chronically hypoxic and pulmonary hypertensive neonatal lambs in the *Alto Andino* have reduced soluble guanylyl cyclase (sGC) protein expression and function, the latter triggered by an elevated reactive oxygen species production by hypoxia. **Objectives**: To evaluate if cinaciguat, a drug that activates oxidized sGC, modifies the small pulmonary artery function. **Methods**: In Putre at 3600 m altitude, six control lambs were treated (i.v.) with vehicle (control group) and six neonatal lambs treated with cinaciguat (BAY-582667) (cinaciguat group) for seven days (35 μg/kg/day). At twelve days of age the lambs were euthanized, the lung tissue was dissected extracting small pulmonary arteries of third or fourth branch from the main trunk. Arteries having internal diameters between 150-400 μm were cut into segments of ~2 mm in length. The arteries were mounted on an isometric force transducer on a myograph. The arteries were incubated with different drug concentrations (concentration response curve (CRC)) of vasoconstrictors (KCl [0-125mM], thromboxane [10^{-13}-10^{-5}M]) and vasodilators (sodium nitroprusside, SNP [10^{-10}-10^{-3}M], NS1619, activator of $BKCa^{2+}$ channels [10^{-10}-10^{-6}M]). All procedures were approved by the Bioethical Committee (0643 FMUCH CBA). **Results**: We found lower maximal contraction (P <0.05) in the cinaciguat group with KCl (1.50 ± 0.16 N/m) and thromboxane (92.5 ± 1.3% Kmax; it is the maximal response expressed as a percentage of the contraction induced by 125 mM KCl) compared with controls. Also, cinaciguat group showed a greater (P <0.05) vasodilation to SNP (Rmax = 30.2 ± 6.4% and NS1619 (Rmax = 65.8 ± 4.0%); Rmax is the maximal effect expressed as percentage of the response induced by serotonin [10^{-6}μM]). Treatment of cinaciguat group with sildenafil, [10^{-10}-10^{-5}M], an inhibitor of phosphodiesterase 5, showed higher sensitivity (pD_2 = 8.6 ± 0.12, P <0.05) than the control group (pD2 = 7.16 ± 0.11; pD2 = –log[EC_{50}], EC_{50} is the concentration at which 50% of the maximal response was obtained). We did not find differences between groups when CRC were performed with cinaciguat. **Conclusions**: The reduced contraction in response to KCl is consistent with a lesser muscle layer in the small pulmonary arteries. These results suggest that treatment with

cinaciguat improved vasodilatation and decreased vasoconstriction in lamb small pulmonary arteries, consistent with preliminary observations showing decreased pulmonary vascular resistance *in vivo*.

PC5

Impaired embryo cardiac-placental axis at organogenesis associated with dysregulation of VEGF and its receptors after maternal alcohol consumption

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Maternal alcohol consumption leads to congenital diseases and early pregnancy loss. Previously, we saw delayed embryo growth, increased dysmorphogenesis, abnormal decidua vascularization and altered expression of vascular endothelial growth factor (VEGF) and its receptors after perigestational 10% alcohol ingestion at organogenesis. **Objectives:** Given that VEGF/receptors regulates the embryo cardiac-placental axis development, we analysed the embryo cardiac and trophoblast-placental development and this angiogenic system after maternal alcohol consumption. **Methods:** Ethanol 10% in drinking water was administered to murine CF-1 females for 15 days before and up to day 10 of gestation (treated females (TF)). Control females (CF) were administered with drinking water without ethanol. Western blot, immunohistochemistry (IHC), laser microdissection and qRT-PCR were used. **Results:** The trophoblastic zone of TF presented increased apoptosis (TUNEL), unaltered proliferation and reduced VEGF expression (CF = 0.23 ± 0.02 vs TF = 0.11 ± 0.01, Arbitrary units (AU), n = 4, $P<0.001$). The VEGF receptor 2 (VEGFR2) expression was unaltered, but protein expression of VEGFR1 was elevated. TF had elevated frequency of embryos with irregular-discontinuous endocardium (CF = 14.3% vs TF = 79.6%, $P<0.01$) and disorganized myocardium (CF = 14.3% vs TF = 61.6%, $P<0.01$), without changes in the cardiac frequency. Caspase 8 activation increased (1.8 ± 0.2 fold) and ventricular proliferation diminished (CF = 4.2 ± 0.9 vs. TF = 2.1 ± 1.2 PCNA+ cells/μm^2). The embryonic *vegf* expression of TF increased (CF = 0.85 ± 0.12 vs. TF = 1.13 ± 0.14 AU, $P<0.01$), but VEGF expression was 27 ± 7% lower ($P<0.05$). The VEGFR1 and VEGFR2 expression in TF resulted augmented (1.46 ± 0.29 and 1.44 ± 0.04 fold, respectively, $P<0.05$) compared with CF. **Conclusions:** Murine perigestational alcohol ingestion at organogenesis causes

embryo cardiopathy and abnormal trophoblast development probably due to embryo-placental VEGF/VEGFRs imbalance.

PC6

Advanced maternal age and placental morphofunctional changes: an experimental study in mice

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Advanced maternal age is associated with adverse pregnancy outcomes (e.g. stillbirth, miscarriage). Human and animal studies suggest that defective decidualization and placentation are involved. Despite worldwide tendency to delayed childbearing, there is a lack of studies in this area. **Objective**: We investigated the effects of age on gestational outcomes and placentation. **Methods**: We evaluated three groups (n = 6) of pregnant mice (BalbC, primiparous). The younger group was 2 months old (mo) and older groups were 6 and 12 mo. At 18.5 day post-conception (dpc), animals were euthanized and numbers of foetuses (NF), reabsorption (NR), implantation sites (NIS), and losses (IL) were determined. One placenta per mother was randomly selected and a stereological evaluation conducted, total O_2 diffusive conductance (O_2DC) was estimated ($O_2DC = K \times (S_{MBS}+S_{FC})/2 \times T$, where S_{MBS} = maternal blood space surface area, S_{FC} = surface area of foetal capillaries, T = interhemal membrane thickness and K = Krogh's diffusion coefficient). Group comparisons were drawn using one-way ANOVA (Boferroni *pos-hoc*), null hypotheses were rejected at $P<0.05$. **Results**: There were no differences in foetal weight and NIS between the groups; however, placental weight (mean difference = 0.077 g) and NR (~1.6 fold) are increased in older mothers ($P = 0.01$ and $P = 0.001$, respectively). In younger animals, NIF were higher (~7 fold, $P<0.001$). Stereological assessment of the placenta showed that the total volume of the placenta was increased in older mothers (~2 fold, $P = 0.01$) because of larger labyrinth and decidua compartments. Detailed examination of the labyrinth structure revealed that volume of maternal blood space (MBS) is enlarged (~2 fold) in older mothers ($P = 0.01$) and volume of trophoblast reduced (~40%, $P = 0.02$). These changes affected MBS surfaces areas (larger in older groups) and the T (thinner) in 6 and 12 mo mothers. Additionally, O_2DC was increased in placentas from 6 and 12 mo compared to 2 mo placentas. **Conclusion**: The findings indicate that maternal age influence gestational outcomes and placental development. Increased NR may be linked to impaired placental initial development and the observed hypertrophy of the placenta suggests a compensatory mechanism to support foetal growth.

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PC7

Versican regulates trophoblast motility and cytoskeleton organization

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Versican is a proteoglycan and a danger associated molecular pattern with four different isoforms (V0, V1, V2, and V3). It regulates numerous biological functions, such as proliferation, differentiation, migration, and invasion. Our group showed that versican is produced by extravillous cytotrophoblast (EVT) cells from first trimester healthy placenta and from abnormally invasive placenta (AIP). However, only AIP EVT cells produce V0 and V1 isoforms, which might be a practical tool for pathology diagnosis. Nevertheless, functional studies on versican regulation of trophoblast cells are lacking. **Objectives**: It was aimed to determine versican roles on trophoblast migration. **Methods:** Versican siRNA was performed by plasmid transfection in HTR-8/SVneo cells. Silencing efficiency was accessed by RT-PCR for all versican isoforms and immunofluorescence and flow cytometry for total versican. Cell death was evaluated by Annexin V/Propidium Iodide staining. Falloidin, Ras homolog gene family A (RhoA) and Rho associated coiled-coil containing protein kinase 2 (ROCK2) expression were verified by immunofluorescence and flow cytometry. Scratch and transwell migration assays were employed to verify cell motility. **Results**: Versican V0 and V1 mRNA were detected in HTR-8/SVneo cells, which also presented protein expression in the majority of cells. Versican siRNA resulted in reduced mRNA and protein expression of versican. Versican siRNA did not alter cell survival, but drastically changed cell morphology and cytoskeleton organization, also reducing RhoA (92 ± 3%) and ROCK2 (32 ± 1.6%) expression, and trophoblast migration (72 ± 7%). **Conclusions**: Versican silencing resulted in changes in cytoskeleton organization and reduced RhoA and ROCK2 expression, as well as in cell migration, which implies versican as a key regulator in trophoblast motility and a possible therapeutic target in AIP.

PC8
PPARs and mTOR interact in the rat decidua during early organogenesis

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During embryo organogenesis, before the establishment of a mature placenta, the decidua serves for the embryonic histotrophic nutrition. Peroxisome proliferator activated receptors (PPARs) are nuclear receptors essential for development that regulate metabolic processes. Mammalian target of rapamycin (mTOR) signalling is

relevant in embryo nutrition and growth. **Objectives:** Aiming to assess whether PPARs and mTOR signalling pathways are interrelated in rat decidua during early organogenesis, we studied the effect of *in vivo* inhibition of mTOR, PPARγ and PPARδ signalling. **Methods**: Female Wistar rats received subcutaneous injections of rapamycin (mTOR inhibitor), T0070907 (PPARγ inhibitor), GSK0660 (PPARδ inhibitor) or vehicle during days 7, 8, and 9 of pregnancy. On day 9, decidua was explanted and level of proteins phosphorylated by the mTORC1 pathway (ribosomal protein S6 (RPS6) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)) and by the mTORC2 pathway (glucocorticoid-inducible kinase 1 (SGK1)), as well as adipophilin (a PPAR target)) were evaluated by western blot. **Results:** Rapamycin administration increased decidua PPARγ (36%, $P<0.01$, n = 7), PPARδ (87%, $P<0.001$, n = 7) and adipophilin levels (26%, $P<0.01$, n = 7). Administration of T0070907 inhibited mTORC1 and mTORC2 signalling, as shown by the reduced levels of phosphorylated RPS6 (25%, $P<0.05$, n = 7) and SGK1 (50%, $P<0.01$, n = 7). Administration of GSK0660 inhibited mTORC2 signalling, as shown by the reduced levels of phosphorylated SGK1 (53%, $P<0.001$, n = 7) but stimulated mTORC1 signalling, as shown by the increased levels of phosphorylated 4EBP (73%, $P<0.001$, n = 7). **Conclusions:** A complex interaction of nutrient signalling pathways occurs under mTOR, PPARγ and PPARδ inhibition, leading to stimulation or inhibition of decidua alternative pathways for embryo nutrition.

PC9

Amniotic membrane conditioned medium promotes cell death and inhibits proliferation of hepatocarcinoma HepG2 cells

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The placenta and fetal membranes have recently been proposed as an important stem cells source for regenerative medicine. Stem cells derived from amniotic membrane offer considerable advantages over other stem cells. Not only are amnion-derived stem cells applicable in regenerative medicine, but also have antitumoral properties. Hepatic failure is one of the major cause of morbidity and mortality, and despite the development in therapies, hepatocarcinoma rates are high worldwide. Little is known about the mechanisms involved in the antitumoral effect of the amniotic membrane and their cells. **Objectives:** The aim of this work was to study some aspects of cell death and proliferation induced by amniotic membrane conditioned medium (AM-CM) in hepatocarcinoma cells. **Methods**: We analysed the expression of proapoptotic

proteins (p53, Caspase-3, PARP-1, p21) by qRT-PCR and Western blot, in HepG2 cells treated with AM-CM. We also studied cell proliferation by [^{3}H]thymidine incorporation assay and cell viability by MTT assay. Serum deprivation was used as cell death control. **Results:** We found a significant increase ($P<0.05$, n = 3) in p53 expression (2 ± 0.03 fold), Caspase-3 fragmentation (1.3 ± 0.14 fold), and cleaved PARP-1 (1.5 ± 0.5 fold) -measured by western blot-, after 24, 48, and 72 h of treatment with AM-CM in hepatocarcinoma cells. AM-CM produced a significant increment in p21 (5.7 ± 2 fold), p53 (1.7 ± 0.3 fold) and Caspase-3 (3.6 ± 0.4 fold) mRNA expression. We also observed that AM-CM significant decreased cell proliferation (12 ± 3%) and viability (23 ± 4.4%), compared with control. Finally, immunofluorescence results showed diminished Ki-67 expression in HepG2 cells treated with AM-CM. **Conclusion:** Our results begin positioning amnion-derived stem cells as emerging candidates in anticancer therapy.

PC10

Increased sprouting in gestational diabetes mellitus could result from downregulation of the Netrin-1's anti-angiogenic receptor, UNC5b

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Dysregulated circulating angiogenic proteins have been proposed to underlie gestational diabetes mellitus (GDM). However, angiogenic factors misbalance has not been identified. Netrins, non-classical pro-angiogenic ligands, act through receptors belonging to DCC/Neogenin-1/UNC5 families. **Objectives**: We analysed differential effect of Netrins produced by Wharton Jelly Mesenchymal Stem Cells (WJ-MSC), acting through their receptors on Human Umbilical Vein Endothelial Cells (HUVEC) from GDM pregnancies. **Methods**: Netrins and receptors were evaluated by qPCR and Western blot. WJ-MSC conditioned medium was assessed in tubule formation and chick chorioallantoic membrane (CAM) assays with Netrin-1 blocking antibody. Experiments were performed in cultured cells from normal (N-HUVEC) and GDM (GDM-HUVEC) pregnancies, exposed (0-48 h) to normal (5 mM) or high (25 mM) D-glucose. **Results**: Netrin-1's WJ-MSC promotes angiogenesis *in vitro* and *in vivo* independent of the pathology or the extracellular concentration of D-glucose. N-HUVEC and GDM-HUVEC show similar expression level of both canonical and non-canonical Netrin's receptors. However, only the expression of the anti-angiogenic receptor UNC5b is reduced in GDM-HUVEC (66 ± 0.1%, $P<0.05$, n = 4) compared with N-HUVEC. In N-HUVEC, UNC5b overexpression induces cell retraction of the sprouting phenotype. **Conclusions**: WJ-MSC Netrin-1 secretion does not account for the increased angiogenesis in GDM, but UNC5b decreased protein abundance in

GDM-HUVEC might explain this phenotype. Thus, the stromal/endothelial niche is essential to maintain functional placental angiogenesis.
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PC11 (*abstract available as* OC10)
Effects of recreational use of marijuana during pregnancy: placental morphofunctional changes in mice

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PC12 (*abstract available as* OC1)
FK506 binding protein 52 modulated AP-1 functions in human trophoblast cells

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PC13
Endoplasmic reticulum stress in human umbilical vein endothelial cells from pre-gestational maternal obesity

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Pre-gestational maternal obesity (PGMO) is associated with adverse cardio-metabolic newborn outcome. Our previous results show insulin-desensitization in human umbilical vein endothelial cells (HUVECs) from PGMO. The endoplasmic reticulum stress (ERS) has been related to the development of obesity-associated insulin resistance. However, whether HUVECs from PGMO show ERS is unknown. **Objective:** To assay whether HUVECs from women with PGMO show increased ERS markers. **Methods:** HUVECs were isolated from normal or PGMO pregnancies from the Hospital Clínico UC-CHRISTUS and Hospital San Juan de Dios (Chile). We evaluated the protein level of CCAAT-enhancer-binding protein homologous protein (CHOP), tribbles-like protein 3 (TRB3), and phosphorylation and total protein level of protein kinase RNA-like endoplasmic reticulum kinase (PERK), eukaryotic

translation initiator factor 2-alpha (eIF2α), inositol-requiring enzyme 1-alpha (IRE1α), and c-jun N-terminal kinase 1 (JNK1) by western blot. X-box binding protein 1 (XBP1) mRNA processing was evaluated by PCR. **Results:** Activator phosphorylation of PERK (1.9 ± 0.4 fold) and eIF2α (1.8 ± 0.5 fold), and protein abundance for CHOP (2.5 ± 0.7 fold) and TRB3 (1.9 ± 0.3 fold) were increased (P<0.05, n = 4) in HUVECs from PGMO compared with normal pregnancies. Activator phosphorylation of IRE1α and JNK1 were unaltered, and there was not processing of XBP1 mRNA. **Conclusions:** HUVECs from women with PGMO show ERS by activation of PERK branch, suggesting that PERK branch-associated ERS could result in PGMO reduced foetoplacental endothelial function. The increase of TRB3 protein level suggests this protein's potential role as inductor of insulin desensitization in this type of foetoplacental endothelium.
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PC14
Maternal - fetal communication: role of fetal estrogens in porcine pregnancy

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Pregnancy in the pig is characterized by rapid development and endocrinological changes involving the conceptus and the uterine environment. Progesterone and oestrogens act through their specific receptors. Progesterone receptors (PGRA and PGRB) and oestrogens receptors (ERα and ERβ) have been shown to have different functional activities. **Objectives**: This work was performed to investigate: a) progesterone and oestrogens concentration in serum from mother and placental extracts from maternal and fetal homogenates (HoPM y HoPF), b) PGRA, PGRB, ERα, ERβ expressions in endometrium of non-pregnant sows and porcine placenta of 5, 17, 30 and 70 days of gestation (dg). **Methods**: Genital tracts from pregnant (n = 16) and non-pregnant sows (n = 8) were obtained at the slaughterhouses. Immunohistochemmistry was used to explore PGRA, PGRB, ERα, and ERβ, while progesterone and estrogens concentrations were measured by chemiluminescence. **Results:** At 17 and 70 dg a significant (P<0.05) increase of oestrogens in the HoPF (17 dg = 12 ± 0.65 fold; 70 dg = 3.69 ± 0.18 fold) was observed. Trofoblastic ERβ nuclear immunoexpresion was observed only at 17 and 70 dg. Maternal tissues expressed ERβ in endometrial glands until 17 dg while PGRA was expressed at all studied stages. **Conclusions**: Although progesterone is the hormone that maintains gestation, the results suggest that foetal oestrogens binding to trophoblastic ERβ promotes the synthesis and release of signal molecules related to maternal immunotolerance and subsequent placental remodelling.

PC15
IL-1β, IL-2, IL-4 and IL-10 profile during porcine gestation

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During gestation, a dialogue is carried out between the conceptus and the endometrium involving the immune system in order to minimize the embryo rejection probabilities. The porcine placenta is epitheliochorial, non-invasive, adecidua, folded, and diffused. **Objectives**: Concentration of interleukins 1β (IL-1β), 2 (IL-2), 4 (IL-4), and 10 (IL-10) in serum from mother and porcine placental extracts from different gestation periods was determined. **Methods**: Crossbred female placental samples (n = 25) of 17, 30, 60, 70, and 114 days of gestation (dg) and non-pregnant uterus (n = 5) were used. Interleukins determination was performed by ELISA. **Results**: IL-1β, IL-2, and IL-4 showed two peaks of concentration at the placental interface (P<0.001) at 30 dg (127, 915, and 2574 pg/ml, respectively) and 70 dg (254, 2298, and 5261 pg/ml, respectively) with significant decrease at term (IL-1β <8.2 pg/ml, IL-2 163.2 pg/ml, IL-4 803 pg/ml), the only period in which they increased in serum (IL-1β 306 pg/ml, IL-2 1477 pg/ml, IL-4 3930 pg/ml). In serum IL-10 increased at 17 (11.6 pg/ml), 60 (15.6 pg/ml), and 114 (19.4 pg/ml) dg, whereas placental tissue concentrations during gestation were unaltered. **Conclusions**: At 30 and 70 dg there are profound placental structural changes that allow the exponential growth of placenta and foetuses, respectively, and IL-1β, IL-2, and IL-4 present at the interface would favor placental remodelling. Its significant increase in serum at the end of gestation would facilitate the delivery and the expulsion of the placentas. Significant IL-10 increase in serum at 17, 60 and 114 dg could indicate its immunoregulatory role at a systemic level during the swine gestation.
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PC16
Placental *Trypanosoma cruzi* infection is restricted by nitric oxide production by endothetial nitric oxide synthase isoform

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Human placenta avoids *Trypanosoma cruzi* (*T. cruzi*) infection by different mechanisms and the production of nitric oxide (NO) is one of them. **Objective:** To

elucidate the importance of NO production in the parasitic load in placental tissue and the relevance of endothelial nitric oxide synthase (eNOS) isoform as a major NO producer. **Methods**: Placentas from caesarean delivery from clinically and serologically healthy women and Trypomastigotes of *T. cruzi* isolated from a congenital case (named Lucky) were used. Placental villi explants were co-cultured in RMPI 1640 for 24 h with or without (control) 1 x10^6 trypomastigotes of Lucky (TcII -VI strains). Cultures were treated with NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) at 0.1 and 1 mM. Quantification of eNOS expression by immunohistochemistry and Western blot, nitrite level in media supernatant with Griess technique, and parasitic load by qPCR with Taqman probes were assayed. **Results:** Placental explants in the presence of *T. cruzi* showed a significant increment of eNOS protein abundance (7.2 ± 0.94 fold, $P<0.05$) mainly at the syncytiotrophoblast, but a non-significant increase of NO production. Explants treated with 0.1 and 1 mM L-NAME showed that the parasitic load in placental tissue was higher (3.01 ± 1.04 and 6.10 ± 2.56 fold, respectively). **Conclusions:** These results suggest the important role of eNOS expression and NO production in placental infection, taking into account that it is not the only factor involved to limit the infection by *T. cruzi*, but is a leading one.
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PC17 ((*abstract available as* OC7)
Evidence for oxygen-mediated regulation of aqp4 expression in human placenta

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PC18
Insulin therapy fails to reverse the human foetoplacental endothelial dysfunction in gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) occurs with maternal hyperglycaemia and foetoplacental endothelial dysfunction. Women with GDM subjected to diet (GDM*d*) present with normal glycaemia at term; however, foetoplacental endothelial dysfunction is still present. Some of the women with GDM*d* fail to control glycaemia and are subjected to insulin therapy (GDM*i*). **Objective**: to determine whether GDM*i* reverses foetoplacental endothelial dysfunction in GDM*d*. **Methods**: Human umbilical vein endothelial cells (HUVECs) were isolated from normal, GDM*d* or GDM*i* pregnancies from Hospital Clínico UC-CHRISTUS and Hospital San Juan de Dios (Santiago, Chile). Kinetics of saturable L-arginine transport (V_{max}, K_m) was measured in Krebs solution. Intracellular content of L-citrulline and NO level were measured by HPLC and fluorescence, respectively. Endothelial nitric oxide synthase (eNOS) and cationic amino acids transporter isoform 1 (hCAT-1) expression was evaluated by Western blot and real time-qPCR. Experiments were performed in the presence or absence of insulin (1 nmol/L, 8 h). Vascular reactivity assays were in human umbilical vein rings challenged with insulin and calcitonin gene-related peptide (CGRP, 0.1–1000 nmol/L). **Results**: In the absence of insulin a higher (P<0.05, n = 5-6) maximal transport capacity (V_{max}/K_m) for L-arginine (2.7 ± 0.2 fold), NOS-generated L-citrulline cell content (7 ± 1 fold), and NO level (5.7 ± 0.6 fold) were found in cells from GDM*i* and GDM*d* compared with normal pregnancies. Insulin reversed the GDM effect. Similar results were found for protein abundance and mRNA expression for eNOS (1.6 ± 0.1 and 3.6 ± 0.4 fold, respectively) and hCAT-1 (4.9 ± 0.5 and 5.0 ± 0.5 fold, respectively). Insulin and CGRP caused concentration-dependent relaxation in umbilical vein rings from normal pregnancies (effective half-maximal concentration (EC_{50}) = 1.4 ± 0.14 and 0.2 ± 0.02 nmol/L, respectively), an effect impaired in GDM*d* (EC_{50} = 5 ± 0.5 and 0.6 ± 0.06 nmol/L, respectively) and GDM*i* (EC_{50} = 11 ± 1.1 and 1.6 ± 0.16 nmol/L, respectively). **Conclusion**: insulin therapy in women with GDM*d* results in normal maternal and foetal glycaemia, an outcome that is not enough to restore GDM*d*-associated human foetoplacental endothelial dysfunction.
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PC19 (*abstract available as* OC5)

Angiotensin II induces decidualisation markers and chemoatractants in human endometrial stromal cells and regulates trophoblast migration

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PC20
Shiga toxin type 2 affects human trophoblast first trimester cells

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Shiga toxin type 2 (Stx2) is the main virulence factor of Shiga toxin producing *Escherichia coli* (STEC). STEC are pathogens involved in food-borne diseases and are responsible for Hemolytic Uremic Syndrome development. We have previously demonstrated that Stx2 induce miscarriage and premature delivery in rats. **Objective:** The aim of this study was to evaluate the effects of Stx2 on human first trimester trophoblast cells. **Methods:** Swan71 and HTR-8 cells were exposed to different concentrations of pure Stx2 (1- 0.001 μg/ml) with or without lipopolysaccharide (LPS) 5 μg/ml in medium without serum (arrested conditions). Cell viability was evaluated at 24 h of Stx2-treatment by neutral red uptake. Migration was studied at 8 and 24 h by the wound-healing assay. Moreover, protein expression of matrix metalloproteinase 2 (MMP-2) was determined in the Swan71 supernatants at 24, 48, and 72 h in a gel zymography. **Results:** Stx2 did not affect cell viability in the HTR-8 and Swan71 lines even in the presence of LPS although impaired Swan71 cell migration after 24 h of treatment. Preliminary results showed that 1 μg/ml of Stx2 decreased the MMP-2 expression in Swan71 cells (39.9 ± 11.6%). **Conclusion:** These data indicate that Stx2 could affect trophoblast invasion and cause alterations in the maternal-placental interface and complications during gestation.

PC21 (*abstract available as* OC6)
RNA-Seq analysis reveals candidate genes that may explain neural tube defects in mouse embryos lacking SR-BI

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PC22

Maternal obesity associates with foetoplacental vascular dysfunction involving endoplasmic reticulum stress and altered insulin vascular reactivity

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Pathophysiological mechanisms involved in obesity include insulin resistance and endothelial dysfunction. It has been reported that endoplasmic reticulum stress (ERS) plays a key role in these mechanisms. **Objective**: To determine possible alterations in the insulin pathway and ERS in the foetal-placental circulation in pregnancies where the mother was with pregestational obesity. **Methods**: Human umbilical vein endothelial cells (HUVECs) were isolated from pregnancies where the mother was with normal weight (HUVECs-N) or pregestational obesity (HUVECs-Ob) from the Hospital Clínico UC-CHRISTUS (Santiago de Chile), and Hospital Guillermo Grant Benavenente (Concepción, Chile). Cells were incubated with insulin (0.1-10 nmol/L, 8 hours) in the absence or presence of tauroursodeoxycholic acid (TUDCA, 100 μmol/L, 24 hours) (ERS inhibitor). Expression and phosphorylation of endothelial nitric oxide synthase (eNOS) and protein kinase RNA-like endoplasmic reticulum kinase (PERK), and synthesis of nitric oxide (NO) and reactive oxygen species (ROS) was evaluated. The effect of insulin and ERS on foetal placental reactivity was measured in KCl preconstricted (12.5 mmol/L) human chorionic veins rings in a wire myograph. **Results**: HUVECs-Ob showed lower (P<0.05, n = 5) eNOS (53 ± 10%) and PERK (47 ± 8%) protein abundance; however, eNOS, but not PERK activity was increased (1.3 ± 0.1 fold), compared with HUVECs-N. The synthesis of NO and ROS in HUVECs-Ob was increased (2.0 ± 0.7 and 2.6 ± 0.6 fold, respectively) compared with HUVECs-N. TUDCA and insulin did not alter these parameters. Chorionic vein rings from obese pregnant women show lower maximal contraction with U46619 (62 ± 6%). Pre-incubation of vessel rings with insulin or TUDCA decreased contraction caused by U46619 (59 ± 7 and 60 ± 10%, respectively) in vein rings from normoweight mothers. However, in vessels from pregnant women with obesity insulin and TUDCA increased the U46619-induced contraction (1.6 ± 0.1 and 1.3 ± 1 fold, respectively), compared with obese controls. **Conclusions**: Maternal obesity in pregnancy results in endothelial dysfunction, altered response to insulin, and activation of factors associated with ERS in the foetoplacental circulation.

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PC23
PPARs and mTOR interact in the rat decidua during early organogenesis

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During embryo organogenesis, before the establishment of a mature placenta, the decidua serves for the embryonic histotrophic nutrition. Peroxisome proliferator activated receptors (PPARs) are nuclear receptors essential for development that regulate metabolic processes. Mammalian target of rapamycin (mTOR) signalling is relevant in embryo nutrition and growth. **Objectives:** Aiming to assess whether PPARs and mTOR signalling pathways are interrelated in rat decidua during early organogenesis, we studied the effect of *in vivo* inhibition of mTOR, PPARγ and PPARδ signalling. **Methods**: Female Wistar rats received subcutaneous injections of rapamycin (mTOR inhibitor), T0070907 (PPARγ inhibitor), GSK0660 (PPARδ inhibitor) or vehicle during days 7, 8, and 9 of pregnancy. On day 9, decidua was explanted and level of proteins phosphorylated by the mTORC1 pathway (ribosomal protein S6 (RPS6) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)) and by the mTORC2 pathway (glucocorticoid-inducible kinase 1 (SGK1)), as well as adipophilin (a PPAR target)) were evaluated by western blot. **Results:** Rapamycin administration increased decidua PPARγ (36%, $P<0.01$, n = 7), PPARδ (87%, $P<0.001$, n = 7) and adipophilin levels (26%, $P<0.01$, n = 7). Administration of T0070907 inhibited mTORC1 and mTORC2 signalling, as shown by the reduced levels of phosphorylated RPS6 (25%, $P<0.05$, n = 7) and SGK1 (50%, $P<0.01$, n = 7). Administration of GSK0660 inhibited mTORC2 signalling, as shown by the reduced levels of phosphorylated SGK1 (53%, $P<0.001$, n = 7) but stimulated mTORC1 signalling, as shown by the increased levels of phosphorylated 4EBP (73%, $P<0.001$, n = 7). **Conclusions:** A complex interaction of nutrient signalling pathways occurs under mTOR, PPARγ and PPARδ inhibition, leading to stimulation or inhibition of decidua alternative pathways for embryo nutrition.

PC24
Study of zika virus infection in human placenta explants

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Zika Virus (ZIKV) is a *Flavivirus* that has been strongly associated with microcephaly in newborns when pregnant women become infected. Infection of the placental barrier may, therefore be a critical limiting step in the intrauterine infection of the foetus. The availability of relevant *ex vivo* models that replicate the events that occur at the placental barrier is of critical importance in our search for effective countermeasures to protect the foetus. **Objectives**: To verify the replication kinetics of two strains of Zika virus (African (AFR) and Brazilian (BR)) and Dengue virus, serotype 2 (DENV2), in human placenta tissue explants and perform apoptosis marker assay. **Methods:** Normal human placenta tissues were obtained from caesarean section and Chorionic villi were dissected and cultured on traditional tissue culture plates. Infections with ZIKV BR, ZIKV AFR (MR766) and DENV2 were performed and quantified by qPCR assay. Detection of nuclear DNA fragmentation as a morphological marker of the apoptosis process in histological sections was performed using the TUNEL assay. **Results**: Quantification of viral replication showed that ZIKV (BR and AFR) infected explants of placental tissue, and maintained a productive infection at 24, 72, and 120 h. DENV2 infected the tissue, however, viral load decreased by 72 and 120 h. Both strains of ZIKV obtained higher labelling for apoptosis compared to DENV2. **Conclusions**: We demonstrate that explant tissue from full term human placentas may be a useful model to study ZIKV infection *ex vivo*.

PC25
Hyperosmolar stress affects TRPV-1 expression and the physiological functions of human trophoblast

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Hyperosmolar stress may be an important stressor that alters normal development of embryos or placentation. Transient receptor potential vanilloid 1 (TRPV-1) is activated by hyperosmolarity and participates in many cellular processes such as apoptosis, authophagy, and others. **Objective**: To evaluate the effect of hyperosmolar

stress on cell viability, migration and invasion, and the contribution of TRPV-1 to these processes in first trimester human trophoblast cells. **Methods:** Swan-71 cell line (human trophoblastic cells) was cultured in complete DMEM-F12 and sucrose hyperosmolar solution was added for 24 h with or without capsaicin (CPZ, 1 μM) and capsazepine (CPZ, 10 μM). TRPV-1 protein expression was analysed by Western blot. Cell viability was analysed by MTT, LDH and β-hCG assays. Apoptosis was evaluated by Bax expression, DNA fragmentation and TUNEL assay, and authophagy by monodansylcadaverine (MDC) assay. Migration was assessed by wound healing assay, activity of metalloproteinases (MMPs) by zymography, and invasion was evaluated in transwells pre-coated with Matrigel. **Results**: In hyperosmolar conditions, TRPV-1 expression was increased (1.3 ± 0.1 fold) (P<0.05, n = 6), cell viability and β-hCG secretion was reduced (25 ± 2% and 28 ± 3%, respectively) (P<0.01, n = 8) and LDH levels were not modified. Apoptotic indices were also increased (1.7 ± 0.1 fold) (P<0.05, n = 6), but no significant changes were observed after the blocking of TRPV-1 with CPZ. MDC assay showed no changes among the treatments. Finally, cell migration, MMPs activity, and invasion were decreased (33 ± 4%, 75 ± 8%, and 45 ± 6%, respectively) (P<0.01, n = 6), and these effects were partially reversed by CPZ. **Conclusions**: Our results proposed that hyperosmolarity induces cell apoptosis and alters cell migration and invasion processes. Our findings also suggest that TRPV-1 may be involved in these events.

PC26

Abnormal endometrial expression of complement regulatory protein decay accelerating factor in women with recurrent implantation failure in cycles of assisted reproduction

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The control of complement activation within embryo-maternal environment is absolutely required for embryo survival. Complement system components are expressed in endometrium during embryo receptivity phase. The mechanism involved in the recurrent implantation failure (RIF) in assisted reproduction remains unknown. **Objective:** To assess the expression of complement C3 and complement regulatory proteins: decay accelerating factor (DAF), CD46 and CD59 in the endometria of women with RIF. **Methods:** RIF was defined as the failure of embryo implantation after three *in vitro* fertilization (IVF) cycles in which one or two morphological high-grade embryos were transferred. Endometrial biopsies from 30 patients with RIF were obtained six days after progesterone administration during mock hormonal endometrial preparation cycle for embryo transfer. Endometrial samples from 16 fertile women obtained six days after ovulation were included as controls. Proteins

were localized by immunohistochemistry and their relative expression determined using histological score (Hscore). The mRNA expression was determined by qRT-PCR. To compare the findings non-parametric Mann-Whitney statistical test was applied. **Results:** Delayed glandular dating with advanced stromal transformation was observed in 40% of RIF. According to Hscore and mRNA levels, DAF expression was significantly low in RIF's samples ($P<0.05$). **Conclusions:** Abnormal DAF expression may lead to uncontrolled complement activation within embryo-endometrial environment compromising embryo survival that potentially explains RIF in assisted reproduction cycles.

PC27

High D-glucose–increased nitric oxide generation leads to higher deiodinase 3 mRNA level in human umbilical vein endothelial cells

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Gestational diabetes mellitus (GDM) characterizes by abnormal maternal D-glucose metabolism, and associates with reduced maternal circulating free tyroxine (fT4) and increased nitric oxide (NO) generation in the placenta vasculature. Deiodinase 3 (DIO3) is a thyroid hormones inactivator enzyme that catalyses deiodination of thyroxine (T4) into reverse triiodothyronine (rT3) and triiodothyronine (T3) into 3,3'-diiodothyronine (T2). DIO3 is increased in syncytiotrophoblast and endothelial cells from GDM pregnancies, but the role of NO in this phenomenon is unknown. **Objective**: To evaluate whether D-glucose and NO increase *Dio3* mRNA level in human umbilical vein endothelial cells (HUVECs). **Methods**: HUVECs from normal pregnancies were exposed (24 hours) to D-glucose (5-20 mM) in the absence or presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM). Total mRNA was extracted with Trizol reagent and used for real time PCR to estimate the relative abundance of *Dio3* and *28S* mRNA using the $2^{-\Delta\Delta Ct}$ method. **Results**: *Dio3* mRNA level was higher (1.8 ± 0.2 fold) in HUVECs exposed to D-glucose from 15 to 20 mM (effective half-maximal concentration (EC_{50}) 12 ± 1 mM). L-NAME blocked D-

glucose increase in *Dio3* mRNA level. **Conclusions**: D-Glucose increases *Dio3* mRNA level via a mechanism that involves NO in HUVECs. GDM-increased NO generation could result in modulation of DIO3 expression in HUVECs.
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PC28 (*abstract available as* OC12)
The role of endogenous annexin A1 (AnxA1) in pregnancy

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PC29
Diabetes and fetal programming: effects on nephrogenesis and basement membranes of renal corpuscles of mouse fetus

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Maternal hyperglycaemia can disturb embryonic differentiation and organogenesis, predisposing the offspring to urinary and cardiovascular dysfunctions in adulthood. This phenomenon is known as fetal programming. **Objective**: Using a mouse model of pregnancy complicated by alloxan-induced type 1 diabetes, we are investigating the effects of maternal diabetes on nephrogenesis, particularly on the structure and molecular composition of renal basement membranes. **Methods:** For this, the number and volume of renal corpuscles of 19 days-foetuses from diabetic (FDM) and nondiabetic (FNDM) females were stereologically estimated and compared. Renal basement membranes were evaluated by PAS staining and immunohistochemistry for collagen type IV, laminin and perlecan. **Results:** We found a number of differentiated and undifferentiated renal corpuscles in FDM significantly lower ($P<0.001$, n = 8)

than that in FNDM. Moreover, FDM had a corpuscular hypertrophy. The levels of mRNA of collagen type IV (*Col4a1* and *Col4a3*), laminin (*Lama5*) and perlecan did not change in FDM kidneys, except *Lama1*, which transcript level was elevated (1.3 ± 0.04 fold). However, Western blot result indicated decreased LAMA1 level (34 ± 7%) in FDM kidneys. Furthermore, we found both glomerular and tubular basement membranes thickened, and an increased deposition of collagen type IV, laminin and perlecan. **Conclusions**: These results indicate that maternal type 1 diabetes promotes renal dysmorphogenesis by (*i*) reducing the number of differentiating renal corpuscles leading to a compensatory corpuscular hypertrophy; (*ii*) thickening of glomerular and tubular basement membranes due to an increased local deposition of extracellular matrix glycoproteins and proteoglycans.
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PC30

Role of IL-4 in learning and memory in the progeny gestated under hypothyroidism.

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Thyroid hormones thyroxin and tri-iodotironine are important for the development of the central nervous system (CNS). Hypothyroidism is diagnosed by low levels of T4 and T3, and high level of thyrotropin (TSH) in the serum. The offspring gestated in hypothyroidism suffer detrimental damage in its learning capacity. Little is known about the mechanisms that are behind these impairments. It has been shown that the secretion of interleukin 4 (IL-4) from T cells, in the meninges, is essential for learning and memory processes. **Objectives:** The aim of this work was to study whether IL-4 can improve cognition in the offspring gestated in hypothyroidism. **Methods:** Using a murine model we evaluated the cognitive ability of the progeny gestated under hypothyroidism and the effect of IL-4 over these central nervous system functions. Barnes maze and the Novel Object Recognition tests were used to evaluate learning and memory. **Results:** The analysis of the results showed a significant reduction in the cognitive abilities and in the serum levels of IL-4 of the offspring gestated in hypothyroidism compared to controls. Interestingly, the IL-4 administration to the offspring gestated in hypothyroidism produces a significant increase in their cognitive capacity of the offspring gestated in hypothyroidism. **Conclusion:** These results support that IL-4 could be involved in the mechanisms that impaired cognition in the offspring gestated in hypothyroidism.

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PC31

Evaluation of Treg, NK cells and their subsets in healthy pregnant women: preliminary results

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Several studies suggest that Treg and NK cells play a relevant role in pregnancy development. Different markers have been used to characterize these cells and their subsets. **Objective**: Our aim was to evaluate Treg and NK cells profile in different trimesters of pregnancy. **Methods:** This cohort study recruited ten healthy women at 2^{nd} and 3^{rd} trimester of pregnancy. Circulating Treg cells were evaluated by flow cytometry using the antibodies $CD45^+$, $CD3^+$, $CD4^+$, $CD25^+$, $FOXP3^+$, and $CD127^-$. $CD69^+$ were for activation and $CD39^+$ for suppression. In order to characterize NK cells it was used $CD16^+$, $CD56^+$, and $CD69^+$ for activation, and intracellular perforin and granzyme were also evaluated. **Results:** We did not find significant difference (P = 0.37) in the percentage of Treg cells expressing $CD45^+CD3^+CD4^+CD25^+FOXP3^+CD127^-$ between 2^{nd} and 3^{rd} trimester of pregnancy (0.19 ± 0.11 versus 0.36 ± 0.26%, respectively). There were no differences regarding activation and suppression markers, neither in the percentages of Treg cells expressing $CD25^{bright}$ or $CD25^{dim}$, $FOXP3^{high}$ or $FOXP3^{low}$. We observed lower (P = 0.01) percentage of $CD16^+CD56^+$ cells (3.5 ± 0.25 versus 5.75 ± 1.39%, respectively) and of $CD56^+16^{dim}$ cells (3.2 ± 0.20 versus 5.24 ± 1.21%, respectively) in the 2^{nd} trimester, compared with the 3^{rd} trimester of pregnancy. There were no other significant differences between the groups. **Conclusion**: These preliminary results reveal increased percentage of circulating NK cells, especially $CD56^+16^{dim}$ cells subset as the pregnancy progresses.

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PC32

Insulin-like growth factor binding protein-1, galectin-9, vimentin, desmin and α-actin expression in decidualized stromal cells from canine and feline placental labyrinth

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In endotheliochorial barriers the syncytium directly faces maternal endothelium. However, in cats and dogs some mesenchymal maternal cells persist and differentiate into a decidualized phenotype. Feline decidual giant cells (FDGC) have been partially characterized. Presence of a canine homologous cell population, once controversial, was recently confirmed. **Objectives:** The aims of the present study were to further characterize FDGC regarding a decidual marker as insulin-like growth factor binding protein (IGFBP1), galectin-9 and cytoskeletal proteins, and to contribute to describe the mesenchymal/vimentin+ cells in canine labyrinth. **Methods:** Canine and feline placental samples (n = 5 each) were evaluated by indirect immunohistochemistry with anti-vimentin, desmin, α-actin, IGFBP-1 antibodies. Anti-galectin-9 was analysed in canine samples. Methodological variations were introduced according to the antibody used. **Results:** IGFBP-1 was highly expressed in the cytoplasm of FDGC. Remarkably, expression was also detected in endometrial fibroblasts closer to the compact wall of decidualized cells, between the spongy zone and labyrinth. In canine labyrinth, scattered large vimentin and desmin positive cells were found; they also were galectin-9 positive, as we reported in FDGC (Conrad *et al*., Am J Reprod Immunol. 75:317-325, 2016). IGFBP-1 was strongly expressed in canine maternal endothelia, and α-actin in close cells. **Conclusions:** Results suggest that feline decidualizing cells express IGFBP-1 before morphological differentiation occurs, and that canine stromal cells also express this decidualizing marker. Desmin expression in canine labyrinth was in accordance to that in mice and humans placenta. Contrary to results reported in baboons, which stated an inversely proportional association between IGFBP-1 and α-actin, stromal canine cells co-expressed both markers.
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PC33

Maternal fat overfeeding programs lack of response to lipid catabolism regulators in the livers of foetuses and offspring, possible implications for lipid overaccumulation

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Leptin induces liver lipid catabolism, increasing acetyl CoA oxidase (ACO) and carnitin palmitoyl transferase-1 (CPT1) expression, through peroxisome proliferator activated receptor ✔ (PPAR✔) activation. We previously found liver lipid overaccumulation and no response to leptin-induced lipid catabolic actions in foetuses from rats fed with an overload of fat (SFD). **Objective:** to analyse whether foetuses and offspring from the SFD group respond to the lipid catabolic effects of the PPAR ✔

activator clofibrate. **Methods:** Female rats were fed with standard (controls) or saturated fat diet (28% fat) since they were 6 week-old (SFD rats). After 8 weeks, they were mated with control males. Control and SFD rats were euthanized at gestational day 21 or allowed to deliver and their offspring euthanized at 140 days of age. Offspring and foetal livers were cultured (3h) (n=6) with or without clofibrate (0.1 M). Lipid levels (triglycerides (TG), phospholipids (PL), free fatty acids (FA) and cholesteryl esters (CE)) were assessed by TLC. ACO and CPT1 expression was analysed by PCR. **Results**: In livers from control foetuses, clofibrate decreased lipid levels (females: TG, FA**,** and EC 25%, males: PL and FA 30%, $P<0.05$, n = 6) and increased ACO mRNA levels (20%, $P<0.05$, males and females). Clofibrate decreased lipid levels (FA: 24%, TG: 44%, EC: 45%, $P<0.05$) in livers from male and female control offspring. Differently, foetuses and offspring from SFD rats showed no response to PPAR activation in their livers. **Conclusions:** Maternal fat overfeeding induces unresponsiveness to PPAR activation in foetal livers that persists in the adult offspring, anomalies probably involved in the intrauterine programming of lipid overaccumulation.

PC34

The offspring gestated in hypothyroxinemia increases Th17 response when suffers experimental autoimmune encephalomyelitis

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Hypothyroxinemia is a highly common thyroid hormone deficiency condition, characterized by a decrease in T_4 serum with normal levels of T_3 and thyroid stimulating hormone. We have shown that gestational hypothyroxinemia increases the autoimmune response in the offspring suffering experimental autoimmune encephalomyelitis (EAE). EAE is a murine model for multiple sclerosis. Given that Th17 cells play a key role in the development and severity of EAE we hypothesize that the progeny gestated in hypothyroxinemia will have greater capacity to secrete interleukin 17 (IL-17) causing a strong EAE. **Objectives:** The aim of this work is to analyse Th17 cell population and their capacity to secrete IL-17 in the progeny gestated under hypothyroxinemia that suffers EAE. **Methods:** We analysed both *in vitro* and *in vivo* the number of Th17 cells and the level of IL-17 in the central nervous system, spleen, lymph nodes, and small intestine in mice gestated in hypothyroxinemia that suffer EAE at different time points. Moreover, we analysed *in vitro* the capacity of $CD4^+$ T cells to differentiate to Th17 cells. **Results**: We found that mice gestated in hypothyroxinemia have increased IL-17 level and higher number

of Th17 cells after five days of EAE induction in small intestine, spleen, lymph nodes, and central nervous system. T $CD4^+$ cells have higher capacity to differentiate to TH17. **Conclusion**: Our results indicate that Th17 response is higher in mice gestated in hypothyroxinemia and suggest that it can be in part responsible of a higher EAE phenotype.
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PC35 (*abstract available as* OC8)

microRNA-130 and microRNA-122 alteration are related to lipid metabolic impairments in the foetal liver of rats with gestational diabetes mellitus

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PC36 (*abstract available as* OC4)
***Trypanosoma cruzi* exosomes increases susceptibility to parasite infection in human placental chorionic villi explants**

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PC37
Circulating NKT cells in patients with gestational diabetes: preliminary findings

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Many cells and mechanisms have been implicated in gestational diabetes mellitus (GDM) pathophysiology. Natural Killer T cells (NKT), which represent 0.2% of all circulating T lymphocytes, are a heterogeneous population; they have some properties of T lymphocytes and others of NK cells. In general, human NKT cells express CD3+CD16+CD56+, and more specifically Valfa+Vbeta+. These cells have been associated with obesity and increased insulin resistance. **Objective:** Our aim was to characterize the profile of NKT cells in peripheral blood samples of women with GDM in the third trimester of pregnancy. **Methods:** This case-control study included 18 women at 28-36 weeks of gestation: 8 with GDM and 10 with healthy pregnancy. Peripheral blood NKT cells were evaluated by flow cytometry, using both monoclonal antibodies: CD3+CD16+CD56+ and Valfa+Vbeta+. We compared the percentages of

NKT in both groups using Student's *t* test. $P<0.05$ were considered significant. **Results:** We found no significant differences in the percentages of NKT cells expressing CD3+CD16+CD56+ in the two groups (0.22 ± 0.13 versus 0.24 ± 0.32%, control and GDM groups, respectively). The percentages of NKT cells Valfa+Vbeta+ were similar in healthy women and GDM patients (0.08 ± 0.04 versus 0.17 ± 0.24%, respectively). **Conclusion:** These preliminary results suggest that circulating NKT subpopulations may not be involved in the pathogenesis of GDM.
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PC38 (*abstract available as* OC2)
The blood brain barrier of the offspring gestated in hypothyroxinemia has higher permeability to macromolecules and to the transmigration of immune cells to the central nervous system

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PC39
***Trypanosoma cruzi* infection in human placentas *in vitro ex vivo* induces the production of MMP-9 and pro-inflammatory cytokines**

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Congenital Chagas has become a global health problem due to the migration of pregnant women with Chagas from endemic to non-endemic countries. Pregnant women have a predominance of Th2 cytokines. But, pregnant women with Chagas with low incidence of transmission to foetus have a predominance of pro-inflammatory Th1 cytokines. **Objective:** To evaluate the levels of pro-inflammatory cytokines and matrix metalloproteinase-9 (MMP-9) activity in placental tissue during parasite invasion *in vitro ex vivo* placental explants culture model. **Methods:** Chorionic villi explants were co-cultured for 4 and 24 h without (control) or with 1 $x10^5$ trypomastigotes (Tulahuen strain). Real time PCR was employed to quantify *T. cruzi* DNA load in explants. Histochemistry analysis was done for tumour necrosis factor alpha (TNFα), interferon gamma (IFNγ), and macrophage migration inhibitory

factor (MIF) expression. In culture supernatants, TNFα and MIF were quantified by ELISA, and MMP-9 activity by zymography. **Results:** *T. cruzi* invasion was verified. TNFα, IFNγ, and MIF increased their expression in syncytiotrophoblast and stromal cells (1.5 ± 0.2, 2.2 ± 0.7, and 3.8 ± 2.1 fold, respectively) of infected explants and in the culture supernatants (TNFα 22.5 ± 11.0 fold and MIF 4.6 ± 1.6 fold) respect to non-infected ones ($P<0.05$, n = 4*)*. MMP-9 activity was also increased (1.2 ± 0.1 fold, $P<0.05$) in infected explants. **Conclusions:** These pro-inflammatory cytokines and MMP-9 could be deleterious or helpful to *T. cruzi* invasion, depending of the level of the placental immune response. It is suggested that the placenta could be the cause of the Th1 profile reported in non-transmitter pregnant women with Chagas.
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PC40
Intrauterine embryo position is altered by obesity prior to implantation

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Diverse signalling pathways, including adrenergic receptor beta 2 (ARβ2), control intrauterine embryo positioning since implantation at a wrong place causes adverse effects on pregnancy outcome. However, the mechanisms underlying remain unclear and even less if obesity affects them. Using cafeteria diet-induced obesity as animal model, we found that obesity causes aberrant uterine fetal distribution and macrosomia on gestational day (gd) 18.5. **Objective**: Determine if the foetal aberrant distribution is consequence of reabsorptions or due to alterations in the foregoing embryo spacing and whether β2AR signalling is involved. **Methods:** Post implantation loss rates (PIL) and embryo positions just after (gestational day (gd) 5.5) and before implantation (gd4.5) were studied in cafeteria diet-induced obese rats and controls. Uterine ARβ2 expression (qPCR and western blot) and localization (immunofluorescence) was analysed on gd4.5. **Results:** PIL was similar in control and obese rats; however, aberrant uterine embryo distribution was detected on gd5.5 and gd4.5 in obese animals, indicating that obesity alters embryo positioning before implantation. On gd4.5, the total number of embryos ($P<0.001$, n = 7 per group) and the percentage of blastocysts were lower (65.42 versus 40.32%, χ^2: $P<0.001$) in obese rats than controls, while the percentage of morulae was increased (20.56 versus 43.55%, χ^2: $P<0.001$) in obese animals. ARβ2 localization was similar in control and obese rats, showing intense label at the myometrium and weak expression at the epithelium. ARβ2 mRNA and protein expression was up regulated (1.44 ± 0.22 ($P<0.05$) and 2.3 ± 0.43 ($P<0.001$) fold, respectively) in uterus from obese rats. **Conclusions:** Alterations in the ARβ2 signalling before implantation may be one of the mechanisms underlying the aberrant uterine embryo distribution observed in obese animals. Treatments that reverts this alteration may be useful to prevent implantation

problems and the consequent adverse effects on pregnancy outcome described in obesity.

PC41 (*abstract available as* OC3)
Versican expression and roles in hydatidiform moles

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PC42
Reck expression is induced in placentas from preeclampsia and reduces migration, invasion, and endovascular remodelling of first trimester human trophoblast

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In normal pregnancies trophoblast cells invade the maternal decidua to reach and modify the spiral arteries. However, in preeclampsia this capacity of the trophoblast is reduced, emerging as proposed cause of this syndrome. Reversion-inducing-cysteine-rich-protein with kazal motifs (Reck) is a plasma membrane GPI-anchored protein that inhibits different metalloproteinases, inhibiting migration, invasion, and angiogenesis. **Objectives**: To determine the role of Reck on migration, invasion, and endovascular remodelling capacity of human trophoblast and its expression and localization in human placentas from normal and preeclampsia pregnancies. **Methods:** Expression and localization of Reck in the human first trimester trophoblast cell line HTR8/SvNeo and in placentas from normal and preeclampsia pregnancies were evaluated by western blot and immunofluorescence. Cells were transfected whit the expression vectors for human Reck or shRNA against Reck. Migration, invasion, and endovascular remodelling capacity was assayed by the Boyden chambers migration/invasion assays as well as the endovascular remodelling of *in vitro* pre-stablished endothelial-vascular tubes. **Results:** Reck protein was detected at the plasma membrane of HTR-8/SVneo cells. Knockdown cells for Reck showed increased ($P<0.05$, n=3) migration (1.4 ± 0.1 fold), invasion (2.2 ± 0.2 fold), and

integration on pre-formed tubes (1.4 ± 0.1 fold). These phenomena were reduced after overexpressing this protein. Reck was also detected in the syncytiotrophoblast in human placentas, and preeclampsia resulted in higher protein abundance (1.4 ± 0.2 fold, $P<0.05$, n=5) compared with placentas from normal pregnancies. **Conclusion:** Reck is a protein expressed from early in pregnancy in human trophoblast where it could play a role in the pathogenesis of preeclampsia.
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PC43

Human maternal supraphysiological hypercholesterolemia leads to endothelial dysfunction of the placental microvasculature

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Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring foetal development. However, maternal supraphysiological hypercholesterolemia (MSPH) leads to endothelial dysfunction in the umbilical vein. **Objective:** To determine the effect of MSPH on the endothelial function of the placental microvasculature. **Methods:** Pregnant women (from the Hospital Clínico UC-CHRISTUS, Santiago de Chile) with total cholesterol ≤280 mg/dL or >280 mg/dL at term were considered as MPH (n = 8) or MSPH (n = 4) respectively. Arteries and veins rings were prepared from the placental microvasculature (300-700 μm lumen), mounted in a wire myograph, and the response to calcitonin gene related peptide (CGRP, 0.01-100 nmol/L, 5 min) in KCl-preconstricted vessels (32 mmol/L) was measured. Nitric oxide (NO)-dependent dilation was estimated in the absence or presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl-ester (L-NAME, 100 μmol/L, 20 min). Human placental microvascular endothelial cells (hPMECs) were isolated by trypsin/collagenase digestion. L-Arginine uptake (500 μmol/L, 3 μCi/mL, 37ºC, 1 minute), L-citrulline formation from L-arginine (60 minutes, 37ºC) (HPLC) was measured in the absence or presence of L-NAME. Arginases activity was measured in hPMECs by determination of urea formation from L-arginine (50 μmol/L, 60 minutes, 37°C). Protein expression of eNOS, human cationic amino acid transporter 1 (hCAT-1) and 2 (hCAT-2), and arginase 2 was evaluated by Western blot. **Results**: The maximal relaxation (R_{max}) to CGRP was reduced ($P<0.05$) in MSPH arteries (R_{max} MSPH: 0.1 ± 0.1%, R_{max} MPH: 28 ± 3%) and veins (R_{max} MSPH: 1.4 ± 0.2%, R_{max} MPH: 32 ± 3%). The protein abundance of hCAT-1, hCAT-2,

arginase 2, and total NOS was unaltered in hPMECs from MSPH compared with MPH. However, L-arginine uptake (MSPH: 0.16 ± 0.04 vs MPH: 0.28 ± 0.02 pmol/μg protein/minute), total arginases activity (MSPH: 0.96 ± 0.15 vs MPH: 1.6 ± 0.07 pmol urea/μg protein/minute) and total NOS activity (MSPH: 0.3 ± 0.6 vs MPH: 5.6 ± 0.5 pmol/μg protein) were lower (P<0.05) in hPMECs from MSPH compared with MPH. **Conclusion**: MSPH associates with reduced bioavailability of NO leading to lower endothelium-dependent reactivity of the placental microvasculature via a including reduced L-arginine transport, and NOS and arginases activity.
Funding: FONDECYT 1150344/1150377, BF holds Faculty of Medicine, PUC–PhD fellowship.

PC44

Human maternal supraphysiological hypercholesterolemia increases the efflux of cholesterol from placental trophoblast and microvascular endothelial cells

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Cholesterol traffic from maternal to foetal circulation occurs in placental trophoblast and microvascular endothelial cells (hPMECs). Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal development, but maternal supraphysiological hypercholesterolemia (MSPH) leads to endothelial dysfunction and atherosclerosis in foetal vessels. **Objective:** To determine the effect of maternal cholesterol levels on the cholesterol efflux to high-density lipoprotein (HDL) in human trophoblast and hPMECs. **Methods:** Pregnant women with total cholesterol ≤280mg/dL or >280mg/dL at term were considered as MPH or MSPH, respectively. Blood samples and placentas were collected at Hospital Clínico UC-CHRISTUS (Santiago de Chile). Trophoblast and hPMECs were isolated by trypsin/DNAse and trypsin/collagenase digestion, respectively, from MPH or MSPH pregnancies. Cells were pre-incubated (5%, 24 hours, 37ºC) with maternal serum from trimester 1 (T1), 2 (T2), or 3 (T3) of pregnancy (n = 4-8). Cholesterol efflux was determined in cells pre-incubated with [^{3}H]cholesterol (24 hours, 37ºC) that were later incubated (6 hours, 37ºC) with 50 μg/mL HDL in the culture medium as cholesterol acceptor. Efflux was estimated as percentage of total [^{3}H]cholesterol in the culture

medium. HDL used as acceptor of cholesterol was obtained by ultracentrifugation of plasma from nonpregnant women. **Results**: The incubation of MPH trophoblast with maternal MSPH serum increased (P<0.05) the cholesterol efflux (T1: 1.4 ± 0.1 fold, T2: 1.2 ± 0.03 fold, T3: 1.3 ± 0.05 fold) compared with serum from MPH pregnancies (P<0.05, n = 2-4 cell cultures and 4-8 maternal sera). Incubation with maternal serum from MSPH or MPH increased cholesterol efflux in MPH trophoblast (~1.22 fold)) and MPH hPMECs (~1.65 fold) compared with cells incubated with maternal serum from MPH. **Conclusion**: MSPH is a condition that increases the cholesterol efflux in placental trophoblast and microvascular endothelial cells along pregnancy. This phenomenon may contribute to the atherosclerosis described in MSPH offspring.
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PC45

Neonatal high-density lipoprotein from pregnancies with maternal supraphysiological hypercholesterolemia impairs nitric oxide synthase activity in human umbilical vein endothelial cells

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Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy, but some women develop maternal supraphysiological hypercholesterolemia (MSPH) leading to foetoplacental endothelial dysfunction. **Objective:** To determine whether MSPH modify the foetal lipid levels (total cholesterol (TC) and triglycerides) and the function of high-density lipoprotein (HDL) isolated from umbilical cord serum from MPH or MSPH pregnancies. **Methods:** TC and triglycerides were measured in maternal and umbilical cord serum from pregnant women from the Hospital Clínico UC-CHRISTUS (Santiago, Chile) (n = 7). Pregnancies with maternal TC ≤280 or >280 mg/dL at term were considered as MPH or MSPH, respectively. Lipoprotein distribution at the umbilical cord serum was determined by fast performance liquid chromatography. HDL in the umbilical cord serum was isolated by ultracentrifugation. Primary cultures of human umbilical vein endothelial cells (HUVECs) were incubated with umbilical cord serum or HDL from MPH or MPSH pregnancies (15 hours, 37ºC). Nitric oxide synthase (NOS) activity was evaluated by HPLC as N^G-nitro-L-arginine methyl ester–inhibited fraction of L-citrulline cell content. **Results:** TC (58 ± 10 vs 60 ± 9 mg/dL), triglycerides (36 ± 12 versus 35 ± 8 mg/dL) or lipoprotein profile (~45% HDL and 45% LDL-cholesterol content) was

unaltered in umbilical cord serum from MSPH compared with MPH pregnancies. Basal NOS activity was lower (P<0.05) in HUVECs from MSPH compared with MPH (10 ± 2 versus 35 ± 4 pmol/μg protein, respectively). In HUVECs from MPH pregnancies NOS activity was reduced after incubation with umbilical cord serum (12 ± 2 versus 30 ± 4 pmol/μg protein) and HDL (5 ± 1 versus 30 ± 4 pmol/μg protein) from MSPH compared with MPH. **Conclusion:** MSPH is a maternal condition that alters the neonatal HDL biological effects on NOS activity in HUVECs.
Funding: FONDECYT 1150344/1150377, BF holds VRI/Faculty of Medicine (PUC) PhD fellowship.

PC46

Prevalence of maternal supraphysiological dyslipidemia in a group of Chilean pregnant women with gestational diabetes mellitus

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During pregnancy mothers exhibits a physiological dyslipidemia characterized by elevated plasma level of total cholesterol (TC) and triglycerides (Tg), referred as maternal physiological dyslipidemia (MPD). Some pregnancies develop a supraphysiological dyslipidemia (MSPD), which associates with fetoplacental endothelial dysfunction. Gestational diabetes mellitus (GDM) is a pregnancy disease with maternal and fetal hyperglycemia leading to fetoplacental endothelial dysfunction. Although is described that GDM also associates with maternal dyslipidemia, the prevalence of MSPD in GDM pregnancies or the effect of this condition on the fetal vasculature are unknown. **Objective:** To determine the prevalence of MSPD in a group of GDM pregnancies. **Methods:** Prevalence of GDM was estimated in 4732 Chilean pregnant women from the Hospital Clínico UC-CHRISTUS (Santiago, Chile) for the period 2014-2015. Maternal lipids were determined in trimesters 1 (T1), 2 (T2), and 3 (T3) of pregnancy to estimate MSPD prevalence. Pregnancies with TC >280 mg/dL and Tg >275 mg/dL were considered as MSPD. **Results:** The prevalence of GDM was 7.2%, of which a 25.2% showed with MSPD. TC (T1: 173 ± 23 vs 232 ± 17, T2: 206 ± 35 vs 285 ± 15, T3: 226 ± 35 vs 310 ± 25 mg/dL) and Tg (T1: 99 ± 22 vs 203 ± 57, T2: 155 ± 36 vs 250 ± 58, T3: 195 ± 52 vs 349 ± 65 mg/dL) were increased (P<0.05) in all the trimesters of pregnancy in GDM with MSPD compared to MPD. **ConclusionS:** A significant number of GDM pregnancies present with MSPD in the studied population with

increased TC and Tg. Although the consequences of this phenomenon are unknown, it could contribute to the fetoplacental vascular alterations described in MSPD.
Funding: FONDECYT 1150344/1150377, BF holds VRI/Faculty of Medicine (PUC) PhD fellowship.

PC47

Effect of $MgSO_4$ on protein expression and exosomes release from human placental microvascular endothelial cells from late-onset preeclampsia

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Exosomes associate with immune tolerance and regulation of glucose concentration and oxygen tension in normal pregnancies. Late onset preeclampsia (LOPE) results in lower endothelial nitric oxide synthase (eNOS) activity at the foetoplacental circulation leading to endothelial dysfunction. Magnesium sulphate ($MgSO_4$) is a salt used to prevent eclamptic seizures and to restoring vascular tension in patients with preeclampsia. Additionally, a role for adenosine via activation of adenosine receptors has been proposed to modulate this phenomenon in preeclampsia. Since magnesium (Mg^{2+}) level play crucial roles in modulating endothelial function we hypothesize that Mg^{2+} will alter adenosine receptors and NOS protein abundance in human placental microvascular endothelial cells (hPMECs). **Objectives:** To evaluate the effect of $MgSO_4$ on the protein abundance of eNOS, inducible NOS (iNOS), A_{2A} (A_{2A}AR), and A_{2B} (A_{2B}AR) adenosine receptors, and the exosome release from hPMECs from normal and LOPE pregnancies. **Methods:** hPMECs were isolated from placentas from normal (n = 3) or LOPE (n = 2) pregnancies from the Hospital Clínico UC-CHRISTUS (Santiago de Chile). Cells were cultured under standard conditions (37°C, 5% CO_2) in serum free culture medium, and incubated (37°C, 12 hours) in the absence or presence of $MgSO_4$ (0.8-4.0 mmol/L). Exosomes were isolated from conditioned culture medium by ultracentrifugation and sucrose density gradient. Exosomes quantification and size distribution was determined using the Nano Sigth NS300. The protein abundance for eNOS, iNOS, A_{2A}AR, and A_{2B}AR, and the exosomes marker CD63 were analysed by Western blot. **Results:** Exosomes release from hPMECs from

preeclampsia was lower (P<0.05) (7.1 and 10.7 x10^7 particles/µg of total protein) than normal pregnancies (1.3 ± 0.3 x10^8). Incubation of cells with $MgSO_4$ reduced the release of exosomes in cells from normal pregnancies (2 mmol/L: 40 ± 15%, 3 mmol/L: 25 ± 11%), and preeclampsia (2 mmol/L: 28 and 110%, 3 mmol/L: 54 and 128%). Incubation of hPMECs from normal pregnancies with $MgSO_4$ increased A_{2B}AR (~1.7 fold), iNOS (~1.4 fold), and eNOS (~1.5 fold) protein abundance. In cells from preeclampsia $MgSO_4$ increased A_{2A}AR (~1.6 fold), A_{2B}AR (~1.8 fold), and iNOS (~1.6 fold). **Conclusion:** Extracellular Mg^{2+} cause differential modulation on protein expression in the foetoplacental microvascular endothelium from normal and LOPE pregnancies. Since Mg^{2+} reduced exosomes release, these nanovesicles may be involved in this phenomenon in the foetoplacental vasculature.
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PC48

Changes in the carbohydrate expression in cattle and buffaloes infected with the abortigenic protozoan *Neospora caninum*

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Carbohydrate pattern in the placenta was studied in different species showing a great variation between species. The sacharydes of the surface glycoconjugates are important to the adhesion between conceptus and uterus. Besides, reproductive diseases as tritrichomonosis and campylobacteriosis generate changes in this pattern in cattle. **Objective:** The aim of this study was to characterize the saccharide in placenta of cattle and buffaloes after experimental infection with the abortigenic protozoan *Neospora caninum* (*N. caninum*) at early gestation by lectinhistochemistry. **Methods:** Heifers and buffaloes with 70 days of pregnancy were inoculated with 1 ×10^8 tachyzoites of *N. caninum* (inoculated groups; bovine n = 9, buffaloes n = 2) and PBS (control group; bovine n = 2, buffaloes n = 2). Nc-1 (n: 3), Nc-6 Argentina (n: 3) and Nc-Spain 7 (n: 3) strains were used in heifers. In buffaloes only Nc-1 strain was used. Placenta and uterus samples were collected at time of necropsy (28 days after inoculation). These tissues were analysed by histopathology analysis, immunhistochemistry (IHC) for *N. caninum*, lectinhistochemistry (LHC), PCR and

microsatellite genotyping. **Results:** Histopathology analyses showed differences among groups, and no compatible lesions were found in control groups. IHC and PCR test were positives to *N. caninum* in the inoculated groups and negatives in control groups. Microsatellite genotyping assay determined that every group had just the specific strain inoculated. LHC results were different between species. Besides, inoculated groups change the carbohydrate expression. **Conclusion**: *N. canimum* inoculation changes the pattern of saccharides present in the placenta and uterus tissues both in cattle and in buffaloes. This variation could generate alterations in pregnancy in infected animals.

PC49

Maternal supraphysiological dyslipidemia in pregnancy worse vascular response of umbilical vein rings from gestational diabetes mellitus

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Pregnancy associates with increased maternal level of lipids (i.e., maternal physiological dyslipidaemia, MPD). However, when lipid level reaches over the physiological values, maternal supraphysiological dyslipidaemia (MSPD) is recognized. The consequences of MSPD in the foetus include foetoplacental endothelial dysfunction and increased foetal atherosclerosis. Gestational diabetes mellitus (GDM) associates with MSPD and endothelial dysfunction in umbilical veins, the latter caused mainly by maternal hyperglycaemia and hyperinsulinemia. However, the consequences of MSPD in GDM pregnancies in the foetal vasculature have been neglected. **Objectives:** To determine whether MSPD alters endothelial-dependent umbilical vein reactivity in GDM pregnancies. **Methods:** Placentas were collected at Hospital Clínico UC-CHRISTUS (Santiago de Chile). Umbilical veins rings were isolated from four study groups: GDM or normal pregnancies, with or without MSPD. Vein rings were mounted in a wire myograph and the response to insulin (0.1-1000 nmol/L, 5 min) or calcitonin-gene related peptide (CGRP, 0.01-100 nmol/L, 5 min) in KCl-preconstricted vessels (32 mmol/L) was measured. Nitric oxide (NO)-dependent dilation was estimated in the absence or presence of the NO synthase inhibitor N^G-nitro-L-arginine methyl-ester (L-NAME, 100 μmol/L, 20 min). **Results**: GDM showed lower (P<0.05, n = 5-10) NO-dependent maximal relaxation (R_{max}) in response to insulin (GDM: 17 ± 2%, normal: 43 ± 6%) and CGRP (GDM: 15 ± 3%, normal: 30 ± 3%). CGRP-dilation was further reduced in vein rings from GDM with MSPD (MSPD: 12 ± 1%) and in normal pregnancies with MSPD (MSPD: 14 ± 1%),

but not in response to insulin. **Conclusion:** MSPD contributes to GDM-associated endothelial dysfunction by worsening the vascular dilation of umbilical vein rings in this pathology.
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PC50

Modulation of the placental HDL and LDL cholesterol uptake by the maternal lipids level in human trophoblast

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In normal pregnancies, a physiological increase of the maternal plasma cholesterol from trimester 1 (T1) to T2 and T3 satisfies the foetal growth and maturation requirements, condition referred as maternal physiological hypercholesterolemia (MPH) (≤280 mg/dL at term). However, ~27% of pregnant women show maternal plasma supraphysiological hypercholesterolemia (MSPH) (>280 mg/dL at term), a condition that associates with placental endothelial dysfunction and foetal atherosclerosis. Human trophoblast incorporates high density (HDL) and low density (LDL) lipoprotein cholesterol via scavenger receptor class B type I (SR-BI) and LDL receptor (LDL-R), respectively. Whether MSPH changes receptor-mediated cholesterol uptake in the trophoblast is not reported. **Objectives:** To determine whether HDL and LDL cholesterol uptake, and SR-BI and LDL-R expression in human trophoblast are altered by MSPH maternal serum. **Methods:** Placentas were collected at Hospital Clínico UC-CHRISTUS (Santiago de Chile). HDL and LDL were purified from human adult serum by ultracentrifugation in a KBr gradient. HDL and LDL were labeled with the lipophilic dye DiI (3 mg/mL, 18 h, 37ºC). Human trophoblast cells were isolated from whole term placentas by digestion with trypsin/DNAse and Percoll gradient. Trophoblast cells were incubated with high D-glucose DMEM/F12 medium containing 5% maternal serum from T1, T2, or T3 of pregnancy (18 h, 37ºC). Cholesterol uptake was estimated in cells incubated with HDL-DiI and LDL-DiI (0-50 µg/mL, 4 h, 37ºC) by quantification of cellular fluorescence. Protein abundance of SR-BI and LDL-R was evaluated by Western blot. **Results:** HDL-DiI and LDL-DiI uptake was higher (P<0.05, n = 6) in cells incubated with T2 serum from MPH (34 ± 8 and 14 ± 2%, respectively). The protein abundance of SR-BI was increased by T2 serum (2.2 ± 0.2 fold). However, the protein abundance of LDL-R was increased by T2 (4.7 ± 0.5 fold) and T3 (4.8 ± 0.8 fold) sera. Incubation

of cells with T2 serum from women with MSPH decreased the LDL-DiI uptake (10 ± 0.2%) and the LDL-R protein abundance (59 ± 10%). **Conclusion:** Maternal serum from MSPH pregnancies modulates the expression and activity of lipoprotein receptors depending on the trimester of pregnancy.
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PC51

Insulin recovers endothelial dysfunction requiring A_{2B} adenosine receptor activation in human umbilical vein endothelium from late-onset preeclampsia

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Preeclampsia courses with endothelial dysfunction and insulin resistance. Late-onset preeclampsia (LOPE) increases maternal and foetal plasma adenosine levels and A_{2B} adenosine receptor (A_{2B}AR) expression in human umbilical vein endothelial cells (HUVECs) and increases L-arginine transport via the human cationic amino acids transporter 1 (hCAT-1) (coded by *SLC7A1* gene). However, whether A_{2B}AR are involved in LOPE-reduced insulin response is unknown. **Objective**: To evaluate A_{2B}AR involvement on insulin effect on endothelial function in HUVECs from LOPE. **Methods**: L-Arginine uptake (100 μmol/L, 3 μCi/mL, 37ºC, 1 minute), hCAT-1 expression (protein and mRNA) and hCAT-1 transcriptional activity (*Luciferase*/*Renilla* activity) were measured. Protein abundance of A_{2B}AR, phosphorylated p44/42^{mapk} (P~p44/42^{mapk}), protein kinase B/Akt (P~Akt), and endothelial nitric oxide synthase (eNOS) was detected by Western blot. Assays were in the absence or presence (8 hours) of insulin and/or the A_{2B}AR agonist BAY 60-6583 (0.1 nmol/L) and antagonist MRS-1754 (30 nmol/L). Vascular response to insulin (0.1-1000 nmol/L) was measured in KCl (32.5 mmol/L)-preconstricted umbilical vein rings in a wire myograph in the absence or presence of adenosine and/or MRS-1754. **Results**: Insulin increased (P<005, n = 5) L-arginine uptake (2.2 ± 0.9 fold (maximal transport capacity (V_{max}/K_m)), hCAT-1 expression (protein abundance 1.7 ± 0.4 fold, mRNA expression 3.5 ± 0.7 fold), and *SLC7A1* transcriptional activity (2.0 ± 0.5 fold) in cells from normal pregnancies. Insulin also increased P~p44/42^{mapk} (2.1 ± 0.6 fold), P~Ser^{1177}eNOS (2.3 ± 0.6 fold), and total

eNOS protein abundance (1.7 ± 0.4 fold) in these cells. MRS-1754 blocked insulin-increase in the V_{max}/K_m, hCAT-1 expression, and *SLC7A1* transcriptional activity. BAY 60-6583 enhanced insulin effect on total eNOS protein abundance (~30%). LOPE increased the V_{max}/K_m for L-arginine transport (3.7 ± 0.4 fold), hCAT-1 expression (protein abundance 1.6 ± 0.3 fold, mRNA expression 1.6 ± 0.4 fold), A_{2B}AR (1.7 ± 0.5 fold), total eNOS (1.6 ± 0.2 fold), P~Ser^{1177}eNOS (1.9 ± 0.4 fold), and P~Thr^{495} eNOS (1.4 ± 0.2 fold) protein abundance. Insulin blocked LOPE-increased eNOS mRNA, P~Ser^{1177} eNOS, P~Thr^{495} eNOS, and A_{2B}AR protein expression, but did not alter total eNOS protein abundance and hCAT-1 expression. L-Arginine uptake in cells from LOPE was further increased by insulin. MRS-1754 blocked LOPE-increased hCAT-1 and total and P~Thr^{495} eNOS expression only in the presence of insulin. BAY 60-6583 did not alter this hormone's effect. LOPE reduced insulin dilation (half-maximal dilatory effect of insulin (EC_{50}) LOPE/normal = 15 ± 0.7) compared with normal pregnancy, a response that was partially reversed by MRS 1754 (EC_{50} LOPE/normal = 2 ± 0.2). **Conclusion**: The reduced foetoplacental vascular response to insulin in LOPE could be enhanced by A_{2B}AR activation in the umbilical vein endothelium.
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PC52

Sodium/proton exchanger subtype 1 regulates intracellular pH in human umbilical vein endothelial cells from gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a pregnancy-related disease that associates with maternal and foetal hyperglycaemia. It is known that intracellular pH (pHi) modulates proliferation in human umbilical vein endothelial cells (HUVECs). One of the main mechanisms regulating pHi in endothelial cells is the sodium/proton exchanger (NHEs) subtype 1 (NHE1), but its role in GDM has not been addressed. **Objectives**: Our aim was to determine whether NHE1 plays a role in pHi regulation in HUVECs from GDM pregnancies. **Methods:** Primary cultures of HUVECs from normal pregnancies and pregnancies where the mother was with GDM and treated with diet (GDM*d*) (n = 4) were collected from Hospital Clínico UC-CHRISTUS at

the Pontificia Universidad Católica de Chile. The pHi was measured with 2,7-bicarboxyethyl-5,6-carboxyfluorescein (BCECF-AM) probe in a fluorimeter. NHEs and NHE1 activity in the absence or presence of 5-N,N-hexametilenamiloride (HMA) (NHEs inhibitor, 6 min, 0.5 μmol/L) and zoniporide (Z) (NHE1 inhibitor, 6 min, 100 nmol/L), respectively, was estimated by measuring the pHi recovery rate (*dpHi/dt*) by the NH_4Cl acid-pulse technique. **Results:** HUVECs from GDM*d* showed higher ($P<0.04$) pHi value (7.9 ± 0.1) compared with normal pregnancies (7.1 ± 0.07). The *dpHi/dt* in GDM*d* was 2-fold higher ($P<0.05$) than in normal pregnancies. HMA and Z blocked *dpHi/dt* in HUVECs from normal and GDM pregnancies. **Conclusions:** NHE1 activity plays a role in the pHi regulation leading to intracellular alkalization in HUVECs from GDM*d* pregnancies. Funding: FONDECYT 1150377/1150344 (Chile). AS holds CONICYT (Chile) PhD fellowship.

PC53 (*abstract available as* OC9)

NHE1 modulates intracellular pH and cell proliferation in human ovarian cancer

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PC54

Insulin therapy restores the equilibrative nucleoside transporter 1 expression in human umbilical vein endothelial cells from gestational diabetes mellitus

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Human umbilical vein endothelial cells (HUVECs) from diet treated women with gestational diabetes mellitus (GDM*d*) show reduced C/EBP homologous protein 10 (hCHOP)-dependent human equilibrative nucleoside transporter 1 (hENT1) expression. Some patients with GDM*d* are enrolled into insulin therapy (GDM*i*) to achieve optimal glycaemia. *In vitro* insulin recovers GDM*d*-reduced hENT1 expression in HUVECs. However, it is unknown whether insulin therapy restores GDM*d*-reduced hENT1 expression via hCHOP in HUVECs. We hypothesize that insulin restores hENT1 expression in HUVECs from GDM*d* or GDM*i* involving hCHOP. **Objective:** To determine the insulin effect on hENT1 and hCHOP expression in HUVECs from GDM*d* and GDM*i*. **Methods**: HUVECs were isolated from GDM*d* or GDM*i* pregnancies from the Hopsital Clínico UC-CHRISTUS and Hospital San Juan de Dios (Santiago de Chile). hENT1 and hCHOP expression was evaluated by Western blot in the absence or presence of insulin (1 nmol/L, 8 h) and N^G-nitro-L-arginine methyl ester (L-NAME, 100 μmol/L, 8 h). Nitric oxide (NO) level was measured by DAF-FM probe. **Results:** The protein abundance of hENT1 was lower ($P<0.05$, n = 4) in cells from GDM*d* (34 ± 6%), but not from GDM*i* compared with normal pregnancies. Incubation with insulin did not alter its expression in cells from normal, GDM*d* or GDM*i* pregnancies. HUVECs from GDM*d* or GDM*i* showed increased NO level (5.7 ± 0.6 and 5.7 ± 0.5 fold, respectively) compared with normal pregnancies, an effect partially reversed by insulin (49 ± 3% and 51 ± 5%, respectively). L-NAME restored the GDM*d*-reduced hENT1 protein abundance. Incubation of cells with insulin + L-NAME reduced hENT1 protein abundance in cells from GDM*i* (40 ± 11%). hCHOP protein abundance was similar in cells from normal, GDM*d*, or GDM*i* pregnancies, and insulin or L-NAME did not alter hCHOP protein abundance in any group. However, cells exposed to insulin + L-NAME show reduced hCHOP protein abundance in GDM*d* (35 ± 12%) and GDM*i* (53 ± 10%). **Conclusion:** Insulin therapy results in normalization of hENT1 protein expression, a phenomenon that could, at least in part, involve hCHOP in HUVECs.
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PC55
sFlt-1 induces endoplasmic reticulum stress by activating PERK pathway in BeWo cells

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The unfolded protein response is a cellular adaptive response for the endoplasmic reticulum stress which evolved to restore protein folding homeostasis by reducing protein synthesis through phosphorylation of the eukaryotic translation initiation factor 2α (eIF2α). Placental endoplasmic reticulum (ER) stress has been postulated in

the pathophysiology of preeclampsia along with imbalance in the angiogenic (vascular endothelial growth factor (VEGF)) and antiangiogenic (soluble VEGF receptor 1 (Flt-1)) factors. The role of sFlt-1 has not been explored in the induction of ER stress. **Objectives:** We studied the role of sFlt-1 in the induction of the protein kinase RNA-like endoplasmic reticulum kinase (PERK) branch of ER stress. **Methods**: Blood samples from normotensive (n = 40) and preeclampsia (n = 40) pregnancies were collected at the Department of Obstetrics & Gynaecology, AIIMS (New Delhi, India) with approval from Institute Ethics Committee. The level of s-Flt-1 was measured by sandwich ELISA. BeWo cells were incubated with these sera and activation of eIF2α was detected by immunofluorescence, and its expression by RT-PCR and Western blot. **Results**: Maternal level of s-Flt-1 was higher (P<0.01) in serum from preeclampsia (9.2 ± 2.3 fold) compared with normal pregnancies. Protein expression of eIF2α was higher (P<0.05) in BeWo cells exposed to sera from preeclampsia (~6 fold) compared with normal pregnancies. Similar results were found when the mRNA levels were compared between these two groups (P<0.04). **Conclusion:** The increased level of s-Flt-1 detected in the preeclampsia serum induced ER stress in BeWo cells. Therefore, we speculate that increased s-Flt-1 may contribute to induction of ER stress in preeclampsia.

PC56

Hydrogen sulphide producing enzymes are decreased in placentas from preeclampsia

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Preeclampsia is recognized as new onset of gestational hypertension and proteinuria after twenty weeks of gestation. The aetiology of preeclampsia is elusive. It is recognized as a state of accelerated inflammatory events, oxidative stress, and angiogenic imbalance with a failure in endogenous protective pathways, which could be antioxidants, anti-inflammatory and vasodilators. One of such molecules is hydrogen sulphide (H_2S) which is endogenously produced by cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS). **Objectives:** The present study was aimed to compare the expression of CSE and CBS in placentas from women with preeclampsia and from normotensive, non-proteinuric pregnancies. **Methods:** Forty placentas (20 from preeclampsia and 20 from normotensive, non-proteinuric) were collected from women undergoing caesarean section from the labour room of the Department of Obstetrics & Gynaecology, AIIMS (New Delhi, India) (Institute Ethics Committee no. IESC/T-467). The placental tissues were processed for immunohistochemistry and immunofluorescence to analyse the expression of CSE and CBS. **Results:** The strong expression of CSE was localized in endothelium and smooth muscle cells of foetal blood vessels whereas weak expression was observed

in syncytiotrophoblast and stromal cells of placenta. The enzyme CBS was predominantly localized in the syncytiotrophoblast with weaker expression around the foetal vessels and stromal cells of placenta. The expression of CSE and CBS was reduced (P<0.05) in the blood vessels, stromal cells, as well as syncytiotrophoblast of placentas from preeclampsia as compared to normotensive, non-proteinuric controls. **Conclusion:** Down regulation of H_2S producing enzymes (CSE and CBS) in placentas from preeclampsia may suggest that H_2S may be one of the plausible factors involved in the pathogenesis of preeclampsia.

PC57

Matrix metalloproteinase 2 and tissue inhibitor of metalloproteinase 2 mRNA levels in early onset preeclampsia

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Preeclampsia continues to be a leading cause of maternal and perinatal morbidity and mortality. Although its exact aetiology is not clear, it is believed that inadequate trophoblast invasion represents a pathologic factor. Poor early placentation is often associated with early onset preeclampsia (EOPE). Matrix metalloproteinase 2 (MMP2) and its inhibitor tissue inhibitor of metalloproteinase 2 (TIMP2) are metal-dependent endopeptidases degrading extracellular matrix and appear to play critical roles in trophoblast invasion. Thus it is relevant to analyse the status of these markers in patients with EOPE. **Objectives:** The present study was designed to compare the gene expression of MMP2 and TIMP2 in patients with EOPE with that of healthy controls. **Methods:** Thirty each of clinically diagnosed patients with EOPE (age of onset ≤34 weeks) and gestational/maternal age matched normotensive, non proteinuric controls were recruited from the Department of Obstetrics and Gynaecology, AIIMS (New Delhi, India), after taking ethical clearance (IECPG-247/30.03.2016). Gene expression of MMP2 and TIMP2 in plasma samples (n = 30) and mode of delivery matched tissue samples (n = 10) of both patients and controls was analysed by RT-PCR. **Results:** MMP2 mRNA levels in plasma and tissue samples from patients with EOPE were decreased (plasma: 10 folds, tissue: 3.88 folds (P<0.05), but TIMP2 mRNA levels were increased (plasma: 8 folds, tissue: 2.76 folds) (P<0.05) as compared to their gestational and maternal age matched normotensive, non proteinuric controls. **Conclusion:** The decrease in MMP2 and increase in TIMP2 mRNA levels in patients with EOPE may suggest shallow trophoblast cell invasion during placentation.

PC58

Effects of magnesium gluconate and magnesium sulfate on lipid peroxidation and Ca^{2+}-ATPase activity of UV-irradiated syncytiotrophoblast plasma membranes

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During preeclampsia the imbalance between oxidant and antioxidant systems occurs, resulting in damage of cell membranes and alteration of the function of the integral membrane proteins, such as the plasma membraneCa^{2+}-ATPase. The standard treatment to prevent eclampsia is administration of magnesium sulfate ($MgSO_4$). However, its use has certain limitations, which has led to study some other alternatives. Among them, magnesium gluconate ($MgGl_2$), has been shown to act as antioxidant, similarly to $MgSO_4$. **Objectives:** To compare the effects of $MgGl_2$ with those already shown for $MgSO_4$, on lipid peroxidation and Ca^{2+}-ATPase activity of syncytiotrophoblast plasma membranes (SCT) induced by UV exposure. **Methods:** The SCT plasma membranes (microvillous (MVM) and basal (BM)) were isolated by differential centrifugation and discontinuous sucrose gradient ultracentrifugation, and then irradiated with UV light for 30 minutes, in absence or presence of $MgGl_2$ or $MgSO_4$. In another set of experiments, both membranes were irradiated with UV light for 30 minutes, and then incubated 24 hours at 4°C, in the absence or presence of $MgGl_2$ or $MgSO_4$. For all the conditions the SCT membranes were assayed for Ca^{2+}-ATPase activity and lipid peroxidation levels. The lipid peroxidation was determined by measuring both conjugated dienes and TBARS. **Results:** For both SCT membranes, the two salts are able to protect against, and even to restore, the lipid damage produced by the UV light irradiation and, consequently, to provide protection to the Ca^{2+}-ATPase. **Conclusions**: There were no significant differences between the two magnesium salts, suggesting that $MgGl_2$ could be used as an alternative treatment for preeclampsia.

PC59

Connexin 46 hemichannels in Trophoblast derived cells HTR-8/Svneo. Possible role for hypoxia survival?

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Extra Villous Trophoblast (EVT) migration/invasion occurs in a hypoxic environment, as placental circulation has yet to be established. This condition would be interpreted as cellular injury by many cell types, but EVT needs low levels of

oxygen to thrive. Research in other tissues (i.e., lens and tumor cells), have implicated Connexin 46 (Cx46) with resistance to hypoxia, enabling the cell to survive longer periods under hypoxic conditions. Expression of Cx46 in choriocarcinoma cell line (Jar) has been previously described, but it´s expression in normal placenta derived cell lines has not been reported yet. **Objectives:** this study aims to evaluate whether Cx46 plays a role in EVT survival under hypoxic conditions. **Methods:** HTR8/SVneo cells were exposed to hypoxia (1% O_2) for 12 or 36 h and compared with cells grown in 21% O_2 environment. Western blot, indirect immunofluorescence and RT-PCR were used to determine protein and transcript expression, while dye uptake assays were performed to establish hemichannel functionality. **Results**: preliminary results show that HTR-8/Svneo constitutively express Cx46, and exposure to hypoxia increases Cx46 expression up to 60%. **Conclusions**: These results suggest that hypoxia regulates Cx46 expression in HTR8/SVneo cells. As such, Cx46 could play an important role in hypoxia survival of EVT cells. Further experiments are required to establish the influence of Cx46 over functional outcomes such as cell viability and migration/invasion capacity. If so, Cx46 would be an interesting target for preeclampsia studies.

PC60
Diagnostic performance of uric acid for prediction of preeclampsia

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Although it is well-established the relationship between uric acid levels (UA) and preeclampsia, its clinical utility is still controversial. **Objective**: to characterize the diagnostic performance of UA for the prediction of preeclampsia. **Method**s: We conducted a prospective approach in which all patients who attended her pregnancy in the Hospital Posadas during 2014 were studied. Serum UA, urea and creatinine were measured and evaluated throughout gestation. Receiver operating curves (ROC) of the UA ratio between a dosage before and after the 20th week of gestation and the ROC of proteinuria were analyzed and compared. For ROC of UA, a ratio of ≥1.5 was considered as a positive value. **Results**: We analyzed 480 normal pregnancies and 315 pathological pregnancies (40 preeclampsia, 23 gestational hypertension, 31 basal hypertension, 140 diabetes, 71 premature births or premature rupture of membranes, 8 with intrauterine growth retardation and 2 with Lupus). The ROC area of UA for preeclamptic pregnancies was 0.872 (0.805-0.939) with positive and negative predictive values (PPV, NPV)) of 26% and 98.6%, respectively. In all cases, urea and creatinine showed normal values, confirming that patients had no renal compromise. The ROC area of positive proteinuria was 0.823 (0.724-0.922) with PPV: 17.5% and NPV: 98.5%. When both determinations were included the ROC area was 0.871

(0.802-0.940) with PPV: 13.3% and NPV:99.3%. **Conclusion**: UA has a slightly better performance and a higher NPV than proteinuria. Therefore, we suggest that a combination of the UA and proteinuria can be helpful to achieve a better prediction of preeclampsia.

PC61

Melatonin modulates the pulmonary expression of prostanoid agents of chronically hypoxic neonatal lambs

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Neonatal pulmonary hypertension (NPH) is a pathophysiological condition determined by perinatal chronic hypoxia. The main characteristics of this condition are the pathologic pulmonary vascular remodelling, abnormal vasoreactivity and increased oxidative stress, which has been associated with increased thromboxane (TX, vasoconstrictor) and decreased prostacyclin (PGI2, vasodilator) functions. The use of melatonin, a neurohormone with antioxidant, anti-remodelling and vasodilator properties, has been postulated as a treatment for NPH (3), but its effects on pulmonary prostanoids are unknown. **Objectives:** Determine the effects of a postnatal treatment with melatonin on the pulmonary transcript level of the prostanoid pathway in lambs gestated, born and raised under hypobaric hypoxia. **Methods:** 1.4 ml of ethanol (Control, 0.5 mL * kg^{-1}) or melatonin (M, 1 mg * kg^{-1} in 1.4% ethanol at 0.5 mL * kg^{-1}) was administered from postnatal day 4 to 21 to lambs gestated and born at 3600 m. After 1 week of treatment completion, lung tissue was obtained and the transcript level of prostanoid enzymes and receptors were assessed by RT-PCR. **Results:** Melatonin induced expression of cyclooxygenase 1 (COX-1), and the vasodilator pathway prostacyclin synthase (PGIs) - receptor IP. In addition, melatonin decreased the expression of the vasoconstrictor receptors FP and EP3. No changes were seen in COX-2, TXs, PGEs, rTP and rEP1. **Conclusions:** Postnatal treatment with melatonin differentially modulates the prostanoid pathway, towards a vasodilatory balance. These effects may contribute to decrease the pulmonary vascular resistance in NPH.

Funding: FONDECYT 1151119. References: 1. Steinhorn SH., Pediatr Crit Care Med 11: S79–S84, 2010. 2. Peñaloza D & Arias-Stella J., Circulation 115:1132-1146, 2007. 3. Torres F et al., J Pineal Res 58:362-373, 2015.

PC62

Potential role of sodium/proton exchanger type 1 in angiogenesis

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Angiogenesis is the physiological process through which new blood vessels from pre-existing vessels are formed. The pH modulates angiogenesis due the high intracellular proton (H^+) production by endothelial cells as a by-product of glycolysis. Intracellular H^+ must be transported to the extracellular media to maintain cell viability. One the main H^+ transporter is the sodium/proton exchanger type 1 (NHE1) identified to promote cell proliferation and migration. However, its role in angiogenesis is not addressed. **Objectives**: Our aim was to determine whether NHE1 plays a role in angiogenesis. **Methods:** Zebrafish embryos of the *Tg*(*fli1:EGFP*) line were raised. Embryos were kept in E3 medium with 1-phenyl-2-thiourea to suppress pigmentation and staged according to hours post-fertilization. Two CRISPR gRNA sequences targeting zebrafish NHE1 gene (*slc9a1*(zNHE1)) were designed using Zinc Finger Consortium Tool (ZiFiT) and cloned using a pT7-gRNA expression plasmid (CRISPR#1, CRISPR#2). Cas9 mRNA was synthetized in pT3TS-nCas9 expression plasmid. CRISPR gRNAs and Cas9 mRNA were injected through the chorion of one-cell or two-cell stage embryos (n = 60). Equal amounts of control CRISPR gRNA (derived from original pT7-gRNA) were used as negative control. Mutations on *slc9a1* were checked through DNA sequencing. The number of vascular alterations were counted by fluorescence microscopy. **Results:** Zebrafish mosaic zNHE1 mutants generation was confirmed by DNA sequencing. CRISPR#1 and CRISPR#2 mosaic zNHE1mutants showed a higher number of vascular alterations in contrast to control embryos. **Conclusions:** NHE1 is involved in the angiogenesis process in zebrafish.
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PC63

Postnatal melatonin administration increases lung antioxidant capacity in chronically hypoxic neonatal lambs

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Gestational complications under chronic hypoxia such as placental insufficiency, preeclampsia and high-altitude pregnancies may develop pulmonary hypertension in the newborn (NPH) (1). One of the main adverse conditions involved in this pathology is oxidative stress (2). Therefore, melatonin, a neurohormone with antioxidant properties, could modulate lung antioxidant capacity (3). **Objectives**: To determine the effect of a postnatal treatment with melatonin on the expression of antioxidant enzymes and the oxidative stress level in lung tissue. **Methods:** Chronically hypoxic lambs were given vehicle (0.5 ml*kg^{-1}) or melatonin (1 mg *kg^{-1}) from day 4 to 21 of postnatal life. On day 28 lung tissue was obtained and the level of transcript and protein expression of Glutathione Peroxidase 1 and 3 (GPx1 and 3), Catalase (CAT) and Superoxide Dismutase 2 and 3 (SOD) were evaluated by RT-PCR and Western Blot. Additionally, we determined the levels of nitrotyrosine (NT), 4-Hydroxynonenal (4-HNE) and NFR2 by Western Blot and malondialdehyde (MDA) by a commercial kit. **Results**: Lambs treated with melatonin showed a significant increase in mRNA level of the antioxidant enzymes SOD2, SOD3 and GPx3 relative to controls. Further, melatonin increased NFR2 expression, whereas NT levels were significantly decreased. **Conclusions**: Melatonin increased some of the lung antioxidant enzymes in addition to decreasing levels of cellular oxidative stress. Melatonin is postulated as a possible pharmacological treatment to reverse the pathophysiology of NPH and recovered the pulmonary oxidative tone.
Funding: FONDECYT 1151119. References: 1. Steinhorn SH., Pediatr Crit Care Med11(2): S79–S84, 2010. 2. Tabima DM et al., Free Radic Biol Med52(9): 1970-86, 2012. 3. Torres F et al., J Pineal Res58(3):362-73, 2015.

PC64

Panx1 participate in the invasion of *T. cruzi* in cardiac cells

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Panx1 form hexameric plasma membrane channels that participate in inflammation, apoptosis and innate immune response. Panx1 is involved in the infection of obligate intracellular pathogens such as Chlamydia and HIV. **Objectives**: We investigated the participation of Panx1 in the invasion of *T. cruzi* in cardiac cells. **Methods**: The invasion were performed in primary culture of neonatal rat cardiomyocytes exposed to trypomastigotes (MOI: 10: 1) for 4 h. The cells were preincubated prior to the experiment for 30 min with blockers [100 μM ^{10}Panx1, 400 μM probenecid (Pbn). The invasion was evaluated by Dapi fluorescence of amastigotes. The Panx1 expression was evaluated by inmmunofluorescence of cardiac cells exposed to trypomastigotes (10:1) for 4 hrs. The *T. cruzi* viability was evaluated by live/dead cell stain in flow cytometry. **Results**: The invasion was significantly reduced by ^{10}Panx1

1.3±0.5 % and probenecid 2.0±0.5% vs control 16±2.9% conditions. Interestingly, infected cells show greater immunoreactivity to Panx1. Moreover, the exposure of parasites to the blockers did not affect the cellular viability of the parasites. **Conclusions**: These results provide first evidence that Panx1 participates in the invasion of cardiac cells by *T. cruzi*.

PC65

Role of calcium-activated potassium channels in vascular tone regulation of human placenta

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In human placenta, the chorionic vein relaxation induced by insulin depends on calcium-activated potassium channels (KCa), but the role of KCa in vascular tone regulation of placenta is poorly understood. **Objectives:** To determine the effects of KCa inhibitors in vascular tone of human placenta. **Methods:** Chorionic veins rings were dissected and isometric tension was determined through wire myography in absence or presence of endothelium and/or tetra-ethyl-ammonium (TEA, KCa-inhibitor), Tram-34 (IKCa-inhibitor) or iberiotoxin (BKCa-inhibitor). To determine perfusion pressure, a suitable fetal vein and artery pair leading to a peripheral cotyledon, were cannulated and perfused with a Krebs solution (95% O^2/5% CO^2; pH 7-4; 37C). After stabilization, the effect of TEA was determined. Nitric oxide (NO) levels were determined through 4,5-diaminofluorescein-diacetate fluorescence in human umbilical vein endothelial cells (HUVEC) treated (30-min) with KCa inhibitors. **Results:** The incubation (30-min) with TEA, iberiotoxin and Tram-34 induces a relaxation of 14±0.8%, 14±0.4% and 4±0.1%, respectively, in pre-constricted veins. In endothelium-denuded vessels, there is no relaxation induced by TEA, but a constriction of 21±2% after 4 minutes incubation turn significant. In isolated cotyledon, TEA enhanced 2.7-fold perfusion pressure. In HUVEC, TEA, iberiotoxin and Tram-34 increased NO levels 1.7, 2 and 1.8 folds, respectively. **Conclusions:** The inhibition of KCa induces rapid constriction that depends on smooth muscle cells activity, which induces an activation of endothelial cells to release NO and finally lead to relaxation. When TEA is continuously perfunded, the smooth muscle cells constriction predominates over endothelium-dependent relaxation.

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PC66

L-type calcium channel stabilization via Polycystin-1 and AKT pathway

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Mechanical stretch induces L-type calcium channel (LTCC) stabilization in neonatal rat ventricular myocytes (NRVM) via Polycystin-1 (PC1), however the signaling pathway involved is unknown. PC1 is a mechanosensor and a Gi-coupled receptor in NRVM. We propose that PC1 stabilizes LTCC during mechanical stretch through its Gi activity and AKT pathway. **Objectives:** Our aim was to determinate the pathway involving PC1 which stabilizes LTCC in cardiomyocytes during mechanical stretch. **Methods:** We used NRVMs controls and knock down to PC1 (specific siRNA), stimulated with hyposmotic solution (HS) as a mechanical stretch model (2 h) and measured Cav1.2, phosphorilated AKT (pAKT) content by western blot. We used AKT VIII (AKTi, 10 μM), Giβγ inhibitor (βARk, MOI 300) and nifedipine (10 μM). We overexpress the total c-terminal of PC1 (FLM-PC1) and mutated by G-binding sequences (CTM-PC1), MOI 20. We measured cytoplasmic Ca2+ signals in NRVMs with Fluo-3AM. **Results:** AKT was activated by PC1 in NRVMs stimulated with HS. Inhibition of AKT or Giβγ subunits prevented Cav1.2 stabilization during mechanical stretch. Also, the stabilization required Cavβ2 LTCC subunit. Overexpression of FLM-PC1 activated AKT and stabilized Cav1.2, but overexpression of CTM-PC1 blunted it. HS increased cytoplasmic Ca2+ in NRVMs, decreased in cells knockdown to PC1 or in presence of Nifedipine. **Conclusions:** AKT activation via PC1 is crucial to stabilized LTCC during mechanical stretch in NRVMs. The Gi-protein-coupled receptor site of PC1 is associated to AKT activation during mechanical stretch.
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PC67
RECK and angiogenesis in the foetoplacental vasculature in preeclampsia

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Inadequate perfusion of the placenta in preeclampsia results in uteroplacental hypoxia, which is believed to associate with angiogenic abnormalities in the placenta as well as in the maternal vasculature. Reversion-inducing-cysteine-rich-protein with kazal motifs (RECK) is a plasma membrane protein that inhibits different metalloproteinases acting as a key regulator of angiogenesis. **Objective:** To determine whether RECK is expressed in human placentas and in human umbilical vein endothelial cells from preeclampsia and normal pregnancies. **Methods:** Expression and localization of RECK in human placentas were evaluated by western blot and immunofluorescence. **Results:** RECK is expressed in the human placenta in a larger proportion in preeclampsia compared with normal pregnancies (1.4 ± 0.2 fold, $P<0.05$, $n = 5$). RECK protein expression in HUVECs from preeclampsia was higher (1.4 ± 0.1 fold, $P<0.05$, $n = 3$) compared with normal pregnancies. **Conclusion:** RECK expression is induced in foetoplacental endothelial cells from preeclampsia. Future studies include inducing HUVECs to form endothelial tubes under hypoxia. The expression of RECK, VE-cadherin and α_v-integrin (endothelial markers), will be determined.
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PC68

Postnatal melatonin treatment improves pulmonary vascular function even after treatment withdrawal in chronic hypoxic newborn sheep

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Neonatal pulmonary hypertension (NPH) is a condition characterized by vascular remodeling and abnormal vascular reactivity. This is a multifactorial syndrome with a limited efficacy in current therapeutic approaches, with chronic hypoxia and oxidative stress as major determining factors of pulmonary vascular dysfunction (1). Melatonin is a neurohormone with antioxidant properties at pulmonary level (2). Therefore, we hypothesize that a postnatal treatment with melatonin will improve the neonatal pulmonary vascular reactivity. **Objectives**: To test whether a 3 weeks postnatal treatment with melatonin improves the neonatal pulmonary vascular reactivity. **Methods**: Ten lambs were used in this study, five received vehicle (1.4 % of ethanol at 0.5 $mL*kg^{-1}$) and five received melatonin (1 $mg*kg^{-1}$ in 1.4% ethanol at 0.5 $mL*kg^{-1}$) was administered from day 4 to 21 postnatal to lambs gestated and born at 3600 m. After 1 week of treatment completion, lung tissue was obtained and small resistance pulmonary arteries (PA) were mounted in a wire myograph to assess

vascular reactivity. **Results**: Treatment with postnatal melatonin decreased the contractile capacity in response to potassium, the maximum response to thromboxane and endothelin relative to the control group. However, the response to serotonin was similar between groups. In addition, postnatal administration of melatonin increased the endothelium-dependent vasodilation tested with methacholine. In contrast, the muscle-dependent vasodilation induced with a nitric oxide donor was similar between groups. **Conclusions**: Melatonin improves vascular reactivity *ex vivo*, effect that is maintained after treatment withdrawal in neonates with NPH, developed and born in chronic hypoxia.
Funding: FONDECYT 1151119. References: 1. Peñaloza D. & Arias-Stella J., Circulation 115:1132-1146, 2007. 2. Torres F., J Pineal Res 58:362-73, 2015.

PC69

Identification of putative orthologs genes for innexins in pathogenic protozoa *Trypanosoma cruzi*

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Chagas Diseases is a public health problem in Latin America. The etiologic agent of this disease is the protozoan *Trypanosoma cruzi*. Innexin proteins are connexin orthologs in invertebrates. However, the presence of these proteins in unicellular organisms has not been described. **Objetives:** The aim of our study was to investigate whether *Trypanosoma cruzi* have innexin-formed hemichannels. **Methods**: The genome of *T.cruzi* was examined using kinetoplastid genomic on-line resource (TriTrypDB.org). Multiple protein alignments of protozoan parasites*, C. elegans,* and *D. melanogaster* were carried out in the National Center for Biotechnology (NCBI, USA) genomic protein databases. **Results**: We analyzed the genomes of *T. cruzi* and showed the existence of 2 genes encoding putative orthologues of innexin proteins. Theses genes coding for proteins with 4 transmembrane domains and topology-like to innexin proteins. The genes identity with innexin genes of *C.elegans* or *D. melanogaster* were 17.8% and 19.1%, respectively. **Conclusions**: Since innexin proteins are not present in vertebrates, identification of innexin in *T. cruzi* could correspond to a new molecular target for the therapy of Chagas disease.

PC70

High D-glucose could increase deiodinase 3 expressions in syncytiotrophoblast from gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) characterizes by abnormal maternal D-glucose metabolism, and associates with reduced maternal circulating free tyroxine (fT4). Deiodinase 3 (DIO3) is a thyroid hormones inactivator enzyme that catalyzes deiodination of thyroxine (T4) into reverse triiodothyronine (rT3) and triiodothyronine (T3) into 3,3'-diiodothyronine (T2). Role of this phenomenon in GDM is unknown. **Objective:** To evaluate whether Dio3 expression in syncytiotrophoblast from GDM and D-glucose increases Dio3 expression in trophoblast cell line (HTR8-Svneo). **Methods:** Placentas were obtained from Hospital Guillermo Grantt Benavente of Concepción, Chile. Dio3 protein expression was evaluated in Placentas from normal and GD pregnancies by immunohistochemistry (IHC). HTR8-Svneo were exposed (6 hours) to D-glucose (11-25 mM). Total mRNA was extracted with Trizol reagent and used for real time PCR to estimate the relative abundance of *Dio3* and *28S* mRNA using the $2^{-\Delta\Delta Ct}$ method. **Results:** Dio3 is localized in endothelial and syncytiotrophoblast cells, and this protein increased (2.1 ± 0.2 fold) in GD pregnancies in both type cells. *Dio3* mRNA level was higher (1.8 ± 0.2 fold) in HTR8-Svneo cells exposed to D-glucose from 11 to 25 mM (effective half-maximal concentration (EC_{50}) 15 ± 1 mM). **Conclusions:** GD increases Dio3 expression in syncytiotrophoblast cell possibly by high levels of D-glucose.
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PC71

Differential pattern of IL-10 and progesterone receptor B in eutopic endometrium of infertile and fertile women diagnosed with endometriosis

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Endometriosis is an inflammatory disease associated with subfertility/infertility. **Objective**: To study the presence and localization of the anti-inflammatory immunosuppressive cytokine IL-10 and the progesterone receptor isoforms A (PRA)

and B (PRB) in eutopic endometrium (EE) of women with endometriosis. **Methods**: Uterine endometria obtained during the menstrual cycle from women with endometriosis diagnosed by laparoscopy (EE, n = 38; 34.3 ± 6.5 years, body mass index (BMI) = 25.5 ± 5.7 Kg/m^2: fertile (FEE) n = 12, infertile (IEE) n = 26) and from women without endometriosis undergoing tubal sterilization by laparoscopy (CE, n = 29; 37.9 ± 5.8 years, BMI = 26.5 ± 3.1 Kg/m^2). By immunohistochemistry, it was studied IL-10 (integrated optical density (IOD) ImageProPlusv6.3; Ab34843, Abcam) and PRA and PRB (HScore, NCL-L-PGR-312-clone-16 and NCL-L-PGR-B-clone-SAN27, Novocastra, respectively). A $p<0.05$ between medias was considered statistically different by *t*-test or Anova/Tukey. Study approved by Ethical committees. **Results**: IL-10 was immunolocalized in the cytoplasm of endometrial epithelial and stromal cells increasing the IOD to mid-late secretory phase in CE and FEE, but not in IEE which was lower 70% and 66% than FEE and CE, respectively. Using two different antibodies, nuclear staining was detected for PRA and PRB in both cell compartments, being PRB 190% higher in stromal cells from IEE than FEE and CE; in epithelial cells, PR isoforms were similar in the three groups. **Conclusion**: The strong increase of IL-10 during the mid and late secretory phases suggests a protective physiological role to the embryo during its implantation and endometrial invasion. Conversely, in IEE, the poor immunosuppressive response by the decreased IL-10 together with the abnormal PRB pattern might contribute to their infertility.
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PC72

The membrane-anchored MMP inhibitor RECK negatively regulates the endothelial-like differentiation and endovascular remodeling of human trophoblast cells.

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In normal pregnancies trophoblast cells invade the maternal decidua to reach the uterine arteries where acquire an endothelial-like phenotype, replacing the maternal endothelial cells. However, in preeclampsia this capacity of trophoblast is reduced, emerging as a cause of this syndrome. RECK is a plasma membrane protein that inhibits different metalloproteinases, acting as a key regulator of invasion and angiogenesis. We found that RECK is overexpressed by trophoblast in placentas from preeclampsia compared to placentas from normal pregnancies, suggesting a role of

RECK in preeclampsia development. **Objectives**: To determine the role of RECK on endothelial-like differentiation and endovascular remodeling capacity of human trophoblast cells. **Methods:** Human trophoblast cell line HTR8/SvNeo cells were transfected whit the expression vectors for human RECK or a CRISPR against RECK and induced to form tubes in a matrigel-tube formation assay. The number of ramification points and total tube length were determined. The expression of VE-cadherin and α_v-integrin, two endothelial markers, were determined by qPCR. Transfected trophoblasts were also co-cultured with pre-stablished endothelial-vascular tubes *in vitro,* determining the number of integrated trophoblast. **Results:** Knockdown of RECK resulted in increased number of ramifications (1.6 ± 0.1 fold) but reduced total tube length (0.8 ± 0.1 fold). The expression of VE-cadherin (1.6 ± 0.1 fold) and α_v-integrin (1.8 ± 0.2 fold) were also increased, as well as the integration on pre-formed tubes (1.4 ± 0.1 fold) compared to control. These phenomena were reduced by overexpressing RECK. **Conclusion:** RECK negatively regulates the capacity of trophoblast to acquire an endothelial-like phenotype and to modify preexisting vascular tubes, emerging as a key player in the pathogenesis of preeclampsia.
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PC73 (*abstract available as* OC11)
Feto-placental endothelial exosomes modulate high glucose-induced endothelial dysfunction in human umbilical vein endothelial cells

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PC74
ABCA1 regulates insulin-dependent Akt phosphorylation and glucose uptake in skeletal muscle fibers

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ATP-binding cassette transporter A1 (ABCA1) promotes cellular cholesterol efflux to extracellular lipid-free apolipoprotein A. Recent reports indicate that ABCA1 regulates adipose tissue lipid content, glucose tolerance, and insulin sensitivity in adipocytes. Most GLUT4-mediated glucose transport occurs in the transverse tubules (TT) of skeletal muscle, a specialized cholesterol-enriched plasma membrane system. Interestingly, we found that cholesterol levels in TT from skeletal muscle are higher in insulin resistant mice (IR); however, the role of ABCA1 on skeletal muscle glucose metabolism remains largely unexplored. **Objective:** To evaluate the role of the ABCA1 on the insulin-dependent signaling pathway and glucose uptake in muscle fibers. **Methods:** Male C57BL/6J mice were fed for 8 weeks with normal chow diet (NCD) or high fat diet (HFD). NCD-fed mice were electroporated with *sh*ABCA1-RFP or scrambled plasmids. Akt phosphorylation and glucose uptake were assayed by Western blot and 2-NBDG transport in cultured fibers isolated from *Flexor digitorum brevis* muscle (FDB), respectively. **Results:** Compared to NCD-fed mice, both ABCA1 mRNA levels and protein content were decreased in muscle homogenates from HFD-fed mice. In FDB muscle from NCD-fed mice, *sh*ABCA1-RFP *in vivo* electroporation resulted in a reduction of insulin-dependent Akt (Ser473) phosphorylation and total suppression of 2-NBDG uptake compared to fibers electroporated with the scrambled plasmid. **Conclusion:** ABCA1 modulates AKT activation and glucose uptake in skeletal muscle fibers. This process may be involved in IR-Induced by HFD.
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PC75
Placental leptin expression is mediated by NFκB signaling

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Leptin is a key hormone in placental physiology. Previous results demonstrated that estradiol (E_2) regulates leptin expression involving genomic and non-genomic effects. **Objectives:** Considering there is a potential binding site for NFκB between -2850 and -2838bp at the promoter sequence of leptin gene, and taking into account the interaction between ERs and the NFκB factor, we analyzed the involvement of this transcription factor in the effects of E_2 on placental leptin expression. **Methods:** BeWo cells were transiently transfected with the Rel A vector which express the

subunit p65, responsible for the activity of NFκB dimers. We performed experiments with placental explants and BeWo cells treated with E_2 for 48 hours in presence of sulfasalazine (an Ikk inhibitor), also BeWo-Sh2, were treated with doxycycline, western blot and qRT-PCR were used to explore protein and transcript expression. **Results:** The expression of subunits p65 decreased significantly E_2 effects on the transcriptional activity of pL1951 vector. We saw a markedly activity reduced of the basal leptin promoter. Similar results it´s seen with the BeWo-Sh2, which presents reduced level of Erα protein. Suggesting that the overexpression of Rel A would inhibit the interaction of others factors with their response element on the leptin promoter, for example ERs receptors. **Conclusion:** The treatment with the drug reduces E_2 action over the endogenous leptin expression, suggesting the participation of the NFκB dimers in the regulatory effect from this steroid. These provide new evidence about the mechanisms by which the E_2 regulates the expression of placental leptin.

PROGRAM at *a glance*

	APRIL 11	APRIL 12	APRIL 13
8:30 a.m.	Registration	Oral Communications I	Oral Communications II
9:00 a.m.			
9:30 a.m.		**Symposium 5** *Role of thyroid hormones in pregnancy* / **Symposium 6** *Early life programming* / **Symposium 7** *Angiogenesis & vascular remodeling*	Plenary Lecture 6 (*N Illsley*)
10:00 a.m.			
10:30 a.m.			*Coffee*
11:00 a.m.		Posters Communications II (*and coffee*)	**Symposium 11** SCHCF - *Vascular modulators: role of adenosine receptors and thyroid hormones* / **Symposium 12** *Infection, toxins and pregnancy* / **Symposium 13** *Pathogenesis of preeclampsia*
11:30 a.m.			
12:00 p.m.		SCHCF - Plenary Lecture 3 (*S Zamudio*)	
12:30 p.m.			Plenary Lecture 7 (*A Jawerbaum*)
1:00 p.m.		*Lunch break*	
1:30 p.m.			Closing ceremony and awards
2:00 p.m.	Welcome (*L Sobrevia*) - Lawson Plenary Lecture 1 (*D Hill*)		
2:30 p.m.			
3:00 p.m.	**Symposium 1** *Placental dysfunction and pregnancy outcome* / **Symposium 2** *Metabolic & endocrine modulators of pregnancy*	**Symposium 8** SCHCF - *Young scientist* / **Symposium 9** *Frontiers in maternal-foetal research I* / **Symposium 10** *Frontiers in maternal-foetal research II*	
3:30 p.m.			
4:00 p.m.		Plenary Lecture 4 (*G Rice*)	
4:30 p.m.	Posters Communcations I (*and coffee*)		
5:00 p.m.		Posters Communications III (*and coffee*)	
5:30 p.m.	**Symposium 3** *SCHCF - Skeletal muscle function* / **Symposium 4** *Key players at implantation*		
6:00 p.m.		Forum PAA research opportunities (*N Illsley, S Zamudio, I Caniggia*)	
6:30 p.m.		Plenary Lecture 5 (*I Caniggia*)	
7:00 p.m.	SLIMP 2015 Prize Award Lecture (*N Portilho*)		
7:30 p.m.	SCHCF - UMCG Plenary Lecture 2 (*JL Hillbrands*)	SLIMP and SCHCF members meeting	
8:00 p.m.			
8:30 p.m.	*Welcome reception*		
9:00 p.m.		*Congress dinner*	
9:30 p.m.			