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.
Funding: FONDECYT 1141236 (D.B.), PhD fellowship CONICYT 21130444 and School of Medicine PMD-04/16 (N.S.)

http://dx.doi.org/10.1016/j.placenta.2017.01.071

MATERNAL OBESITY ASSOCIATES WITH FOETOPLACENTAL VASCULAR DYSFUNCTION INVOLVING ENDOPLASMIC RETICULUM STRESS AND ALTERED INSULIN VASCULAR REACTIVITY

A. Saavedra [1], S. Rojas [2], A. Haensgen [2], M. Cid [3], M. González [2], M. Farías [1], R. Villalobos-Labra [1], A. Leiva [1], L. Sobrevia [1,4,5].

[1] *Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile;* [2] *Vascular Physiology Laboratory, Department of Physiology, Faculty of Biological Sciences, Universidad de Concepción, Concepción, Chile;* [3] *Department of Obstetrics & Childcare, Faculty of Medicine, Universidad de Concepción, Concepción, Chile;* [4] *Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Seville, Spain;* [5] *University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, Queensland, Australia*

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.

Funding: FONDECYT 1150377/1150344/11100192. RV-L holds a CONICYT-PhD fellowship (Chile).

http://dx.doi.org/10.1016/j.placenta.2017.01.072

PPARS AND MTOR INTERACT IN THE RAT DECIDUA DURING EARLY ORGANOGENESIS

S. Roberti, H. Sato, R. Higa, A. Jawerbaum.

Laboratory of Reproduction and Metabolism, CEFYBO-CONICET, School of Medicine, University of Buenos Aires, Argentina

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.

http://dx.doi.org/10.1016/j.placenta.2017.01.073

STUDY OF ZIKA VIRUS INFECTION IN HUMAN PLACENTA EXPLANTS

M.R. Ribeiro [1,6], J.B. Moreli [2], R.E. Marques [3], M.P. Papa [4], L.M. Meuren [4], P. Rahal [1], L.B. Arruda [4], A.H. Oliani [6], D.C.M.V. Oliani [6], S.M. Oliani [1], A. Narayanan [5], M.L. Nogueira [6].

[1] *Department of Biology, Institute of Biosciences, Letters and Exact Sciences, São Paulo State University (UNESP), São José do Rio Preto, SP, Brazil;* [2] *Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil;* [3] *Brazilian Biosciences National Laboratory (LNBio), National Center for Research in Energy and Materials (CNPEM), Campinas, SP, Brazil;* [4] *Institute of Microbiology, Universidade Federal do Rio de Janeiro, Brazil;* [5] *George Mason University, Virginia, USA;* [6] *Laboratory of Virology Research, Faculdade de Medicina de São José do Rio Preto (FAMERP), São José do Rio Preto, SP, Brazil*

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*.

http://dx.doi.org/10.1016/j.placenta.2017.01.074

HYPEROSMOLAR STRESS AFFECTS TRPV-1 EXPRESSION AND THE PHYSIOLOGICAL FUNCTIONS OF HUMAN TROPHOBLAST

J. Reppetti [1,2], A. Sehayian [1], N. Szpilbarg [1], M. Farina [3], A.E. Damiano [1,2], N. Martínez [1].

[1] *Laboratory of Biology of Reproduction, Institute of Physiology and Biophysics Bernardo Houssay (IFIBIO)- CONICET- School of Medicine, Universidad de Buenos Aires, Buenos Aires, Argentina;* [2] *Department of Biological Sciences, School of Pharmacy and Biochemistry, Universidad de Buenos Aires, Buenos Aires, Argentina;* [3] *Laboratory of Placental Physiopathology, CEFyBO, School of Medicine, Universidad de Buenos Aires, Buenos Aires, Argentina*

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.

http://dx.doi.org/10.1016/j.placenta.2017.01.075

ABNORMAL ENDOMETRIAL EXPRESSION OF COMPLEMENT REGULATORY PROTEIN DECAY ACCELERATING FACTOR IN WOMEN WITH RECURRENT IMPLANTATION FAILURE IN CYCLES OF ASSISTED REPRODUCTION

W.A. Palomino [1], C. Aguirre [1], F. Argandoña [1], M. Ventureira [2], P. Kohen [1], L. Devoto [1].

[1] *Institute for Maternal and Child Research, University of Chile, Santiago, Chile;* [2] *Laboratory of Reproduction and Maternal-Embryo Physiology, IBBEA-UBA/CONICET, FCEN-University of Buenos Aires, Buenos Aires, Argentina*

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 biopses 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.

http://dx.doi.org/10.1016/j.placenta.2017.01.076

HIGH D-GLUCOSE–INCREASED NITRIC OXIDE GENERATION LEADS TO HIGHER DEIODINASE 3 MRNA LEVEL IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

J. Nuñez-Hermosilla [1], A. Armella [1], M. Hernandez [1], D. Gallardo [1], S. Gutierrez [1], P. Fernandez [1], J. Gallegos [1], A. Leiva [2], M. Gonzalez [3,4], C. Escudero [4,5], L. Sobrevia [2,6,7], E. Guzmán-Gutiérrez [1,4].

[1] *Molecular Medicine Laboratory, Faculty of Health Sciences, Universidad San Sebastian, Concepción, Chile;* [2] *Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetric and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago, Chile;* [3] *Vascular Physiology Laboratory, Faculty of Biology Sciences, Universidad de Concepción, Concepción, Chile;* [4] *Group or Research and Innovation in*