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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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.

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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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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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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.

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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