

a role of SDF2 in trophoblast cell survival/apoptosis in ER stress response. The onset of ER stress during pregnancy is related with several pathologies, including preeclampsia. Identifying new factors of this response may contribute to the understanding of gestational diseases.

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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 coursed 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-precontracted (32.5 mM) vein rings using wire myography in the absence or presence of 100 μ M 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 ($87 \pm 5\%$ for insulin, $29 \pm 3\%$ 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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β 2GPI-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 (β 2GPI) is constitutively expressed by trophoblast and anti- β 2GPI antibodies are involved in obstetric disorders associated with APS inducing an inflammatory response in trophoblast. Transglutaminase-2 (TG2) crosslinking activity regulates nuclear factor- κ B signalling and its inflammatory effects. TG2 truncated variants encoded by alternatively spliced mRNA are involved in enzyme regulation.

Objectives: We analysed effects of anti- β 2GPI 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- β 2GPI antibodies effect on cytokine production by ELISA and TG2 variants expression by qPCR.

Results: Sera with anti- β 2GPI antibodies increased IL-6 production and induced TG2 expression in trophoblast (dose dependent effect). In differentiated THP-1 cells exposed to anti- β 2GPI 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 β 2GPI-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.

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POSTER COMMUNICATIONS.

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- β 1 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- β 1 (10 ng/mL) and/or hCG (10 IU/mL) to evaluate TGF- β 1-induced Smad2/3 phosphorylation (Western blot), mRNA level of the TGF- β 1-induced gene SMAD 7 (qPCR), and secretion of metalloproteinase-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- β 1. 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- β 1, whereas no effect was observed upon co-stimulation with hCG. The secretion of MMP-2 decreased ($20 \pm 10\%$) 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- β 1 (30 \pm 15%), but increased (1.4 \pm 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.

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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 10⁵ 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 (β -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 \pm 0.38 vs. 2.46 \pm 0.26 nmoles/min/mg protein $P < 0.001$), IDO protein expression (2.04 \pm 0.16 vs. 1.83 \pm 0.12 fold, relative to β -actin, $P < 0.05$), Kyn production (11.54 \pm 1.43 vs. 6.75 \pm 0.6 μ M Kyn/mg protein, $P < 0.05$), and AhR protein abundance (68.41 \pm 1.44 vs. 61.09 \pm 2.07 fold, relative to β -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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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 (ER α) 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 ER α , vimentin and Ki67 (proliferation marker). Then, some animals were mated to evaluate the reproductive performance and to determine foetoplacental weight and ER α 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 \pm 0.76 vs CAF 8.27 \pm 1.87, $P < 0.05$, n = 7) with a higher vimentin expression in the periglandular stroma (C 0.276 \pm 0.036 vs CAF 0.404 \pm 0.044). CAF group showed higher ER α and Ki67 expression in: luminal epithelium (ER α C 2.2 arbitrary units (AU) \pm 0.3 vs CAF 4.7 AU \pm 0.5, Ki67 C 40.6% \pm 5.1 vs CAF 68.1% \pm 2.2); and glandular epithelium (ER α C 3.6 AU \pm 0.2 vs CAF 8.7AU \pm 1.0, Ki67 C 63.5% \pm 5.1 vs CAF 76.1% \pm 1.4). CAF diet did not alter reproductive performance and foetal weight; however, a decrease of GD21-placental weight (C 0.507g \pm 0.140 vs CAF 0.440g \pm 0.08), and pup weights at birth (C 5.683g \pm 0.199 vs CAF 4.732g \pm 0.343) were detected. Furthermore, the ER α mRNA relative expression was lower (C 2.750 \pm 1.033 vs CAF 1.516 \pm 1.017) on CAF-placentas.

Conclusion: CAF diet affected the uterine morphology and altered placental development with a lower weight of pups at birth.

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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 μ g/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 μ m were cut into segments of \sim 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 BKCa²⁺ channels [10^{-10} – 10^{-6} M]). All procedures were approved by the Bioethical Committee (0643 FMUCH CBA).