PA.59.
TRYPANOSOMA CRUZI INCREASES THE EXPRESSION OF CELLULAR PROLIFERATION MARKERS IN *EX VIVO* INFECTED HUMAN PLACENTAL CHORIONIC VILLOUS EXPLANTS (HPCVE) AND BEWO CELLS

Liempi Ana [1], Droguett Daniel [1,2], Castillo Christian [1], Gómez Fresia [1,2], Negrete Miguel [1,2], Carrillo Ileana [1], Galanti Norbel [1], Kemmerling Ulrike [1]. [1] *Institute for Biomedical Sciences, Faculty of Medicine, University of Chile, Santiago, Chile*; [2] *Facultad Ciencias de la Salud, Universidad de Talca, Talca, Chile*

Objective: To analyze the expression of the cellular proliferation markers PCNA and Ki67 in response to the causative agent of Chagas disease (*Trypanosoma cruzi* (*T. cruzi*)) in human placental chorionic villous explants (HPCVE) and BeWo cells. We have previously shown, that *T. cruzi* induces cellular differentiation in the trophoblast and postulated that the trophoblast turnover may be a local anti-parasite mechanism of the placenta.

Methods: HPCVE and BeWo cells were incubated in the presence or absence of *T. cruzi* trypomastigotes (Y strain) or 10% FBS (positive control) for 24 h. HPCVE were incubated with 10^5 parasites/ml. BeWo cells were challenged with a parasite/cell ratio of 0.1:1. PCNA and Ki67 expression was determined by immunohistochemistry in HPCVE and by immunofluorescence in BeWo cells. PCNA expression was additionally measured by Western blotting. Statistical analysis was performed by ANOVA followed by the Dunnett's post-test.
Effective infection in HPCVE was tested by immunohistochemistry (anti-flagellar calcium-binding protein) and PCR. Parasites inside BeWo cells were identified by their nuclear and kinetoplast morphology.

Results: *T. cruzi* significantly increased the expression of PCNA and Ki67 in HPCVE and BeWo cells.

Conclusions: We conclude that *T. cruzi* induces cellular proliferation in the trophoblast; this process may form part of a "local placental anti-parasite" defense mechanism.

Financed by FONDECYT Projects N°1120230 (UK), N°1130113 (NG) and REDES130118.

PA.60.
PLACENTAL GROWTH FACTOR (PGF) IN CEREBRAL VASCULAR DEVELOPMENT

Rayana Leal Luna [1,2], Vanessa R. Kay [1], Matthew T. Rätsep [1], Kasra Khalaj [1], Mallikarjun Bidarimath [1], Nicki Peterson [1], Albert Jin [1], Anne B. Croy [1,*]. [1] *Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, K7L 3N6, Canada*; [2] *Federal University of Pernambuco — UFPE, Recife, 50670-901, Brazil*

Objective: Preeclampsia (PE) is an important human gestational disease in which concentrations of the angiokine Placental Growth Factor (PGF) in maternal plasma are often lower than in healthy pregnancies. PGF contributes to decidual vascular development and wound healing but is thought to be redundant to Vascular Endothelial Growth Factor (VEGF) during development. Recently, cerebral vascular defects were identified in adult *Pgf*$^{-/-}$ mice. We hypothesized that PGF is expressed during CNS development and has key roles in cerebral vascularization.

Methods: We examined the expression of *Pgf*, (*Vegfa*), *Vegfr1* and *Vegfr2* in hind, mid and fore brains of gestational day (GD)12.5, 14.5, 16.5 and 18.5 C57BL/6 (B6) fetal mice using quantitative PCR. Regional expression of PGF and VEGF was assayed using immunohistochemistry. To assess developmental importance of PGF, *Pgf*$^{-/-}$ and B6 mice were compared by staining hindbrain vasculature at GD10.5 and 11.5 and the circle of Willis (cW) at GD14.5 and postnatal day 7. Functional importance of PGF to cW function and stroke resistance was compared using unilateral carotid occlusion assays.

Results: PGF and VEGF were expressed in all fetal brain regions at all times studied with PGF predominant in forebrain, VEGF predominant in hindbrain and equal midbrain expression. *Vegfr1* expression paralleled that of PGF but *Vegfr2* did not. *Pgf*$^{-/-}$ mice had delayed hindbrain vascularization and cW defects and, in contrast to B6, were vulnerable to stroke after 30 min. unilateral carotid occlusion.

Conclusion: This is the first study to map PGF/VEGF and *Vegfr1/Vegfr2* expression in mouse fetal cerebral tissue during mid and late pregnancy and demonstrates the impact of PGF deficiency during cerebral vascular development. Our data contribute to understanding the roles of PGF in developing brain and raise questions about the consequences of reduced PGF during human fetal cerebral development in gestations complicated by PE.

Supported by NSERC and CNPq

* Corresponding author.

PA.61.
EXPRESSION AND FUNCTION OF EGF /STAT5 AXIS IN PRIMARY TROPHOBLAST AND TROPHOBLASTIC CELL LINES

Stephanie Ospina-Prieto, Wittaya Chaiwangyen, Udo R. Markert, Diana M. Morales-Prieto. *University Hospital Jena, Department of Obstetrics, Placenta-Lab, Jena, Germany*

Objective: Epidermal Growth Factor (EGF) is able to influence positively or negatively a variety of fundamental cell properties including cell proliferation and invasion. Although several studies have demonstrated the role of EGF and STAT5 in the regulation of trophoblast behavior, the interaction between these molecules has not yet been investigated. Aim of this study was to examine the activation of STAT5 by EGF in different trophoblastic cell lines and the effects on cell proliferation, viability and invasion when STAT5 expression is abrogated.

Methods: Expression of STAT5B mRNA in trophoblast models was compared to that of primary cells isolated from placenta tissue by qRT-PCR. Cell viability, proliferation and invasion of trophoblastic cell lines were analyzed by MTS-, BrdU incorporation, and Matrigel assays. STAT5 was abrogated by transient transfection with specific siRNA. Cells were challenged with EGF (100 ng/ml) before and after STAT5 knock-down.

Results: High expression of STAT5B in isolated trophoblast cells was demonstrated, which is similar to the observed in HTR8/SVneo cells. Remarkably, expression in JAR cells was significantly lower. Moreover, EGF-mediated STAT5 activation increased cell viability in both cell lines. STAT5 knock-down resulted in significant decrease of cell viability induced by EGF. Only in HTR8/SVneo cells, invasion decreased after STAT5 silencing and this effect could not be rescued by further addition of EGF.

Conclusions: Our results demonstrate that STAT5 activated by EGF constitutes an important cascade for the regulation of cell viability and invasion in trophoblast cells. Altogether, this study contributed to elucidate the function of the EGF/STAT5 axis in regulating major biological functions of trophoblast cells.

POSTER SESSION B.

PB.1.
RAT YOLK SAC CELL VIABILITY DURING DIABETES

J.R.A. Santos [1], K.S. Souza [2], P.S. Farias [2], A.C.V. Santos [2], D.A. Maria [3], M.B. Aires [2], E.T. Fioretto [2]. [1] *Department of Nursing, Brazil*; [2] *Department of Morphology, Federal University of Sergipe, Sao Cristovao, Sergipe, Brazil*; [3] *Laboratory of Biochemistry and Biophysics, Butantan Institute, Sao Paulo, SP, Brazil*

The visceral yolk sac function seems to be critical during embryo organogenesis until final fetal development in rats and could be affected by conditions like diabetes.

Objectives: The objective of the present study was to assess the fetal weight and visceral yolk sac cell viability in diabetic female rats on gestational day (gd) 15.

Methods: Diabetes was induced by a single injection of alloxan on gd 8 in Wistar rats. On gd 15, rats from the control and diabetic groups were anesthetized and laparotomized to remove the uterine horns for weighing of fetuses and collection of yolk sacs. Flow cytometry was performed to determine mitochondrial activity, cell proliferation, DNA ploidy, cell cycle phases and Caspase 3 activity of yolk sac cells.

Results: Fetal weight was reduced in the diabetic group. More rhodamine negative cells and activated Caspase-3 cells were present in the diabetic

condition. The cell cycle analysis showed variable distribution of cells in all phases similar for both groups. Diabetic yolk sac cells showed low proliferation.
Conclusions: This study showed the negative effect of severe hyperglycemia on fetal development and yolk sac cells viability, as evidenced by reduced fetal weight, reduced mitochondrial activity, low proliferation and high expression of activated Caspase-3.

PB.2.
PROTECTIVE EFFECT OF LEPTIN ON THE APOPTOSIS OF TROPHOBLAST EXPLANTS TRIGGERED BY HIGH TEMPERATURE

A. Pérez-Pérez [1], A. Toro [3], J. Maymó [3], Isabel Corrales [2], J.L. Dueñas [2], C. Varone [3], V. Sánchez-Margalet [1]. [1] *Department of Medical Biochemistry and Molecular Biology, School of Medicine, Virgen Macarena University Hospital, Seville, Spain*; [2] *Obstetrics and Gynecology Service, Virgen Macarena University Hospital, Seville, Spain*; [3] *Department of Biological Chemistry, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina*

Objective: Maternal fever is common during pregnancy and has for many years been suspected to harm the developing fetus. Whether increased maternal temperature produces exaggerated apoptosis in trophoblast cells remains unclear. Since p53 is a critical regulator of apoptosis, we hypothesized that increased temperature in the placenta produces abnormal expression of proteins participating in the p53 pathway and finally Caspase-3 activation.
Leptin, produced by placenta, has demonstrated to promote the proliferation and survival of trophoblast cells. That is why we aimed to study the possible role of leptin in preventing the apoptosis triggered by high temperatures, as well as the molecular mechanisms underlying this effect.
Methods: Fresh placental tissue was collected from normal pregnancies. Placental explants were exposed to increased temperature (40 °C and 42 °C) for different times in the presence or absence of 10 nM leptin. Western blotting was performed on tissue lysate for protein expression of p53 and downstream effector proteins: p21, Bax, Mdm-2 and Caspase-3.
Results: Maximal apoptotic effect of high temperature was observed after 3 h incubation of trophoblasts. Protein expression of p53, p21, Bax, Mdm-2 as well as of cleaved Caspase-3 was significantly increased in explants at 40 °C and 42 °C as compared with explants at 37 °C. Conversely, this increased expression was significantly attenuated by leptin 10 nM at both 40 °C and 42 °C.
Conclusions: These data illustrate the potential role of leptin in inhibiting the excessive apoptosis in villous trophoblasts triggered by high temperature.

PB.3.
GESTATIONAL DIABETES DIFFERENTIALLY MODIFIES THYROID HORMONE TRANSPORTER EXPRESSION IN HUMAN PLACENTAL COTYLEDON

A. Armella [1,2], A. Venegas [1,2], C. Veas [1,2], C.A. Escudero [2,3], M. González [2,4], A.E. Covarrubias [1,2], L. Sobrevia [5], E. Guzmán-Gutiérrez [1,2]. [1] *Molecular Medicine Center, Facultad de Ciencias de la Salud, Universidad San Sebastián, Chile*; [2] *Group of Research and Innovation in Vascular Health (GRIVAS Health), Chile*; [3] *Group of Tumor Angiogenesis (GIANT), Department of Science Basic, Faculty of Sciences, Universidad del Bio-Bío, Chile*; [4] *Department of Physiology, Faculty of Biological Sciences, Universidad de Concepción, Chile*; [5] *Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile*

Overview: Gestational diabetes (GD) is associated with thyroid hormone disorders. The placenta is involved in regulating thyroid hormones transport from the mother to the fetus. It has been described that the normal placenta expresses thyroid hormone transporters (THT), including monocarboxylate transporters 8 and 10 (MCT8 and MCT10), L-amino acid transporters 1 and 2 (LAT1 and LAT2) and organic anion transporter polypeptide systems A1 and A2 (OATPA1 and OATPA2). The GD effect on THT expression is not reported.
Objectives: A. To determine whether GD alters thyroid hormone levels during pregnancy B. To determine whether GD alters the expression of MCT8, MCT10, LAT1, LAT2, OATP1 and OATPA2 mRNA levels in human placental cotyledon.
Methods: We evaluated maternal and newborn clinical variables from normal (n=10) and GD (n=30) pregnant women, and correlated different variables with maternal free T4. RNA extraction was performed with Trizol reagent from human placental cotyledon. cDNA synthesis was performed using reverse transcriptase. MCT8, MCT10, LAT1 and LAT2 mRNA levels were evaluated by real time PCR ($2^{-\Delta\Delta ct}$) from human placental cotyledon from normal (n=8) and GD (n=8) pregnancies.
Results: In GD, we observed an increase in glycemia 2 hours after a glucose load (normal: 106 ± 5 mg/dL, GD: 153 ± 2 mg/dL), and a decrease in free T4 only in the first trimester of pregnancy (normal: 1.36 ± 0.12 pg/mL, GD: 0.91 ± 0.11 pg/mL). In addition, there was a negative correlation between free T4 and glycemia 2 hours after a glucose load. Moreover, GD showed a decrease of ~53% in mRNA levels for MCT10 and 4.2- and 2.3- fold increases for LAT2 and OATPA1 with respect to normal human placental cotyledon, respectively.
Conclusions: Low maternal free T4 in the first trimester of pregnancy could be associated with GD. Moreover, GD alters THT expression in human placental cotyledon.

PB.4.
GESTATIONAL DIABETES DECREASES TERMINAL VILLOUS VASCULATURE IN HUMAN PLACENTA

A. Venegas [1,2], A. Armella [1,2], C. Veas [1,2], C.A. Escudero [2,3], M. González [2,4], L. Sobrevia [5], A.E. Covarrubias [1,2], E. Guzmán-Gutiérrez [1,2]. [1] *Molecular Medicine Center, Faculty of Health Sciences, Universidad San Sebastian, Chile*; [2] *Group of Research and Innovation in Vascular Health (GRIVAS Health), Chile*; [3] *Department of Science Basic, Faculty of Sciences, Universidad del Bio-Bío, Chile*; [4] *Department of Physiology, Faculty of Biological Sciences, Universidad de Concepción, Chile*; [5] *Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Chile*

Gestational diabetes (GD) is associated with alteration of vascularization. Some changes in the placental morphometry from GD have been described, but the effect of GD on the vascularization area in terminal villi and villous vessels is controversial.
Objectives: The aim of this work was to analyze whether GD alters the vascularization area in terminal villi and villous vessels.
Methods: Placentas were obtained from Hospital Guillermo Grant Benavente of Concepción, Chile. Placental samples from normal (n=3) and GD (n=3) pregnancies were rinsed in PBS and fixed in 4% paraformaldehyde. Sections (5μm) of each sample were stained with H&E and Periodic Acid-Schiff reaction (PAS).
Results: The percentage (%) villous area that contained vessels in normal and GD placentas showed no significant difference in villous vascularity between normal and GD placentas. However, GD placentas showed a decrease in the percentage of vessel area with respect to terminal villous area in comparison with normal placentas (25.6 and 32.1 %, respectively). Furthermore, in GD placentas, the vessel number for each terminal villus showed no changes with respect to normal placentas (7.9 and 7.4 vessels/terminal villus, respectively). However, in GD, the percentage of area per vessel in each terminal villus showed a decrease in comparison with normal samples (3.1 and 4.3 % of area per vessel).
Conclusions: GD shows a decrease in the terminal villous vasculature.

PB.5.
DEXAMETHASONE-INDUCED INTRAUTERINE GROWTH RESTRICTION IMPACTS PLACENTAL GENE EXPRESSION IN MICE

J. Aisemberg [1], M.V. Bariani [1], V. Schiariti [1], A.P. Domínguez Rubio [1], A.M. Franchi [1]. [1] *Laboratory of Physiopathology of Pregnancy and Labor, Center for Pharmacological and Botanical Studies (CEFYBO, CONICET), Buenos Aires, Argentina*

Intrauterine growth restriction (IUGR) remains a leading cause of perinatal morbidity and mortality. Current evidence suggests that changes in fetal