there is an increase of hyperpolarization in GDM endothelial cells, compared with healthy endothelium
Conclusion: In foetal endothelial cells from healthy pregnancies the insulin-induced NO bioavailability is dependent of the activity of KCa channels. In GDM there is a decrease in the participation of KCa channels in NO bioavailability, despite the expression of BKCa and hyperpolarization is higher in GDM HUVECs. These results could be related with alterations of mechanism that relates membrane potential and L-arginine/NO pathway in GDM endothelium.

P1.21.
ATTENUATION OF EZH2-MEDIATED H3K27 METHYLATION UP-REGULATES STAT5B EXPRESSION DURING TROPHOBLAST SYNCYTIALIZATION

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Objectives: The transcription factor STAT5B is involved in signal transduction initiated by a number of growth factors and cytokines. Our recent RNA sequencing data revealed that during syncytialization, the expression of STAT5B was up-regulated along with the down-regulation of the expression of EZH2, which is a histone-lysine N-methyltransferase enzyme catalyzing the addition of methyl groups to histone H3 at lysine 27 (H3K27) to silence gene expression. In this study, we examined whether EZH2-mediated H3K27 trimethylation was involved in the regulation of STAT5B expression during trophoblast syncytialization and explored the functional role of STAT5B in placental trophoblasts.
Methods: Cultured human primary placental trophoblasts were used
Results: Syncytialization was accompanied with significant increases in the abundance of STAT5B mRNA, protein and phosphorylated STAT5B as well as decreases in the abundance of EZH2 mRNA and protein ($P<0.05$ or 0.01, vs pre-syncytialization). Inhibition of EZH2 with either its antagonist EPZ-005687 or siRNA-mediated knock-down increased STAT5B expression, whereas overexpression of EZH2 with transfection of vector GV230-EZH2 decreased STAT5B expression in trophoblasts ($P<0.05$ or 0.01, vs scrambled siRNA or empty vector). Chromatin immunoprecipitation assay showed that the enrichments of EZH2 and trimethylated H3K27 at the STAT5B promoter was significantly decreased after syncytialization ($P<0.05$, vs pre-syncytialization). However, we failed to observe any significant changes in the syncytialization markers including e-cadherin, syncytin-1, syncytin-2 and hCG with siRNA-mediated knock-down of STAT5B ($P>0.05$,vs scrambled siRNA). Additionally, we found that siRNA-mediated knock-down of STAT5B decreased the expression of both long and short variants of Adam12 ($P<0.05$, vs scrambled siRNA), a metalloproteinase.
Conclusion: We have found that attenuation of EZH2-mediated H3K27 trimethylation upregulates STAT5B expression during trophoblast syncytialization, which results in increased expression of Adam12 with no effects on syncytialization. The exact role of STAT5B in human placenta awaits further investigation.

P1.22.
CYTOKINES PROFILE IN DIFFERENT POPULATIONS OF EXTRACELLULAR VESICLES DURING THE FIRST TRIMESTER OF PREGNANCY

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Objectives: The aim of this study was to isolate and characterise different populations of EVs from maternal plasma at first trimester of pregnancy and quantify the levels of 27 human cytokines associated with EVs.
Methods: Plasma samples were collected from pregnant women during the first trimester of pregnancy (n=10). EVs were isolated through differential centrifugation, at 2,000 x g for 30 min (pellet 1), 12,000 x g for 45 min (pellet 2); and at 100,000 x g for 120 min (pellet 3). The supernatant after the last centrifugation was termed "soluble fractions". EVs were characterised by size distribution, abundance of proteins associated with EVs (i.e. CD63, Flotilin-1, and TSG101), negative control for Grp94, and morphology, according to the recommendations of the International Society of Extracellular Vesicles, using Nanoparticle Tracking Analysis (NTA), Western blot analysis and electron microscopy, respectively. The concentration of 27 human cytokines in the EVs and the soluble fractions were established by cytokine array analysis (Bio-Plex Pro™ Human Cytokine 27-plex Assay).
Results: Specific changes in the levels of cytokines, in different population of vesicles, and in the soluble fractions were identified. The levels of IL-10, IL-6,IFN-γ and TNF-a were significantly higher ($p<0.05$) in the exosome fraction (pellet 3) compared to the values observed in pellet 1 and pellet 2 (macro and microvesicles fractions). The levels of IL-10, IFN-γ and TNF-a were significantly higher ($p<0.05$) in the soluble fractions compared with the exosomal fraction. No significant difference in the level of IL-6 in the exosomal and soluble fraction was observed.
Conclusion: This study established that cytokines are packaged within exosomes (in which these molecules are protected), suggesting a novel mechanism of action through which exosomes can lead to distal interactions.
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P1.23.
EXPERIMENTAL GESTATIONAL DIABETES INDUCES A PRO-INFLAMMATORY AND PRO-OXIDANT ENVIRONMENT IN MATERNAL AND FETAL LIVERS

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Objectives: Gestational diabetes (GDM) is a prevalent disease associated with a pro-oxidant and pro-inflammatory environment and with adverse consequences to both the mother and the offspring. The fetal liver is highly exposed to metabolic abnormalities, potentially leading to alterations in its development, which would affect the metabolic function in adulthood.
The aim of this work was to evaluate whether pro-inflammatory and pro-oxidant markers are increased in maternal and fetal livers in GDM rats.
Methods: GDM was spontaneously induced by intrauterine programming in the offspring (F1) of diabetic rats (F0 diabetic rats were obtained through neonatal streptozotocin administration). In control and GDM rats, maternal and fetal livers were explanted on day 21 of gestation for further evaluation of pro-oxidant and pro-inflammatory markers (nitric oxide production (nitrates/nitrites measurement), lipoperoxidation (TBARS measurement), and the protein expression of metalloproteinase-2 (MMP2), connective tissue growth factor (CTGF), and Manganese Superoxide Dismutase (Mn-SOD)) as well as the levels of PGC-1alpha, a PPAR co-activator that regulates pro-oxidant/pro-inflammatory processes.
Results: In the maternal liver of rats with GDM the levels of nitrates/nitrites were increased (254%, $p<0.001$ vs GDM), as well as the levels of TBARS (114%, $p<0.01$ vs GDM). In the liver of male and female fetuses of rats with GDM was observed an increase in the levels of nitrates and nitrites (34%, $p<0.05$ vs GDM), TBARS (35%, $p<0.01$ vs GDM), MMP-2 (22%, $p<0.01$ vs GDM), CTGF (25%, $p<0.01$ vs GDM) and Mn-SOD (80%, $p<0.001$ vs. GDM). PGC-1alpha expression was found reduced (45%, $p<0.05$ vs GDM) in fetal livers of rats with GDM.
Conclusion: In a GDM model induced by intrauterine programming, increased markers of oxidative stress and a pro-inflammatory environment are observed in livers from pregnant rats and fetuses, in the latter possibly related to reduced levels of PGC1-alpha. These alterations are likely to affect the liver function in the offspring's later life.