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Objectives: Pregnant women with sickle cell anemia (SCA) exhibit a higher incidence of pain episodes, infections and various complications. The placenta is an important organ during gestation and may undergo changes in its DNA methylation profile through the presence of maternal diseases. Thus, in this work we tested the hypothesis that pregnant women with SCA may present changes in the DNA methylation profile in the placenta compared to a control group of healthy pregnant women.
Methods: We included 8 pregnant women with SCA and 7 healthy pregnant women. The pregnant women were followed at a referral center, at the University of Campinas, Brazil and clinical data retrieved from medical chart and systematic placental sampling shortly after childbirth. DNA extraction was performed from placenta villous tissue and followed by bisulfite conversion and subsequent hybridization in the Methylation BeadChip. To analyze the differentially methylated positions (DMPs) was used the limma package. The p-values were adjusted (adjP) by the Benjamini-Hochberg procedure. To select DMPs were considered beta-values $\geq$ 15% and adjP <0.05.
Results: The analysis revealed 396 DMPs, of which 291 were hypermethylated and 105 were hypomethylated. 214 genes were identified, which were enriched in the following biological processes: neurogenesis, generation of neurons and cyclic-nucleotide-mediated signaling (FDR < 0.05). Among the most importantly methylated genes we can highlight: *GALR2*, *PTGFR*, *CSMD3* and *OPCML*, which are involved in cell surface receptor signaling pathway, regulation of cell proliferation, development of dendrites and cell adhesion. These findings suggest possible pathways involved in the pathophysiology of the disease during pregnancy.
Conclusion: We observed that the methylation profile of DNA is altered in the placental tissue of pregnant women with SCA compared to the control group, suggesting that SCA can affect DNA methylation levels of placentas, which could contribute to placental dysfunction or fetal diseases.

P2.52.
CONTRIBUTION OF MIRNAS MIR-21 & MIR-126 TO THE EARLY ENDOTHELIAL PROGRAMMING IN RESPONSE TO FETAL GROWTH RESTRICTION.

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Objectives: Fetal growth restriction (FGR) is associated with intrauterine chronic hypoxia and, endothelial dysfunction that would result from an altered eNOS expression mediated by epigenetic mechanisms. Previously have demonstrated the contribution of DNA methylation and histone post-translational modifications in this FGR-induced eNOS programming; however, no studies have determined the role of hypoxia-inducible microRNAs. (i.e. miR-21 and miR-126), as well as the potential epigenetic effects on other genes involved in the NO-dependent vasodilator pathway.
Methods: Levels of miR-21 and miR-126, as well as, eNOS, DDAH1, Nrf2 y ARG2 mRNA were determined in primary cultures of umbilical artery endothelial cells (HUAEC) from FGR (n=7) and control (n=7) pregnancies by qPCR. Additionally, control subjects were exposed to hypoxia (1% O_2, for 6 to 48 h) and the expression of the previously described miRNA and mRNA quantified. Finally, we determined the mRNA expression of miR-21 prediction targets (eNOS, DDAHH1) following the transfection of HUAEC-control with 30 nM miR-21 mimic precursor.
Results: FGR HUAEC showed a decrease of miR21 levels along with higher levels of miR-126 and eNOS, but lower expression of the pro-NO genes (DDAH1, Nrf2). Control HUAEC exposed to *in vitro* hypoxia (1% O2, 6 h) showed a transient increase in pro-NO genes (eNOS, DDAH1) along with a decrease in miR-21, whilst the level of miR-126 were not affected by hypoxia. The overexpression of miR-21 using a miR-21 mimic in control HUAEC led to a decrease in both eNOS and DDAH1 mRNA levels to 0 and 6 hours of hypoxia.
Conclusion: Hypoxia-related miRNAs, miR-21 and miR-126, are differentially expressed in HUAEC from FGR pregnancies and their expression is associated with heterogeneous levels of pro-NO genes. The negative regulation of pro-NO enzymes by miR21 suggests that the decreased miR-21 levels in FGR contribute to an epigenetic-mediated short-term up-regulation of eNOS in the endothelium.

P2.53.
INSPECTION OF THREE-DIMENSIONAL MORPHOLOGY OF THE MURINE PLACENTA BY HIGH RESOLUTION CONTRAST-ENHANCED MICROCT

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Objectives: Genetic engineering of the mouse genome identified many genes that are essential for embryogenesis. Remarkably, the prevalence of concomitant placental defects in embryonic lethal mutants is highly underestimated and indicates the importance of detailed placental analysis when phenotyping new individual gene knockouts. The placenta should therefore be appropriately evaluated when placental defects are suspected. Conventionally, the relative size of different placental layers is evaluated by histological evaluation. Here, we introduce high-resolution contrast-enhanced microfocus computed tomography (CE-CT) as a non-destructive, high-throughput technique to evaluate the 3D placental morphology.
Methods: Using a novel contrast agent, Zirconium-substituted Keggin polyoxometalate (Zr-POM), the soft tissue of the placenta (i.e. different layers and cell types, and its vasculature) was imaged with a resolution of 3.5μm voxel size. This novel approach allowed us to visualize and study early and late stages of placental development. Using CE-CT, placental parameters (i.e. volumes, volume fraction, ratio of different placental layers and volumes of specific cell populations) were quantified and correlated with histological stains of the corresponding sections.
Results: CE-CT allowed for the assessment of early embryonic development including chorioallantoic attachment and embryo turning. Quantification of the placental volume by CE-CT indicated an increase during gestation, which could be attributed to an increase in the volume of the labyrinth. In addition, the substantial difference in cell morphology between glycogen and spongiotrophoblast cells residing in the junctional zone allowed for a quantification of their relative proportions. The CE-CT technique was validatedby evaluating placentas from two different mouse strains, 129S and C57BL/6J mice and by confirming the placental phenotype of mice lacking phosphoinositol 3-kinase (PI3K)-p110α. Finally, Zr-POM-based CE-CT could be used as a tool to quickly detect placental defects in pathologies characterised by embryonic resorption and placental fusion.
Conclusion: Taken together, Zr-POM-based CE-CT offers a novel quantitative 3D methodology to investigate placental development or pathologies.

P2.54.
GROUP B STREPTOCOCCUS PROVOKES BIOMECHANICAL AND BIOCHEMICAL ALTERATIONS IN TROPHOBLAST CELLS: A RAMAN SPECTROSCOPY AND ATOMIC FORCE MICROSCOPY-BASED STUDY

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Objectives: Atomic force microscopy (AFM) allows for nanometer-scale investigation of cells and their biophysical properties, while raman