ERV3 knock-out in the human choriocarcinoma cell-line Jeg-3 using CRISPR/Cas9 technology.
Methods: The CRISPR-associated protein 9 nuclease (Cas9) from Streptococcus pyogenes is part of the bacterial immune mechanism used to protect the bacteria from foreign nucleic acids. By expression of a guide RNA (gRNA) the nuclease is directed to the target sequence and consequently is able to induce sequence specific double strand breaks.
A specific gRNA was cloned into the pSPCas9(BB)-2A-GFP plasmid expressing Cas9 from S. pyogenes and GFP. Jeg-3 cells were transfected with the construct and single cell clones were isolated by fluorescence activated cell sorting (FACS). Mutations within the ERV3 gene were detected by sequencing. Knock-out of the ERV3 protein was analyzed using western blot.
Results: Transfection of Jeg-3 cells with the CRISPR/Cas9 plasmid induced different ERV3 mutations. We were able to isolate six Jeg-3 clones with deletions from 1 to 186 nucleotides and one clone with a heterozygote point mutation. Two of these mutations induced a frameshift resulting in a loss of ERV3 protein expression.
Conclusions: Using CRISPR/Cas9 technology we were able to induce different mutations within the ERV3 gene in Jeg-3 cells. A stable knock-out of ERV3 in Jeg-3 cells might help to investigate its functional significance for cancer progression, proliferation or cell fusion. Functional assays using the ERV3 knock-out cell-line will give insights into the impact of ERVs for cancer and placental development.

P2.15
SUCCESSFUL ISOLATION OF VIABLE FIRST TRIMESTER ENDOTHELIAL CELLS FROM PLACENTAL EXPLANTS AFTER THREE DAYS IN CULTURE

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Objectives: Incidents *in utero* can have profound health effects in later life. Cardiovascular disease risk in the offspring has been well established after maternal malnutrition and other health hazards leading to fetal malnutrition, causing permanent metabolic changes that contribute to the development of future disease. This process, called fetal programming, is likely to be due to epigenetic changes. Improper development of the placenta affects fetal development as it gives rise to reduced oxygen and nutrient supplies and hence fetal programming. Various studies have shown that offspring born from pregnancies with an improper placental development have an almost doubled risk of cardiovascular disease in later life.
First trimester placenta endothelial cells might represent the ultimate model to study epigenetic changes due to changes in the first trimester environment resulting in an increased risk to develop cardiovascular disease.
It is currently unclear how long endothelial cells within first trimester placental explants remain viable when blood is no longer present in placental vessels. However, considerable treatment length will be needed to obtain persistent and measurable epigenetic changes in the isolated endothelial cells.
Methods: First trimester placental explants (week 6-11, n = 20) were cultured for 24-96h at 3% O_2 after which they were minced, treated with Collagenase/Dispase, followed by collection of live cells by Ficoll gradient separation. CD31 magnetic beads were used to isolate endothelial cells. Immunocytochemistry was used to assess the purity of isolated endothelial cells by staining for VE-cadherin (endothelial cells), Vimentin (fibroblasts) and Cytokeratin-7 (trophoblasts). Viability was measured by a cell viability assay.
Results: Up to 72h in culture a consistent 75±10% of cells isolated were found to be viable endothelial cells, while viability percentages drastically decreased at 96h.
Conclusion: Treatment to induce epigenetic changes in first trimester endothelial cells can be performed up to 3 days in culture.

P2.16
KARYOTYPES OF TROPHOBLASTIC CELL LINES

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Cell lines have been used for many years as in vitro models to study tropboblast functions such as invasion, migration or intracellular signaling. Widely used cell lines have been developed either by virus transfection, like HTR8/SVneo, from choriocarcinoma like JEG-3, BeWo or JAR cells or by hybridization of their mutants with primary cells such as ACH-1M59 or ACH-3P.
These cell lines are extensively studied functionally, but are still widely anonymous genetically. In some cell lines like BeWo chromosome study with Q-band technique showed a modal chromosome number at a hypotetraploid level. Extracellular trophoblast cells become hyperdiploid after they leave the cell cycle and differentiate into the invasive phenotype. Meanwhile, there are higher resolution techniques available. Therefore, our aim was to characterize the HTR8/SVneo, JEG-3, JAR, BeWo, ACH-1M59 and ACH-3P cells on a molecular cytogenetic level in order to better understand functional alterations, but also to distinguish these cells from each other.
Methods: The cell lines HTR8/SVneo, JAR, JEG-3, BeWo, ACH-3P and AC-1M59 were karyotyped through **M**ultiplex-**F**luorescence **I**n **S**itu **H**ybridisation (M-FISH). In short, chromosomes were arrested in metaphase with colcemide and chromosomes were stained specifically using 5 fluorochromes in different combinations. 15-20 metaphase spreads of a single passage were analysed and chromosomal aberrations were identified with ISIS software.
Results and conclusion: The composite karyotypes demonstrated relatively stable, clonally-recurring aberrations for each cell line. HTR8/SVneo is a hypotriploid cell line (3n-); JEG-3, JAR and BeWo are triploid (3n) while ACH-1M59 and ACH-3P were tetraploid (4n). It also confirmed that HTR8/SVneo is female while JEG-3, JAR, BeWo, ACH-1M59 and ACH-3P have male origin.
The cell lines exhibit specific stable aberration patterns. The composite karyotypes summarize all clonal (at least 3x per metaphase) aberrations. Cell lines tend (particularly in long term culture) due to lack of selection pressure in culture to accumulate a number of structural and numerical aberrations. This is also reflected in the examined cell lines. HTR8/SVneo is among the examined cell lines, the only with female karyotype, the sex chromosomes of all cell lines show additional aberrations as partial deletions or were involved in translocations. These karyotypes may function as identification marker. The spectrum of structural and numerical aberrations is typical for immortal cell lines.

P2.17
ANNEXIN A1 LOCALIZATION AND RELEVANCE IN HUMAN PLACENTA FROM PREGNANCIES COMPLICATED BY GESTATIONAL DIABETES MELLITUS. PRELIMINARY RESULTS

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Objective: Current data indicate that the endogenous homeostatic anti-inflammatory role of Annexin A1 (ANXA1) contributes to the resolution of inflammation. In this study, we aimed to investigate whether ANXA1/receptors are expressed in the human placenta in women with gestational diabetes mellitus (GDM).

Methods: This cross-sectional study included 20 pregnant women, who underwent a 75g oral glucose tolerance test (GTT-75g) during pregnancy for GDM diagnosis. Pregnant women were classified as Nondiabetic (ND; normoglycemic, GTT-75g; n=10) and gestational diabetic (GDM; abnormal GTT-75g and increased HbA1c blood levels; n=10). Glycemic control was evaluated by HbA1c levels (high-performance liquid chromatography). ANXA1 and receptors FPR1 and FPR2 protein expression were analyzed by immunohistochemistry in formalin-fixed, paraffin-embedded placental samples and Western blot.
Results: In ND pregnancies, strong ANXA1 immunoreactivity was seen in syncytiotrophoblast cytoplasm and nuclei of syncytial knots. Leukocytes inside fetal vessels were also immunostained. In the maternal compartment, decidual cells exhibited nuclear staining. FPR1 was observed in the nuclear area of mesenchymal and fetal endothelial cells of the chorionic villi; cytoplasm of endothelial cells was also reactive. Weak immunostaining was seen in decidual cells. No FPR2 immunostaining was found in either of the two compartments. Only neutrophils inside fetal/maternal vessels expressed FPR2. In GDM pregnancies, immunoreactivity was weaker than those seen in ND gestations but followed a similar pattern. Nuclear staining was prevalent in both ND and GDM placentas. Densitometric analysis of the immunoblots, having as reference b-actin as load control, corroborated the lower ANXA1 staining in the villi in GDM pregnancies ($p< 0.01$).
Conclusion: Our preliminary results show lower levels of ANXA1 in chorionic villous of placentas from GDM pregnancies. GDM placentas have higher levels of inflammatory cytokines. In this context, we hypothesize ANXA1 is playing a role in the inflammatory/anti-inflammatory regulatory mechanisms in the chorionic villi, which may be crucial in gestational diabetic diseases. *Supported by CAPES and FAPESP

P2.18
GENDER-SPECIFIC PLACENTAL EXPRESSION OF CANNABINOID RECEPTOR ONE (CB1R) IN BABOON MODEL OF OBESITY

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Introduction: Maternal obesity is associated with offspring behavioural and cardiovascular developmental problems through poorly understood mechanisms. Endocannabinoids (eCB) are lipid derivatives, linking metabolism to mental and cardiovascular health. CB1R – a G-protein-coupled receptor- is the main target of endogenous and exogenous cannabinoids (e.g. marijuana). We previously reported a seemingly paradoxical decrease of CB1R ligands' concentrations, decreased mRNA, but not CB1R protein expression in placentas of obese baboons (increased food consumption model) (Placenta 2013 34(11):983) and decreased placental protein expression of CB1R and CB2R in obese pregnant women carrying male fetuses (AJOG, 2014, 210(1):S94).
Aim: To explore the role of dietary fat composition (higher fat/higher carbohydrate diet) vs. overeating pattern in placental CB1R expression.
Material and Methods: Baboons *(Papio spp)* were fed a diet of 45% fat (HF) while controls (CTR) ate 12% fat from at least 9 months prior to conception through pregnancy until 0.9 gestation. Four HF and four CTR placentas of male fetuses were evaluated. Commercially available CB1R antibodies were applied for immunohistochemistry, Histoscore of the receptor expression was calculated using ImageScope™ v11.1.2.752 by Aperio.
Results: CB1R is expressed in fetal endothelium, cytotrophoblast and syncytiotrophoblast. The expression of CB1R protein was decreased in the villous tree of HF animals (214.11 ± 1.28 vs 209.22 ± 0.98; CTR vs. HF, respectively, $p=0.05$, Mann-Whitney U test). The placental (203.00 ± 12.49 g vs. 226.23 ± 7.13 g) and fetal (810 ± 17.76 g vs 849. 59 ± 24.47 g) weights did not differ between the groups.
Conclusion: Availability of the cytoplasmic pool for placental CB1R is regulated by a high fat diet, but not increased food intake in this baboon model of maternal obesity. Decreased placental CBR1 availability might alter eCBs regulated fetal vascular reactivity (37 RSoA, 2014). Supported by NIH R24OD010916 and P51 RR013986.

P2.19
MATRIX PROTEOGLYCAN CHANGES IN HUMAN PLACENTA AFTER GESTATIONAL DIABETES

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Background & objectives: During gestational diabetes mellitus (GDM) the feto-placental vasculature is exposed to hyperglycaemia and a pro-inflammatory environment. Proteoglycans (PG) are extracellular matrix (ECM) proteins located in the surrounding of cells influencing proliferation, migration and adhesion, and modulating cell phenotype.
As endothelial cells and smooth muscle cells (SMC) are in intimate contact and their interaction is fundamental for several cardiovascular processes, we hypothesize that the intrauterine, diabetic environment modifies matrix proteoglycan expression in feto-placental endothelial and smooth muscle cells leading to vascular dysfunction.
Methods: Primary arterial endothelial cells (AEC) and SMC were isolated from human term placentas of healthy (control) and GDM pregnancies (GDM) and cultured. Matrix proteoglycan expression was analyzed by RT-qPCR. Molecules with highest fold-changes were analyzed by ELISA, WB and IHC.
Results: Gene expression of PG versican was higher in GDM AEC by 41.1-fold ($p<0.001$) compared to control AEC. Analysis of the 4 versican isoforms (V0-V3) revealed that V0 was 300-fold upregulated and V3 was exclusively expressed in GDM AEC ($p<0.001$). Expression of fibrillin1 and syndecan was also higher in GDM AEC by 5.6-fold and 4.1-fold, respectively ($p<0.001$). Versican protein levels were lower in GDM AEC conditioned media (-0.48-fold, $p<0.05$), whereas in cell layer no difference was found. Higher protein levels of fibrillin1 were found in GDM AEC compared to control AEC (1.9-fold, $p<0.05$). V0 was downregulated in GDM SMC compared to control SMC (-0.65-fold, $p<0.005$). Preliminary data suggest that GDM SMC secret higher levels of versican than control SMC. IHC showed higher levels of versican in control compared to GDM human placenta.
Conclusion: Matrix proteoglycans have altered expression in GDM AEC and SMC. Since matrix proteoglycan misregulation is associated with vascular dysfunction, these results suggest that diabetes induces vascular changes already *in utero*.

P2.20
THE RELATIONSHIP BETWEEN MATERNAL BODY MASS INDEX AND EXOSOMAL CONCENTRATION ACROSS GESTATION

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Background: Obesity is one of the most powerful drivers for the onset and development of several pregnancy complications with short and long term consequences for both the mother and child. Recently, we have established the exosomal profile across normal pregnancies and pregnancies with complications. The aims of this study were to establish the relationship between maternal Body Mass Index (BMI) and exosomes present in maternal circulation during gestation and to determine the contribution of placental exosomes to the total exosomes present in maternal circulation across pregnancies.
Methods: Plasma samples were obtained from women with normal pregnancies at different times during gestation (10-38 weeks) from the Ochsner Baptist Medical Center (New Orleans, USA) and classified according to the Body Mass Index (BMI) into three groups: normal (n=15, BMI 18.5-24.9 Kg/m^2), overweight (OW, n=15, BMI 25-29.9 Kg/m^2) and