P1.1

HYDROGELS FROM DECELLULARIZED BOVINE PLACENTA AND AMAZONIAN CRUDE EXTRACTS TO INCREASE WOUND HEALING ACTIVITY

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Objectives

Create tissues and organs grafts from decellularized biomaterials is an approach in bioengineering for repairing processes repair, such as in wound healing. To improve those biomaterial function, some compounds derived from plant and microorganisms can be useful, and Amazonian biodiversity can provide several active compounds. Herein, we aim in potentialize decellularized bovine placenta hydrogel with crude extracts from Amazonian plants with recognized wound healing activity.

Methods

For this study, mid-term bovine cotyledons were obtained from slaughterhouse, decellularized with crescent concentration of SDS, digested with pepsin/HCl and polymerized with alginate and calcium chloride. For the crude extracts, Amazonian plants leaves will be dried, and homogenized in distilled water during five days. The biomass will be filtered, freeze-dried and lyophilized. Afterwards, this hydrogel and crude extracts will be homogenized and the cytocompatibility will be test by in-vitro culture with NIH3T3 fibroblasts cells. Then, skin wound induction assay will be performed in rodent to test wound healing increased activity.

Results

As partial results, bovine cotyledon was decellularized with maintenance of natural structure and major extracellular matrix components. Hydrogel was better polymerized with of 12% alginate addition, when had better structure, support, and maintenance for NIH3T3 fibroblasts cells. Also, crude extracts from Amazonian plants were produced and tests for solubilization in hydrogels is in progress.

Conclusion

Altogether, hydrogel was produced, been cytocompatible, and structurally viable to be ready for solubilize the crude extracts and to perform the skin wound induction assay.

P1.2

Placenta Homeopatic Remedies and Complementary Medicine in squamous cell oral cancer treatment: a case report

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Objectives

We want to present a possible association between Viscum album (the classical antroposophic remedy for supporting cancer diseases) and the method of creating placenta homepatic dilutions as specific genetic homepaty (genetic isotherapy) for cancer treatment.

Methods

Data regarding clinical and biochemical parameters were extracted from the actual patients' files.

Results

A 77 y.o. man with no previous clinical history except for a long smoking habit (20 years) and dispnea from 3 months went throughout a complete resolution of a lung mass in the anterior basal segment with pleural-based area of parenchymal consolidation and air bronchogram characterized by spiculated and irregular borders.

Conclusion

Further investigations seem worthwhile to establish the possible role of placental homoepatic remedies and their association with viscum album in the cancer integrative medicine approach.

P1.3

ENDOTHELIAL IMMATURITY IN THE PLACENTA IN HYDROPS FETALIS

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Objectives

CD15 expression has been used as a marker of endothelial immaturity in the placenta and is linked to the setting of antenatal hypoxia. As such, expression should be increased in cases of fetal anemia, especially when severe enough to cause hydrops fetalis (HF). This has not been previously studied and the current study tested this hypothesis.

Methods

Placentas from cases of HF and control cases (27 of each, matched by gestational age) underwent CD15 immunohistochemical staining. Results were quantitatively assessed for the proximal vasculature (chorionic plate and stem vessels) and distal vasculature (villous vessels).

Results

HF cases included hemoglobin (Hb) Bart (*n*=14), anemic HF other than Hb Bart (*n*=4), and non-anemic HF (*n*=9), with a gestational age range (mean ± SD) of 25-38 (29 ± 4) weeks. Compared to controls, the mean number of CD15+ vessels in the distal vasculature was higher in Hb Bart (*P*=0.001), anemic HF other than Hb Bart (*P*=0.001), and non-anemic HF (*P*=0.04). The mean number in Hb Bart was not significantly different from anemic HF of non-Bart (*P*=0.8), but was higher than that for non-anemic HF (*P*=0.008). No significant difference in staining of the proximal vasculature was noted.

Conclusion

Placentas belonging cases of HF demonstrate increased expression of CD15 in villi, regardless of etiology. Highest expression was seen in cases resulting from anemia, suggesting expression is, at least partly, driven by hypoxia and may reflect an attempt to compensate for this. Additional studies are needed to determine if increased expression occurs in all villi, supporting hypoxia as a driving mechanism, or is more related to villous immaturity.

P1.4

High frequency of persistent placenta previa in pregnancies conceived by assisted reproductive technology treatment. What is the cause?: A retrospective cohort study.

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Objectives

In general, over 90% of women with a low-positioned placenta including low-lying placentas (LLP) and placenta previa in the second trimester are not at risk in the third trimester due to a phenomenon called placenta migration in which the placenta migrates upwards during pregnancy. Singleton pregnancies conceived by assisted reproductive technology (ART) treatment are known to be at high risk of persistent placenta previa. However, the mechanism is unclear. Our investigation aimed to determine whether the high frequency of placenta previa is due to the inadequate placental migration function.

Methods

A total of 4943 nulliparous singleton pregnancies from January 2008 to January 2024 in our hospital were retrospectively evaluated. Nulliparas diagnosed with LLP or placenta previa in the second trimester were selected and compared between ART and non-ART pregnancies for persistent LLP and placenta previa.

Results

In the third trimester, 248 nulliparas (5.0%) were diagnosed with placenta previa or LLP. Nulliparas with a history of endometriosis were excluded. A total of 90 nulliparas who gave birth in our hospital with second trimester ultrasound data were assessed in this study.

Maternal age in non-ART pregnancy group (n = 57) was significantly younger than in the ART pregnancy group (n = 33). There was no significant difference in the migration rate in LLP (non-ART 94.4% vs. ART 93.3%) or the percentage of persistent low-positioned placenta after diagnosis of placenta previa in second trimester (82.1% vs. 72.2%) between the two groups.

Conclusion

The study showed no significant difference in migration rate between non-ART and ART pregnancies. However, the migration rate in cases of placenta previa was much lower than in previous reports. The low migration rate in the non-ART group may be due to the low number of low-risk pregnancies and the high number of referrals for placenta previa.

P1.5

EVIDENCE FOR THE ANTI-INFLAMMATORY EFFECT OF ASPIRIN ON HYPERTENSIVE PREGNANCIES

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Objectives

Pregnancy-related hypertension disorders are a leading cause of fetal and maternal morbidity and mortality. In preeclamptic women, uricemia increases by 1.5 times after the 20th week of gestation, suggesting an association with maternal inflammation. Low-dose aspirin (LDA) has been proposed to prevent early preeclampsia (PE), but its mechanism is still uncertain. We aimed to evaluate the effect of LDA during pregnancy.

Methods

We conducted a prospective study, approved by the Institutional Review Board, on 301 pregnant women who attended their pregnancies at Hospital Posadas in 2023. 192 of these women were stratified into 4 groups according to their risk of developing PE or fetal growth restriction using the Fetal Medicine Foundation algorithm. Those at high risk were prescribed LDA (150 mg/day) from the 14th to the 35th week of gestation. Uricemia (mg/dl) was measured as a marker of endothelial inflammation.

Results

Out of 55 women at risk who received LDA, 7 developed gestational hypertension (GH) and 10 PE. 9 of these preeclamptic women presented a BMI>25, and 2 of them developed early-onset PE.

LDA-treated preeclamptic women compared to those untreated (n=34) improved their gestational age at birth *(36.6±1.8 vs 34.5±4.0 weeks, P<0.03)* and birthweight *(2765±491g vs 2471±1236g, P<0.04)* and showed lower uricemia after the 20th week of gestation *(5.02±0.78 vs 5.74±1.29, P<0.05)*. However, they increased the days of hospitalization *(5.3±1.6 vs 4.0±1.6, P<0.03)*. Interestingly, LDA-treated GH women showed higher uricemia levels than those untreated *(n=79), (4.35±1.09 vs 3.8±0.96, P<0.05).*

Conclusion

Aspirin treatment improves hypertensive pregnancies. Therefore, the higher uricemia levels observed in LDA-treated GH women may indicate that aspirin prevents preeclampsia development in these patients. In preeclamptic women who received LDA, lower levels of uricemia after the 20th week of pregnancy, suggest that aspirin may have an anti-inflammatory effect. The poor response to aspirin in obese women may be related to their chronic inflammation.

P1.6

GLYCOSYLATED FIBRONECTIN AS A NOVEL PREDICTOR FOR THE MATERNAL AND FETAL COMPLICATIONS OF PREECLAMPSIA

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Objectives

The aim of this study was 1) to evaluate glycosylated fibronectin (GlyFn) as a novel predictor for preeclampsia (PE)-related maternal and fetal complications, and 2) to compare the predictive value of GlyFn versus that of the soluble Fms like tyrosine kinase-1/placental growth factor (PlGF) (sFlt-1/ PlGF) ratio.

Methods

This was a secondary analysis of a prospective multicenter cohort study involving 524 pregnancies either suspected of (but not confirmed with) PE, with gestational hypertension (GH), or with confirmed PE and/or hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome (PE/HELLP). In all patients sFlt-1, PlGF and GlyFn were measured at the time of inclusion. Logistic regression was conducted to study the association between GlyFn and PE-related complications. A traditional model (incorporating gestational age at biomarker measurement, parity, diastolic blood pressure and proteinuria at inclusion), to which GlyFn and the other biomarkers were added, was used for multivariable analysis.

Results

In GH and PE/HELLP, GlyFn levels were significantly elevated (P=0.002 and P=0.02, respectively), compared to women with suspected PE. GlyFn correlated positively with sFlt-1 and the sFlt-1/PlGF ratio, and negatively with PlGF. GlyFn alone performed better than the traditional model with regard to the prediction of maternal but not fetal complications, and this was also true for sFlt-1, PlGF and their ratio. Combining the traditional model with GlyFn increased the C-index for maternal complications further, and on top of the traditional model the GlyFn/PlGF ratio yielded the best results for predicting fetal complications in the overall cohort, while in women with a GA<37 weeks this was also true for the prediction of maternal complications.

Conclusion

GlyFn is a novel biomarker for PE diagnosis and its complications, and its role might be particularly relevant in patients with PE below 37 weeks of gestation.

P1.7

Elevated phosphatidylglycerol in cord blood of infants born with congenital heart disease is associated with altered lipid profile in placenta

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Objectives

Congenital heart disease (CHD) is the primary cause of birth defect, affecting 1% of live births. Genetics explain only 20% of sporadic cases. Most infants with complex CHD will develop neurodevelopmental disorders. Abnormal vascularization in CHD placenta may lead to heart defect partly due to reduced nutrient transfer. Changes in lipid composition in the placenta affects placental mitochondrial function and lipid delivery to the fetus impacting the fetal development. We hypothesized that the lipid profile in umbilical cord blood is altered in CHD infants in association with changes in placental mitochondrial phospholipids.

Methods

Term placenta and paired umbilical cord blood were collected at birth from complex CHD (n=7) and matched control (n=10) neonates. Changes in fatty acid composition of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), cardiolipin (CL), diacylglycerols (DG), and triacylglycerols (TG) were determined in placenta and cord venous plasma by lipidomic analysis using LC-MS/MS. Significant differences between groups were evaluated by Mann-Whitney multiple tests (adjusted $p<0.05$).

Results

CHD placenta showed decreased PC, PE and TG containing long chain polyunsaturated fatty acid (LCPUFA n-3) [PC (18:2/22:5), PE (18:2/22:5), TG [TG (58:8), TG (60:10)] and increased DG (40:6) and several TG with low LCPUFA. PG and CL were unchanged. In contrast, CHD cord plasma contained elevated PG [LPG (18:0), PG (16:0/16:0), PG (16:0/18:0), PG (20:0/20:0)], and PS (16:0/18:1). Those lipids are known as precursor of cardiolipin in mitochondrial membranes. DG (40:6) and LPG (16:0) in placenta were positively correlated with total PG in cord plasma.

Conclusion

Decreased PC and PE containing LCPUFA in CHD placenta, which are key in placental metabolism and omega 3 transfer to the fetus, may negatively impact cognitive functions in CHD infants. PG

accumulation in umbilical cord plasma of CHD suggests a compensatory mechanism trying to regulate a possible defect of mitochondrial cardiolipin synthesis in fetal heart.

P1.8

Correlation between placental CD105 Endoglin and Matrix metalloproteinase -9 in normal human pregnancy.

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Objectives

Endoglin (CD105) (Eng) and matrix metalloproteinase-9 (MMP-9) proteins are constitutively expressed in normal human placenta throughout gestation. The study determined the correlation between Eng and MMP-9 proteins in normal human pregnancy.

Methods

Placentas from normotensive women with uncomplicated pregnancy in the first, second and third trimester were collected. Chorionic villi were isolated. Eng and MMP-9 proteins of the tissue homogenate were analyzed using ELISA kits from R&D Systems. Independent T test and Spearman's correlation were performed. $P<0.05$ considered significant.

Results

The mean $\pm$ SD expression for Eng protein were 61.73 $\pm$ 28.97, 46.36 $\pm$ 27.55 and 28.58 $\pm$ 11.76 ng per 100 mg tissue; and for MMP-9 protein were 18.59 $\pm$21.51; 28.60 $\pm$ 33.00; and 23.90 $\pm$ 10.63 ng per 100 mg tissue for first (n=68), second (n=67) and third trimester (n=80), respectively. Eng protein showed a negative correlation with gestational age in days (GAD) (r^2 = -.528, p =<.001, n=215), while MMP-9 protein showed a positive correlation with GAD (r^2 = .290, p =<.001, n=215). The correlation between Eng and MMP-9 proteins was negative but significant (r^2 = -.334, p =<.001, n=215).

Conclusion

The finding of reduced expression of Eng with increasing GAD may be associated with vasorelaxation as pregnancy advanced, because reduced Eng expression is associated with vasorelaxation. The increased expression of MMP-9 with an increase in GAD may be involved in degradation of extracellular matrix, and in facilitating pregnancy- associated vascular remodeling of the spiral arteries. Eng is a membrane bound protein. However, we have homogenized the tissue and used the homogenate to determine Eng and MMP-9 protein expressions. The negative correlation seen between Eng and MMP-9 protein in this study provides a new perspective on MMP-9 activity during human pregnancy. If confirmed in future studies, the interaction between these two proteins may be exploited to develop therapeutic strategies in preventing preeclampsia.

P1.9

SYNCYTIOTROPHOBLAST-DERIVED EXTRACELLULAR VESICLES IN FETAL GROWTH RESTRICTION

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Objectives

Fetal growth restriction (FGR) is the main cause of stillbirth and it is associated with severe future morbidity but only 30% of cases are diagnosed. Syncytiotrophoblast-derived vesicles (STB-EVs) are released by the placenta to maternal circulation, and they can work as circulating, non-invasive placental biopsies. With this study we aimed to 1) validate a method for isolation of STB-EVs from placental explants; 2) characterize differences in protein expression in STB-EVs in early FGR when compared to healthy pregnancies.

Methods

Immediately after cesarean section (before labor onset), we collected 6 placentas from patients with early FGR (ISUOG 2020 criteria), and 6 healthy controls. We extracted villous tissue from each sample and cultured placental explants in 12 well plates with media at 37ºC, 8% O2 and 5% CO2 for 48 hours. Media was analyzed for expression of b-hcg, PlGF and sFLT1. STB-EVs were isolated by differential ultracentrifugation (fraction enriched in medium/large EVs, 10K; and fraction enriched in small EVs, 150K). Transmission electron microscopy, nanoparticle tracking analysis and Western-Blot were used to characterize these vesicles according to MISEV guidelines. Samples containing 100 µg protein were processed by S-trap plate protocol and analysed on the NeoVanquish-Orbitrap Ascend system over a 1 hour gradient (DIA mode). Results were analysed with Perseus and STRING 12.0 ($p < 0.05$ was considered significant).

Results

Per mg of tissue, b-hcg, sFLT1 and PlGF were not significantly different between groups. Vesicle characterization is shown in figures 1-3.

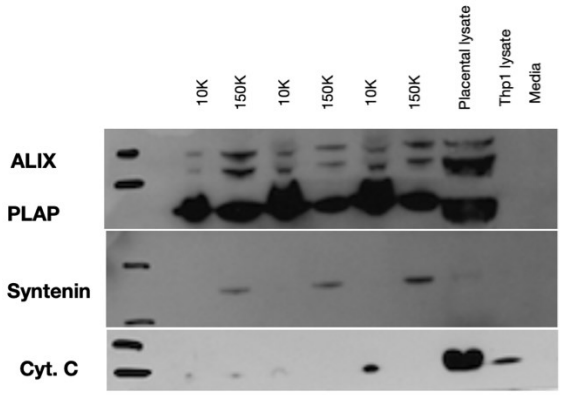


Fig. 1A - Western-Blot in reducing conditions. Antibodies used: ALIX (3A9) 200 µg/µl, 1:200 (Santa Cruz); PLAP, 1.6 µg/µl, 1:250 (in house antibody); syntenin (EPR8102) 200 µg/µl, 1:1000 (Abcam), cytochrome c (A-8) 200 µg/µl, 1:10000 (Santa Cruz). Secondary antibodies: anti-mouse 1:1000 (Cell Signalling) for ALIX, PLAP and cytochrome c; anti-rabbit 1:1000 (Cell Signalling) for syntenin

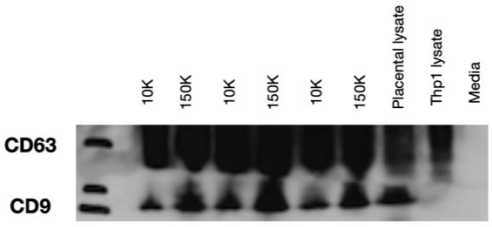


Fig. 1B - Western-Blot in non-reducing conditions. Antibodies used: CD63 200 µg/µl, 1:1000 (Santa Cruz); CD9 (Alb 6) 100 µg/µl, 1:1000 (Santa Cruz). Secondary antibody: anti-mouse 1:1000 (Cell Signalling)

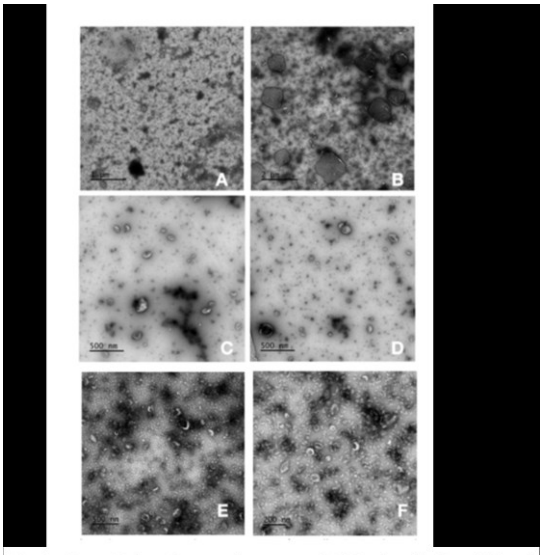


Fig. 2 - Transmission electron microscopy of 10K pellets (A, C and D) and 150K pellets (B, E and F). A and B are images with wide view

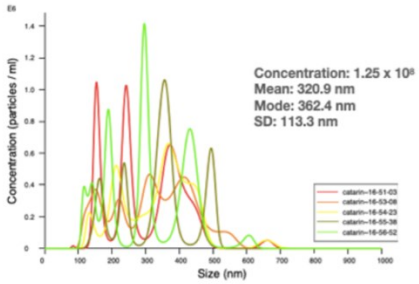


Fig. 3A - Nanoparticle tracking analysis results for the 10K pellet isolated from placental explants (1:200 dilution). We can confirm that the sample concentration is in the desired range. Mean particle size is 320.9 ± 113.3 nm which is consistent with the presence of medium/large EVs

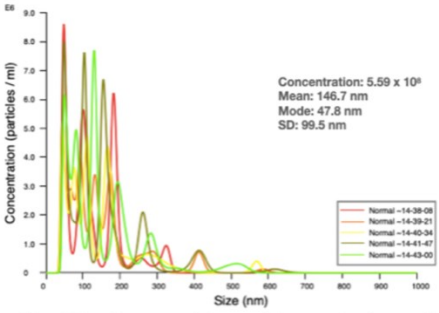


Fig. 3B - Nanoparticle tracking analysis results for the 150K pellet isolated from placental explants (1:500 dilution). We can confirm that the sample concentration is in the desired range. Mean particle size is 146.7 ± 99.5 nm which is consistent with the presence of small EVs

Proteomic analysis revealed different protein expression profiles for FGR and healthy pregnancies (figures 4, 5).

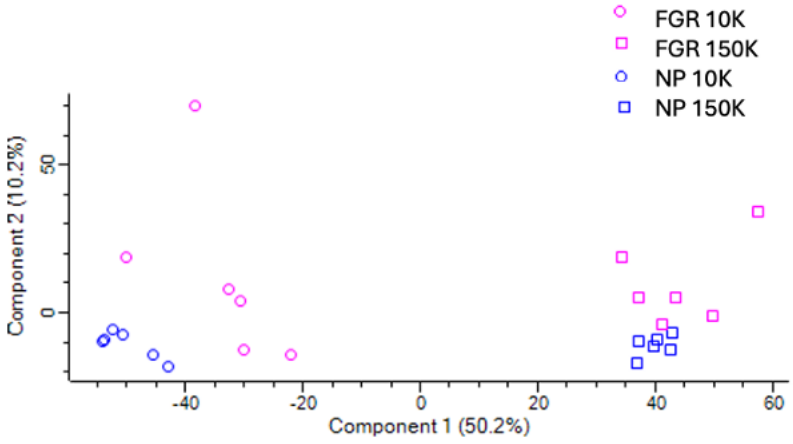
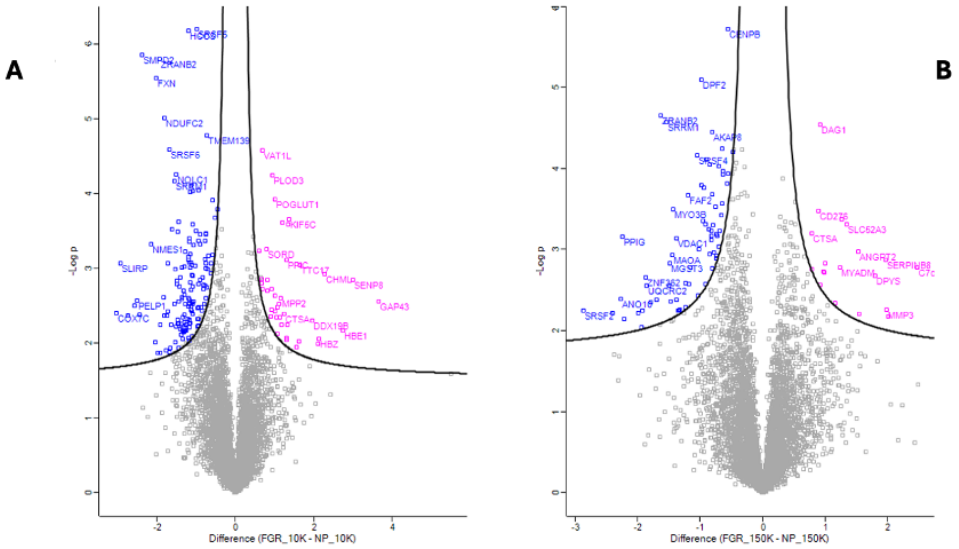


Figure 4: Principal component analysis of FGR and healthy pregnancies



Figures 5A and 5B: Volcano plot showing differential protein expression in medium/large vesicles (A) revealed 201 proteins differentially expressed. The same analysis for small vesicles (B) revealed differential expression of 86 proteins

Pathway analysis revealed most differentially expressed proteins are related to adenine transport and mitochondrial function.

Conclusion

STB-EVs released from placentas with early FGR exhibit a distinctive protein signature that is consistent with the pathophysiology of the condition. Further studies are needed to test this signature in maternal peripheral blood.

P1.10

In-Vivo Placental Volumes and Placental Growth Trajectories are Abnormal in Fetuses with Congenital Heart Disease

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Objectives

To compare *in vivo* placental growth in fetuses with CHD compared to healthy controls.

Methods

Pregnant women with fetal diagnosis of CHD and healthy low-risk pregnancies were enrolled in a longitudinal observational study. Fetal MRI was obtained up to two timepoints during pregnancy on a 1.5T MRI scanner. Placentas were manually segmented using ITK-SNAP software. All fetuses with CHD underwent a fetal echocardiogram within 48 hours of MRI and were categorized by cardiac physiology. Placental volume z-scores were created from the controls.

Results

451 MRIs were analyzed from 286 pregnant women, 114 mothers with fetal CHD (190 scans), and 172 controls (261 scans). After correction for gestational age, fetal sex, maternal race, and maternal education, placental volumes in CHD were significantly smaller than placentas from controls, ($p=0.015$). CHD placental volume z-scores were approximately 2SD below the normal mean ($p=0.01$). Z-score placental growth trajectories showed worse placental growth in fetuses with single ventricle CHD with aortic obstruction compared to 2 ventricle CHD without aortic obstruction. Smaller placental volume z-scores were associated with smaller head circumference ($p=0.02$) and weight ($p=0.01$) at birth, and lower middle cerebral artery pulsatility index ($p=0.045$).

Conclusion

This is the first study to identify that *in vivo* congenital heart disease placentas are significantly smaller and the growth trajectories are abnormal compared with healthy controls. Congenital heart disease placental growth rate declined in the final 4 weeks of gestation, a phenomenon not seen in controls. Smaller placental volume z-scores were associated with smaller head circumference and weight at birth suggesting the importance of the placental circulation in outcome.

P1.11

Impaired Feto-Placental Growth in Congenital Heart Disease Detected Prenatally via Quantitative MRI

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Objectives

Fetuses with congenital heart disease (CHD) have small placentas, but it remains unknown whether and to what extent growth restriction of the placenta contributes to growth restriction of the fetus. The objectives of this study were to evaluate fetal body volumes in fetuses with CHD compared to healthy fetuses, identify differences in the fetal:placental (F/P) volume ratio between groups, and investigate how fetal body volumes and F/P volume ratios correlate with birth anthropometrics.

Methods

Pregnant women with a fetal diagnosis of CHD and healthy low-risk pregnancies were prospectively enrolled. Fetal MRI was obtained up to two timepoints during pregnancy. The fetal-placental ratio was calculated using 3D-MRI fetal body volumes and placental volumes.

Results

55 control (55 scans) and 56 CHD subjects (80 scans) were enrolled. Fetal body volumes in CHD were significantly smaller compared to controls ($p<0.001$) with larger F/P volume ratios ($p<0.001$). Further, there was increasing divergence of both the fetal body volumes and F/P volume ratios between groups. CHD fetal body volumes were significantly correlated with birth weight ($p=0.01$), but not with birth head circumference (HC). There was a negative association between F/P volume ratios and birth HC in d-transposition of the great arteries (dTGA; $p = 0.04$) and hypoplastic left heart syndrome (HLHS; $p = <0.01$), with larger F/P volume ratios correlating with smaller birth HC.

Conclusion

Fetuses with CHD have impaired F/P growth when compared to healthy fetuses. This is further supported by evidence of progressive growth failure of the placenta occurring in the third trimester, usually a period of exponential fetal growth. Worsening F/P volume ratios in dTGA and HLHS are associated with smaller HC at birth, suggesting that fetuses with these defects may be particularly vulnerable to the impact of placental failure on brain growth in the third trimester.

P1.12

Association of preconception maternal metabolic factors with placental lipid profile, and its impact on birth weight in the NiPPeR study

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Objectives

Placental lipid metabolism regulates maternal-fetal transfer of lipids, which act as building blocks and bioactive signals critical for fetal growth and development. Placental lipids are influenced by maternal metabolic status during pregnancy. However, the influence of preconception maternal metabolic health, which could impact early placental programming, is poorly understood. We hypothesised that preconception maternal metabolic factors influence placental lipid supply and metabolism, as reflected in term placental lipid profiles, with consequent impacts on birthweight, and that these relationships are modifiable by a nutritional intervention starting preconception.

Methods

Term placentas were collected from 383 NiPPeR trial participants, randomised to either a standard micronutrient control supplement or an intervention supplement (myo-inositol, probiotics, additional micronutrients) taken from preconception to delivery. Placental lipid profiles were assessed using targeted liquid-chromatography-mass-spectrometry and clustered by Weighted-Correlation-Network-Analysis into 16 lipid clusters. Multiple linear regression examined associations between (1) preconception plasma metabolic factors and placental lipid clusters, (2) placental lipid clusters and birthweight centile (gestational-age and sex-standardised) following stratification into control and intervention groups, adjusting for key covariates.

Results

Among controls, preconception fasting glucose, total triglycerides and lipoprotein(a) each associated with several placental lipid clusters, independently of maternal BMI. Following mutual adjustments of these factors, preconception triglycerides remained positively associated with clusters containing acylcarnitines, ceramides, fatty acids, lysophospholipids, phosphatidylcholines, sphingomyelins, triacylglycerols; while preconception lipoprotein(a) negatively associated with clusters containing ceramides, lysophospholipids, phospholipids, sphingomyelins. Additionally, lysophospholipid-containing clusters associated most strongly with birthweight centile and partly mediated the link between

preconception triglycerides/lipoprotein(a) and birthweight. These associations either attenuated or reversed in the intervention group.

Conclusion

Preconception maternal plasma total triglycerides and lipoprotein(a) influence placental lipid profiles, including increased lysophospholipids, which in turn associate with higher birthweight. Nutritional supplementation starting preconception may moderate links between suboptimal maternal metabolic status and dysregulated offspring growth, potentially breaking intergenerational metabolic disease risk transmission.

P1.13

A global approach to advancing prediction of preeclampsia pathogenesis

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Objectives

Preeclampsia, a pregnancy-related complication, is a major cause of maternal and fetal morbidity and mortality. Currently, there are no precise methods to predict, prevent, or treat the disease.

Multi-omics methods such as single-cell RNA-sequencing or spatial transcriptomics and proteomics can be used to study and understand preeclampsia pathophysiology. These methods enable the identification and validation of biomarkers leading to early detection and ultimately therapy of preeclampsia. Recently published results from these so-called multi-omics studies point to the histone acetyltransferase P300 and its activation possibly leads to placental senescence profiles and ultimately to preeclampsia.

Methods

BeWo cells were used as a model for cytotrophoblasts and syncytiotrophoblasts. These were cultured for 15 passages in cell culture medium with physiological glucose concentration (5.5 mM) instead of being cultured in standard cell culture medium (with 17.1 mM glucose concentration). DMSO was used as a negative control, while BeWo cells were differentiated into syncytiotrophoblasts with the cAMP agonist forskolin. They were then treated with CTB (P300 agonist).

Additionally, first trimester placental explants were treated with CTB and DMSO as control for 24 hours. P300 protein was stained performing immunofluorescence staining and PAI1 was stained with immunohistochemistry.

Results

It was shown that treatment of the BeWo cell line with p300 agonist CTB led to a decrease in mitochondrial activity just like BAM15. In addition, a lower cell viability could be detected after treatment with CTB. Senescence markers elevated from single nucleus RNA-seq multi-omics studies in placental tissue and maternal serum from preeclamptic women were elevated at the RNA expression level.

Additionally, treatment of first trimester placental explants with CTB confirmed the activation of P300 and an increase in the senescence marker PAI1.

Conclusion

P300 may play a role in the pathophysiology of preeclampsia, causing premature senescence of trophoblasts, similar to that of preeclampsia.

P1.14

Swine trophoblast organoids as a robust, physiologic model of porcine placental development

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Objectives

Although all eutherian mammals have a placenta and trophoblasts, this organ has evolved independently, leading to divergent placental structures between species. For example, while the human placenta is highly invasive, characterized by trophoblast cells eroding the maternal endometrium and blood vessels to access to the maternal circulation, the porcine placenta attaches to the endometrium, never fully invading maternal tissue. Although human placentation has been extensively studied, research in non-human placentae has been relatively limited due to the lack of reproducible, scalable models. Here we sought to develop and characterize a novel organoid model of porcine placentation.

Methods

We derived swine trophoblast organoids (sTO) from term porcine placentae (n = 5 independent). Expressional analyses were performed using both bulk RNAseq and single cell RNAseq from 3 independent sTO lines. Single cell data was further integrated with Visium spatial transcriptomics of mid-gestation porcine placentae and predictive scoring was used to assign sTO cellular identities.

Results

RNA-seq revealed that sTO express many canonical trophoblast markers including Cytokeratin-18, HAND1, PEG1, pregnancy-stimulated-genes, and 3b-HSD, the latter of which was confirmed via progesterone ELISA. Spatial transcriptomics identified numerous, novel markers of the porcine uterus and placenta, which allow for highly specific separation of histological structures at the maternal-fetal interface. Using predictive scoring of the integrated single cell and spatial transcriptomics data, we identified unique gene signatures of the porcine maternal-fetal interface and placental areola that are also present in sTO cell populations.

Conclusion

Our data suggest that sTO spontaneously differentiate into various cell populations within the porcine placenta making them a valuable model for the study of porcine placentation. Further, our data represent a resource for the study of porcine placental development as they represent a spatially refined whole transcriptomic dataset of the porcine maternal-fetal interface *in situ*.

P1.15

Correlative three-dimensional X-ray histology (3D-XRH) as a tool for quantifying mammalian placental structure

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Objectives

Mammalian placentas exhibit unparalleled structural diversity, despite sharing a common ancestor and principal functions. The bulk of anatomical studies in placental research has used two-dimensional (2D) histology sectioning, allowing differential staining/labelling of tissue features and permitting significant advances in our understanding of mammalian placental structure. However, 2D histology sectioning may be limited if it does not provide accurate information of three-dimensional (3D) tissue architecture. Our objectives were therefore to employ a workflow to retain 3D tissue architecture information prior to 2D histology for placental tissue, and use this to quantify mammalian tissue architecture.

Methods

We used 3D-XRH, which involves scanning a formaldehyde-fixed, paraffin embedded (FFPE) tissue block with 3D X-ray microscopy (microCT) prior to histological sectioning to generate a 3D image volume of the embedded tissue piece. The subsequent 2D histology sections can then be correlated back into the microCT image volume to couple histology staining (or immunolabelling) with 3D tissue architecture. 3D-XRH is non-destructive and requires no additional sample preparation than standard FFPE histology sectioning, however the image volume provides 3D morphometric data and can be used to guide microtomy. As such, 3D-XRH introduces additional information to standard histological workflows with minimal effort or disruption.

Results

Using 3D-XRH, we were able to 3D reconstruct and morphometrically quantify blood vessels in porcine placenta, maternal septa in bovine placenta, chorionic villi in equine placenta, and the tissue layers of a canine placenta, all while retaining 2D histological H&E or immunostaining information.

Conclusion

Here, we propose correlative 3D X-ray histology (3D-XRH) as a tool with great potential for resolving mammalian placental structures. The wealth of information derived from 2D histological sectioning in the biomedical, veterinary, and comparative reproductive sciences provides a rich foundation from which 3D-XRH can build on to advance the study of placental structure and function.

P1.16

Role of peripheral serotonin on placental development.

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Objectives

In loving memory of Francine Côté

Serotonin (5-HT) is a well-known neurotransmitter in the central nervous system (CNS), yet only 5% of the body's 5-HT is found in the brain, where it is synthetized by tryptophan hydroxylase (Tph) 2. Most of the 5-HT (~95%), peripheral 5-HT, is synthesized by Tph1 in the gastrointestinal tract.

Our team has developed a mouse model deficient in peripheral 5-HT: Tph1 KO mice. This model has highlighted a large number of functions of peripheral 5-HT, notably in iron homeostasis, in erythropoiesis or in osteoclast differentiation.

In 2007, Francine Côté showed that maternal peripheral 5-HT is essential for proper embryo development. We aim to investigate whether peripheral 5-HT influences placental development and how this may impact embryonic development.

Methods

We compared peripheral 5-HT-deficient Tph1 KO mice with control (WT) mice at different days of gestation (embryonic days E11.5, E12.5, E13.5). We analyzed their phenotype and performed RNA sequencing on WT and Tph1 KO placentas.

Results

Compared with WT embryos, Tph1 KO embryos were significantly smaller and presented a delay in development. We observed more embryonic necrosis in Tph1 KO mice. Some placentas engulfed their embryos, a phenotype observed exclusively in Tph1 KO mice. Macroscopically, placentas did not differ between Tph1 KO and WT mice. However, histologically, we saw alterations of the junctional zone in Tph1 KO placentas, compared to WT. There was a decreased expression of junctional zone-specific genes in Tph1 KO placentas, and also of labyrinth zone-specific genes only at E13.5, compared to WT.

Using RNA seq analysis, we explored several possible causes for these alterations in Tph1 KO placentas. Among the hypotheses tested, our preliminary results suggest immune dysregulation and T cell activation in Tph1 KO placentas.

Conclusion

Overall, our data suggest that lack of maternal peripheral 5-HT leads to altered placental development, possibly linked to immune dysregulation.

P1.17

Differential decidual CD8+ memory T cell responses in spontaneous labor versus induction of labor at term

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Objectives

This prospective study aimed to investigate the differential CD8+ memory T cell responses in the decidua of nulliparous women experiencing spontaneous labor (SL) compared to those undergoing induction of labor (IOL) at term.

Methods

Decidua basalis and parietalis samples were collected postpartum from nulliparous women who underwent either SL or IOL at term. Multicolor flow cytometry was employed to assess seven CD8+ memory T cell types, including central memory (CM), effector memory (EM), terminally differentiated effector memory (TEMRA), tissue-resident memory (TRM), follicular helper memory (FHM), stem cell memory cells (SCM), and the expression of co-stimulatory molecules CD28 and CD27, as well as co-inhibitory molecules TIM-3 and PD-1. CD8+ memory T cell activation status was evaluated through CD69 expression. Statistical analysis included the Mann-Whitney U test, and FlowSom analysis was conducted.

Results

Cross-sectional analysis revealed higher levels of CM CD69 in the decidua basalis of the IOL group compared to the SL group ($p=0.04$). Conversely, FHM PD-1 expression was elevated in the SL group ($p=0.001$), along with higher SCM TIM-3 expression ($p=0.005$). In the decidua parietalis, SCM TIM-3 expression was higher in the SL group ($p=0.03$), and FHM PD-1 expression was also higher in the SL group ($p=0.0004$). No differences between study groups in other investigated parameters have been revealed.

Conclusion

These findings suggest differential CD8+ memory T cell responses in the decidua between spontaneous labor and induction of labor at term. Understanding these immunological differences may provide insights into the mechanisms underlying parturition and could potentially inform strategies for managing labor induction.

P1.18

Understanding the morpho-functional features of embryo-maternal communication in canine placenta through in vivo and in vitro studies; implications for canine placenta as a translational model.

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Objectives

Deriving from convergent evolution, the canine endotheliochorial placenta exhibits various species-specific morpho-functional features, including the formation of decidual cells situated closely to maternal vessels. Dogs have a shallow/restricted trophoblast invasion, unlike the more invasive haemochorial placentation in humans, which makes them an interesting model for investigating the modulation of trophoblast invasion driven by the decidua. Decidual cells arise from the mesenchymal-epithelial transition of maternal stromal cells, constituting the sole cellular population in the canine placenta expressing the nuclear progesterone receptor (PGR). Interfering with PGR signaling during normal or antigestagen-induced parturition triggers the luteolytic cascade, inducing PGF2a release from the trophoblast, and leading to luteolysis and placentolysis. Our research focuses on comprehending the underlying feto-maternal communication for targeted control of pregnancy outcomes in dogs. Additionally, it lays the groundwork for utilizing canine decidualization as a potential model for translational studies on decidual-derived modulation of trophoblast function.

Methods

We integrated morphological, functional, and molecular-biological aspects of canine decidualization and feto-maternal interaction. In vivo assessments encompassed histological, immunohistochemical, and electron microscopic (TEM) observations. Observations from functional omics studies are implied.

Results

Using TEM-based 3D modeling, we identified a canine decidual cells layer tightly surrounding maternal vessels, contrasting with the decidua tissue layer observed in more invasive placentae. A reduction in PGR signaling during late pregnancy, linked to a decrease in the number of decidual cells is suggested, accompanied by an increase in local placental cortisol availability regulated at the fetal level. The potential of the endotheliochorial model of placentation for studying pathological shallow invasion in more invasive placentae is implied. Possible factors include endothelins, thrombospondins, selectins, or FLRT1/sFLT1.

Conclusion

Our research offers a comprehensive insight into the significance of P4/PGR signaling in canine decidualization. Several factors prevalent in canine-specific decidualization and shallow trophoblast invasion have previously been linked to preeclampsia.

P1.19

Comprehensive Analysis of decidua in ectopic pregnancies reveals role of ATG2A

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Objectives

Ectopic pregnancy (EP) is one of major an early pregnancy complication. It has been reported that the endometrium of an ectopic pregnancy has no response between the embryo and the endometrium at the time of implantation, resulting in impaired decidualization.

The present investigation involved a thorough RNA-seq analysis of decidualized stromal cells in normal and ectopic pregnancies. The results indicated a decrease in the expression of autophagy-related 2A (ATG2A) in decidualized stromal cells in ectopic pregnancies.

We examined the possibility of ATG2A's involvement in the primary endometrial cell (ESC) decidualization.

Methods

Ten patients were enrolled for RNA-seq analysis. Five of the patients had an intrauterine pregnancy (IUP) as the control group, and the other five had a tubal EP. All were drug-free, spontaneous conceptions. Additionally, the ESC specimens who had undergone total hysterectomy for benign gynecological diseases were used to investigate the role of ATG2A in decidualization.

ATG2A expression has been assessed using immunohistochemical analysis. To examine the effect of ATG2A knockdown on decidualization in vitro, ESCs were transfected with ATG2A siRNA and differentiated into decidua using P4 and dibutyryl-cAMP (P4/db-cAMP) to evaluate decidua of markers.

Results

ATG2A expression in endometrial stromal cells and epithelial cells was detected by immunohistochemical analysis during the menstrual cycle.

ATG2A knockdown cells showed reduced expression of decidua markers, prolactin, IGFBP1, FOXO1, and HAND2 in comparison to control cells.

Conclusion

Knockdown of ATG2A inhibited P4/cAMP-induced decidualization, suggesting that ATG2A is involved in the pathogenesis of ectopic pregnancy and in physiological decidualization for pregnancy.

P1.20

Co-culturing of endometrial cells to study the physiology of the uterus and the process of embryo implantation

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Objectives

This study aims to explore the potential of using human endometrial 3D-cell coculture to investigate reproductive mechanisms.

Methods

Endometrial biopsies (n=3; CAAE 31433520.1.0000.5467) were digested in DMEM-collagenase II (0.1 mg/mL)-DNAse I (0.05 mg/mL). The gland fragments were collected after filtering; some were redigested, expanded, and characterized. Endothelial cells were selected from the cells that passed through the filter using a column with CD105 magnetic beads. The remaining cells were considered stromal cells. Cultures proceeded using DMEM/F12 medium (20% FCS, antibiotics, 17β estradiol 10 nM, progesterone 0.01µg/mL) at 37°C and 5% CO_2. Stromal cells (1 x 10^5 cells) were combined with an ECM mixture (fibronectin/collagens) and placed on the intermediate filter of an 8-µm pore size insert. The glands or isolated epithelial and endothelial cells were plated at the surface of the gelled ECM. Gland cells were cultured with chorionic gonadotropin (hCG 0.1 ng/mL). Epithelial cell-ECM cultures received trophectoderm from aneuploid blastocysts. The endothelial cell layer-ECM was treated with DMEM/F12 -10% serum from endometriotic women, replacing FCS. After 48 hours, all samples were fixed in 2.5% glutaraldehyde or 2% paraformaldehyde for morphology and immunohistochemistry reactions.

Results

Fibroblasts/decidual cells show health morphology in the ECM, and mitoses are seen. The morphology of the gland fragments was preserved. Epithelial and endothelial cells form a lining layer over the ECM. Under hCG treatment, gland cells significantly increase MIF and VEGF immunostaining levels, suggesting a protagonist role in the maternal-fetal dialogue. Trophectoderm shows adherence to the endometrial epithelial layer. Endometrial cells respond to endometriotic serum, possibly through the interaction of endothelial-stromal cells.

Conclusion

Our results highlight an in vitro model that allows us to use uterine cells organized in tissue-like configuration to study the endometrial physiology and embryo implantation signaling in an interactive cellular 3-D network. **Supported** by FAPESP, CAPES and CNPq

P1.21

IFNγ mediated effects of decidual stromal cells in villitis of unknown etiology

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Objectives

Villitis of unknown etiology (VUE) is an inflammatory placental pathology characterized by maternal CD8+ T cell infiltration into the villous stroma. Decidual stromal cells (DSC) interact with immune cells at the maternal fetal interface to promote tolerance during pregnancy; however, their role in VUE is unknown. This study aimed to investigate how VUE inflammation affects DSC function and phenotype.

Methods

4 high grade VUE and 4 gestationally age matched control placentae underwent whole transcriptome analysis using Digital Spatial Profiling technology (NanoString). Formalin fixed paraffin embedded tissues were sectioned, stained with fluorescent antibodies against Vimentin (DSCs), Cytokeratin 7 (trophoblasts), and CD45 (immune cells), alongside RNA probes for Illumina sequencing and downstream gene/pathway analysis. Human endometrial stromal cells were decidualized for 7 days using 10 nM β2-estradiol, 1 µM medroxyprogesterone acetate, and 500 µM 8-bromo cyclic AMP. Decidualization was confirmed by prolactin (PRL) secretion via Elisa. On day 5 and day 7, DSCs were treated with interferon gamma (IFNg) for 48 hrs and PRL and CXCL10 secretion were assessed via ELISA to evaluate inflammation's impact on DSC secretion.

Results

Pathway analysis revealed significant upregulation of IFNg signaling in VUE DSCs (p=7.94E-16), and our top differentially expressed genes are induced by IFNg signaling, including Beta-2-microglobulin, HLA-A, HLA-C, HLA-B, C1QB, IFI30, and STAT1. Exogenous IFNg treatment on days 5 and 7 of decidualization increased T cell attracting chemokine CXCL10 and maintained normal levels of PRL.

Conclusion

While villous tissue inflammation is central to VUE, our findings suggest an important yet weakly definde role for DSCs in VUE immuno-pathophysiology. IFNg signaling in DSCs is significantly upregulated during VUE, and exogenous IFNg treatment leads to CXCL10 upregulation, possibly promoting heightened T cell trafficking to the maternal fetal interface. Together, our data indicates that aberrant IFNg signaling via DSCs could contribute to VUE immunopathogenesis.

P1.22

Effects of BaP-coated CeO_2 nanoparticles on the human placenta: involvement of the AhR pathway

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Objectives

Epidemiological studies have established a correlation between maternal exposure to air pollution and adverse pregnancy outcomes. Indeed, various xenobiotics in maternal blood can cause functional disruption of the human placenta. Cerium dioxide nanoparticles (CeO_2 NP) are among emerging air pollutants found in maternal blood. Their use in diesel fuels and cigarettes due to their catalytic properties, has raised concerns about their impact on human health, particularly when combined with other pollutants from common emission sources such as polycyclic aromatic hydrocarbons (PAH). Benzo-a-pyrene (BaP), a prototypical PAH defined as a reprotoxic mutagenic carcinogen as well as an endocrine disruptor, is metabolized and bioactivated via the aryl hydrocarbon receptor (AhR) pathway. This pathway contributes to the harmful cellular toxicity of many pollutants, but is also involved in the regulation of physiological and detoxication processes. This study aimed to investigate the biological impact of BaP-coated CeO_2 NP on the human placenta, focusing on AhR activation and stress signaling pathways.

Methods

BaP-coated CeO_2 NP were produced, characterized and incubated with placental villi and villous cytotrophoblasts obtained from term human placentas. Cellular uptake was confirmed by confocal and transmission electron microscopy. Cellular toxicity, trophoblast differentiation capacity, AhR activation and downstream stress signaling pathways were assessed using molecular techniques, including XRE plasmids.

Results

While there was no significant difference in cell toxicity, exposed trophoblasts displayed notable variations in AhR activity and in trophoblasts differentiation when exposed to BaP-coated CeO_2 NP compared to co or single exposures. BaP-induced stress pathways in trophoblasts were driven by activation of the AhR pathway, and co-exposure with CeO_2 NP attenuated this effect.

Conclusion

These findings highlight the modulation of the effects induced by BaP when stably coated on CeO_2 NP, potentially modifying its metabolization kinetics and thus its biopersistence, giving a closer reflection of environmental reality.

P1.23

Environmental Exposures and Metabolomics: Unraveling Impacts on Placental Inflammatory Signaling

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Objectives

Environmental/plasticizer compounds and biochemicals such as non-esterified and esterified lipid species are associated with endocrine disruption, inflammation, higher pre-pregnancy weight/obesity, fetal development, and placental inflammatory-related pathways such as STAT3 and MAPK which can regulate trophoblast amino acid transport activity. Prior research has used targeted methods to measure compounds, potentially overlooking exogenous-endogenous interrelationships. To address this gap, we used untargeted metabolomics to examine the relationship between metabolomic profiles and placental inflammatory signaling that could help identify specific pathways by which environmental and metabolic factors affect placental function.

Methods

Our analysis included 89 mother-offspring pairs from uncomplicated pregnancies in the Healthy Start cohort. From fasting serum collected at 18 weeks gestation, we measured untargeted metabolomics through liquid chromatography mass spectrometry and collected placental villus samples at delivery. Traditional western blotting was used to identify total and phosphorylated protein in placental inflammatory pathways ($STAT3^{Tyr705}$/STAT3, $JNK1^{Thr183/Tyr185}$/JNK1, $JNK2^{Thr183/Tyr185}$/JNK2, p38 MAPK, pro-caspase 1, and IL-1β). Data were processed and analyzed to identify metabolomic clusters and their associations with clinical data and placental inflammatory pathways, employing WGCNA for profiling, mummichog for enrichment, and Pearson correlation for association assessment.

Results

The analysis identified a profile of 138 compounds enriched for fatty acid activation of derivatives/analogs suggestive of arachidonic acid metabolism by enzymes in pro-inflammatory pathways. This profile correlated significantly with higher gestational weight gain and non-esterified fatty acids levels. Notably, 13% of central profile compounds were identified as plasticizers/environmental (e.g., 2-Ethylhexyl sebacate, 3-Methyl-2-cyclohexen-1-one, Diisononyl cyclohexane-1,2-dicarboxylate), and were significantly positively correlated with higher levels of placental STAT3, p38 MAPK, IL-1β (R^2 range 0.05-0.15).

Conclusion

Our findings highlight a metabolomic profile enriched with fatty acids/environmental compounds that was associated with increased placental inflammatory activity. This profile correlated with higher gestational weight gain and non-esterified fatty acids, offering insights into how environmental factors and metabolic responses interact and influence placental signaling.

P1.24

Cannabis smoke exposure alters murine placental development

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Objectives

About 5-10% of pregnant women have reported using cannabis for relief of common pregnancy symptoms. Evidence suggests that the use of cannabis during pregnancy is correlated to dysfunctional placentation and poor pregnancy outcomes, such as intrauterine growth restriction (IUGR). The bioactive components of cannabis, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), have been shown to impact trophoblast differentiation. Despite the fact that smoking is the most popular mode of prenatal cannabis consumption, the majority of existing research has only examined the individual impacts of injected cannabinoids on placental development and function in vivo. Therefore, we sought to characterize the impacts of cannabis cigarette smoke on placental development and function using a murine smoke exposure model. ***We hypothesize that cannabis smoke exposure adversely impacts placentation and function.***

Methods

Pregnant outbred CD1 mice were exposed to smoke from cannabis cigarettes (14% THC: 1% CBD) for 30 min/day between E6.5 to E18.5. Food intake and body weights were recorded during pregnancy. Gene expression and protein expression were assessed in placentae at E18.5.

Results

While fetal body weights were unchanged, placental weights were decreased. We observed a 0.5-fold decrease in transcripts of both ectoplacental cone cell *(Tfap2c)* and spongiotrophoblast cell (*Tpbpa)* markers, as well as the angiogenesis marker (*Vegf)*. Glycogen trophoblast cell marker (*Pcdh12*) was decreased by 25%, whereas trophoblast giant cell markers (*Ctsq*, *Pl2)* were decreased by 25% and 75%, respectively, suggesting altered placental development. Moreover, mRNA expression of mitochondrial transcription factor (*Tfam)* exhibited a 2-fold increase with cannabis exposure. Finally, we demonstrated decreased protein expression of the lipid peroxidation marker, 4HNE, with no changes to SOD1 protein expression in cannabis-exposed placentas.

Conclusion

Overall, our results suggest that cannabis-induced cellular stress is associated with altered placental differentiation. This may have implications for placental function and fetal development.

P1.25

Vasoactivity of cannabis components in human placental arteries

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Objectives

Due to increasing legalization of cannabis, its use during pregnancy is on the rise, despite its association with fetal growth restriction and potential unknown risks for offspring. Active components of cannabis like Cannabidiol (CBD) and delta-9-TetraHydroCannabinol (THC) might affect the endocannabinoid system, which consists of cannabinoid receptors 1 (CB1) and 2 (CB2), endocannabinoids, and several enzymes.

Methods

We quantified CBD- (n=9) and THC (n=3)-induced relaxation in U46619-preconstricted 3rd-order arteries from healthy human placentas using wire-myography with and without antagonists for CB1 (1M AM251) and CB2 (1M AM630). Vehicle was used as a control (n=6). Relaxant responses are expressed as percentage of U46619-preconstriction. We also explored THC-induced vasoconstriction with and without AM251 and AM630 (n=4). Constrictor responses are expressed as percentage of constriction to 100 mM KCl.

Results

CBD dilated healthy placental arteries by 36±4% (pEC_{50}:7.1±0.5). AM251 attenuated this effect to 17±6% ($P<0.05$) without altering the pEC_{50} (6.5±0.4), while AM630 altered neither CBD-induced dilation (40±7%) nor pEC_{50} (6.3±0.7). However, when combining both inhibitors, the relaxant response to CBD was virtually abolished ($P<0.05$ vs. AM251 alone). Vehicle was without effect. THC did not exert any dilator effect in the presence of vehicle or AM630. Yet, it enhanced U46619-induced constriction in the presence of AM251 by 37±11% (pEC_{50}:7.1±0.2). This constrictor effect was abolished when combining both inhibitors. In agreement with this observation, in the absence of preconstriction, THC also induced vasoconstriction in the presence of AM251 (14±6%, pEC_{50}:6.3±0.6), while no effect was seen in the presence of vehicle, AM630 or AM251+AM630. CBD did not induce constrictor effects.

Conclusion

CBD relaxes healthy human placental vessels via CB1 and CB2, while THC induces vasoconstriction in the presence of CB1 blockade, potentially via CB2. These data may help to understand potential risks of cannabis use in pregnancy.

P1.26

Placental exposure to urban air pollution leads to disrupted placental microarchitecture and Hofbauer cell polarisation towards a M1 pro-inflammatory phenotype

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Objectives

Exposure to urban air pollution during early pregnancy is associated with increased risk for adverse pregnancy outcomes, such as preeclampsia (PE). The biological mechanisms by which air pollution exposure during or prior to pregnancy contribute to the development of PE remain largely unexplored. Studies deciphering the biological mechanisms contributing to the toxicity of air pollution during pregnancy are urgently needed. Placental macrophages, or Hofbauer cells (HBCs) are a fetal population of innate immune cells present in the placenta that regulates immune tolerance. In normal pregnancy, HBCs are polarised towards an anti-inflammatory M2 phenotype, in contrast to a more pro-inflammatory M1 phenotype.

Methods

We used the placenta perfusion model to study the influence of urban traffic-derived air pollution particles of size < 2.5 µm (PM2.5) on the placenta. In addition, we investigated the PM2.5 effects on HBC polarisation. The PM2.5 were collected at a site representative of urban traffic in Malmö, Sweden. Placental tissue was evaluated with electron microscopy and immunohistochemistry was used to investigate HBC polarisation.

Results

Placentas exposed to urban PM2.5 displayed a disrupted collagen structure with loose fibroblasts, compared to control placentas with dense morphology with intact collagen and fibroblasts surrounding fetal vessels. The endothelial cells lining fetal vessels displayed swollen mitochondria with damaged cristae and cytotrophoblast showed dilated rough ER surrounding swollen mitochondria with damaged cristae. Urban PM2.5 particles could be detected inside the placental syncytium and inside HBCs. The PM2.5 exposure had no impact on CD163 expression, a resident marker of HBCs. It led to a 60% decrease in CD206 positivity, while CD209 expression remained unchanged; indicating altered M2 polarisation. Additionally, exposure to urban PM2.5 increased expression of pro-inflammatory M1 markers CD40 ($p=0.02$) and CD80 ($p=0.03$) in HBCs.

Conclusion

Exposure to PM2.5 causes disrupted placental tissue morphology and skews M2 polarisation of HBCs towards a pro-inflammatory M1 phenotype.

P1.27

Expression of the PPARG gene in the human placenta: more exons than expected.

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Objectives

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-dependent transcription factor of the nuclear receptor family. In various tissues, PPARγ controls cell differentiation, proliferation or fusion. In particular, its essential role in the development and functions of the placenta is now established. To date, ten exons of this gene have been described. The involvement of these different isoforms in the functions of the placenta and trophoblast remain unknown. In addition, international databases show a large number of gene's transcripts. As translation is mainly regulated by the 5'UTR sequences of the mature mRNA, the objectives of this study were to: 1) carry out a sequence analysis of this region on the transcripts presents in NCBI database, 2) quantify the expression of the new exons identified in the placental villi (PV) and villous cytotrophoblasts (VCT) during the formation of the syncytiotrophoblast.

Methods

The sequences of PPARG RNA transcripts included in NCBI were extracted, analyzed and their 5'UTR region was compared with each other and with data from the literature. Nine primers targeting the newly identified exons were designed and validated by sequencing. PCR and RT-qPCR analyses were performed on PL and VCTs isolated from elective caesarean section of term placenta.

Results

In silico analysis revealed four new sequences named X1, X3, X4, and X5 that had not been described previously. Analysis of their expression showed that X1 was weakly expressed by VCTs and PV. The others were consistently expressed during syncytiotrophoblast formation. Only X4 was overexpressed by the syncytiotrophoblast. The expression of X3 and X5 in PV and VCTs was not significantly different.

Conclusion

This study showed that the PPARG gene therefore consists of 14 exons and not 10 as previously described. Expression of these new exons appears to be cell type specific, indicating strong regulation of PPARγ isoform expression and specific function for these isoforms.

P1.28

Sex-influenced DNAme profiles of isolated human placental cell types

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Objectives

Sex differences in function and morphology of the human placenta can lead to sex differences in pregnancy outcomes. X-chromosome inactivation (XCI) is the primary mechanism for dosage compensation between the sexes, and in somatic cells, is strongly associated with X-chromosome promoter DNA methylation (DNAme), but in the placenta a reduction in this promoter DNAme has been reported. The placenta is a complex organ consisting of cells of different cellular origins, but the sex differences in specific cell types have not been investigated.

Methods

We investigated the sex-influenced DNAme profiles of 94 term placentas (XX=50, XY=44) including 18-19 samples each of endothelial, stromal, Hofbauer cells, cytotrophoblasts and chorionic villi from previously collected Illumina Infinium HumanMethylationEPIC arrays.

Results

Sex-stratified X/Y and autosomal chromosomes were analyzed to identify sex differences associated with sex chromosome complements. DNAme distribution of the X-chromosomes differed by cell type, reflecting differing developmental origins The XX placental cell types derived from the extraembryonic mesoderm (endothelial/stromal cells) and trophectoderm (cytotrophoblast) showed distinct DNAme distributions from each other and from Hofbauer cells, which shared a similar distribution with somatic cells. Surprisingly, the typical DNAme at promoter-associated CpG islands on the X-chromosome of XX cells was completely absent for endothelial/stromal cells and present only at low levels in cytotrophoblasts/chorionic villi, suggesting that XCI escape may occur in the absence of promoter DNAme. Sex-influenced DNAme at autosomal loci was mainly observed in endothelial cells, and included ZNF300, which has been reported to be involved in placental development and morphology.

Conclusion

We demonstrated that X-chromosome DNAm profiles in XX cells are different by cellular origins. The lack of promoter DNAm in association with XCI escape in extraembryonic-mesoderm-derived cells suggests an origin distinct from the other cell types. This work may provide insight into the sex differences and cellular origins of the human placenta.

P1.29

Differential Methylation in the Placenta Associated with Maternal Socioeconomic Status: The SPAH Study

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Objectives

Low maternal socioeconomic status (SES) is associated with adverse perinatal outcomes, including fetal growth restriction and preterm birth. It has been theorized that SES disadvantage becomes biologically embedded during the highly sensitive period of gestation; however, the cellular and molecular mechanisms that facilitate this phenomenon are still being elucidated. The placenta, which serves as the interface between the fetal and maternal environment, has been postulated to play a role in this process. DNA methylation (DNAme) is a well-studied epigenetic mark that influences gene expression. However, the effect of SES on placental DNAme remains unclear.

Methods

Illumina EPIC (850K) array data from 507 placentas was processed as part of the Stress, Pregnancy, and Health study (SPAH), a diverse cohort from Chicago, USA, which included measures of socioeconomic Prestige, Resources, and Disadvantage. Using R, extensive quality control checks were performed and data was normalized using noob/dasen combined methods. Principal component analysis was run using the R package irlba on epiphenotypical, clinical, and demographic variables. Linear models were run using the limma R package and included fetal ancestry, gestational age, fetal sex, maternal diabetes, preeclampsia, and cell composition estimates as covariates.

Results

35 CpGs were differentially methylated (FDR < 0.05, $|\Delta\beta| > 0.02$) in association with Prestige score, comprising of clinician-estimated occupational and educational prestige. With Resources score, encompassing assessment of wealth and income, no CpGs were differentially methylated. For Disadvantage score, which included access to support and disability measures, 9 CpGs were differentially methylated. The differentially methylated CpGs mapped to genes related to metabolic pathways, including drug metabolism. The potential effect of cell composition still needs to be further explored.

Conclusion

Our results suggest that some aspects of low maternal SES may be associated with altered DNAme in the placenta. However, the mechanisms mediating this association are not known and will require further investigation.

P1.30

Placental DNA Methylation Associated with Early Childhood Growth Trajectories and Obesity Risk

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Objectives

DNA methylation (DNAm) changes in birth tissues have been associated with health outcomes in offspring. Our study aimed to investigate associations of placental DNAm with early childhood growth outcomes.

Methods

Genome-wide DNAm of 681 placentas was assessed using Illumina Infinium EPIC arrays. The study examined two early childhood growth outcomes included body mass index (BMI) trajectories from birth to age 4 (high-, moderate-, and low-BMI trajectories) identified previously in the study population and overweight/obesity (OWO) at age 4 defined using the CDC growth chart. Regression models and differentially methylated region (DMR) analysis with adjustment for confounders were conducted to identify individual CpG sites and DMRs associated with the outcomes, respectively. The false discovery rate (FDR) method was used to adjust for multiple testing.

Results

No individual CpG sites were significantly associated with the growth outcomes of interest after adjusting for multiple testing (all FDR > 0.05). However, we identified 109 DMRs consisting of 693 CpG sites and 167 DMRs consisting of 861 CpG sites significantly associated with BMI trajectories and OWO at age 4, respectively (FDR < 0.05 and mean difference > 2%). Five of these DMRs were shared by both growth outcomes, containing 42 CpG sites. These CpG sites are mainly located in or near five genes, *MNX1*, *CDX2*, *GFPT2*, *ZBTB46-AS1* and *GDA*. Three of these genes are mapped to biological pathways that are potentially related to obesity, such as the pathways maturity-onset diabetes of the young (*MNX1*), alanine aspartate and glutamate metabolism (*GFPT2*), and purine metabolism (*GDA*).

Conclusion

We identified placental DMRs associated with early childhood growth outcomes, further supporting the intrauterine origin of obesity. Future studies are warranted to identify prenatal exposures causing these epigenetic changes and the biological mechanisms for their impacts on postnatal growth.

P1.31

Arginine Methyltransferase PRMT1 Equipoises Epigenetic Balance in Trophoblast Progenitors to Prevent Early Pregnancy Loss

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Objectives

1-3% of all implantation-confirmed conceptions suffer from idiopathic recurrent pregnancy loss (RPL). Idiopathic RPLs are devastating to patients and a better understanding of underlying mechanisms is necessary for better diagnosis and treatment. Here, using Idiopathic RPL tissues, patient-specific human trophoblast stem cells (human TSCs), and *Prmt1*-mutant mice, we tested the importance of PRMT1 in mammalian trophoblast development and progression of pregnancy.

Methods

We studied PRMT1 expression in placental tissues from pregnancies associated with idiopathic RPL and established patient specific human TSCs to define correlation of PRMT1 loss with idiopathic RPL. We performed loss-of-PRMT1 function studies in human TSCs and developing mouse conceptuses to define the importance of PRMT1 in trophoblast development. For mechanistic analyses we performed Cleavage Under Targets and Release Using Nuclease (CUT&RUN)-sequencing to identify PRMT1 targets genes in trophoblast progenitors. We tested chromatin features including histone H4 arginine 3-asymmetric di-methylation (H4R3Me2a) on the chromatin loci of PRMT1 target genes.

Results

We show that a subset of idiopathic RPLs is associated with strong reduction of PRMT1 expression in trophoblast progenitors. Using patient-specific and PRMT1-depleted human TSCs, we provide evidence that PRMT1 shapes the gene expression program in trophoblast progenitors to ensure their self-renewal and to prevent premature differentiation. CUT&RUN-sequencing in primary mouse and human trophoblast progenitors revealed an underlying mechanism in which PRMT1 directly regulates transcription of a conserved set genes through H4R3Me2a modification, thereby orchestrating cell-type specific transcriptome. Finally, using Prmt1-mutant mice we show that trophoblast-progenitor specific function of PRMT1 is essential for developmental progression beyond placenta primordial stage.

Conclusion

We provide evidence that PRMT1 is an epigenetic governor to orchestrate mammalian trophoblast development and implicate loss-of-PRMT1 function as a molecular cause in early pregnancy loss.

P1.32

16p11.2 Microdeletion Induces Sex-Specific Defects in Placental Development in Mice

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Objectives

Neurodevelopmental disorders (NDDs) are extremely common. The prenatal environment significantly influences NDD risk which may include how NDD risk genes affect placental function. However, placental disruptions in mouse models of NDD risk genes remain unexplored. Our objective was to investigate placental development and function in the 16p11.2 microdeletion model (16p del). 16p del in humans is associated with NDDs including autism spectrum disorder, attention deficit hyperactivity disorder, and intellectual disability. Mice modeling this deletion display sex-specific behavioral phenotypes relevant to NDDs, mirroring the male bias observed in human NDDs. We hypothesized that 16p del would alter placental growth and function with more severe phenotypes observed in male placentas.

Methods

To test this, wildtype female mice were bred with hemizygous 16p del males, and placental weight, fetal weight, placental morphology, and placental gene expression were assessed.

Results

Male 16p del placental mass was increased at embryonic day 16 (E16) and E18 compared to litter-matched wildtypes; females showed no group differences. This increased 16p del male placental weight may have been related to increased E16 decidual proportion we found in 16p del males, a phenotype typically associated with reduced fetal growth. Indeed, both male and female 16p del embryos had reduced body weight at E14 and E16, but females recovered body weight to wildtype levels on E18. There were no group differences in E16 female placental subregion morphology. Unexpectedly, E16 16p del male placentas exhibited comparable expression to wildtypes in two of three 16p11.2 genes tested. E16 16p del female placentas, on the other hand, showed the expected 50% expression level in all three genes. E16 16p del male placentas also had increased expression of growth factors *Igf-1* and *Vegf*.

Conclusion

The sex-specific placental abnormalities in 16p del mice likely impact postnatal development and may contribute to early developmental origins of male bias in NDDs.

P1.33

Circular RNAs (circRNAs) cause DNA damage and characterise accelerated ageing in stillbirth placenta

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Objectives

We aimed to determine whether circ_A (identity commercial in confidence) accelerates placental ageing by causing DNA damage, using placenta from 37-41+ weeks' gestation and stillbirth.

Methods

Placenta samples (n=26 term uncomplicated; n=3 unexplained stillbirth, 23, 26 and 34 weeks' gestation) were assessed for DNA damage using an alkaline Comet Assay. Expression levels of circ_A, and its linear transcript gene_A, were quantified using qPCR. Physical interaction of circ_A with DNA was confirmed by DNA:RNA ImmunoPrecipitation (DRIP). The effect of circ_A knockdown in HEK293T cells was assessed following transfection with either a siRNA (designed to knockdown circ_A) or a scrambled siRNA control, both at 5, 10 and 20 nM final concentrations using Lipofectamine RNAiMax. DNA damage was then assessed by Comet Assay. Statistical analyses were undertaken using SPSS Statistics Software.

Results

Compared with earlier gestations (37, 38, 39 and 40 weeks'), DNA damage was increased in placentae from 40 (p_{37}=0.0381, p_{38}=0.0204) and 41+ (p_{37}=0.0103, p_{38}<0.0001, p_{39}=0.0017, p_{40}=0.0125) weeks' gestation, and in stillbirth ($p_{37,38,39,40}$<0.0001).

Compared with earlier term gestations, expression of circ_A, but not its linear transcript, was increased in placentae from 40 (p_{37}=0.0339, p_{38}=0.0352) and 41+ (p_{37}=0.0019, p_{38}=0.0012, p_{39}=0.0272) weeks' gestation, and in stillbirth ($p_{37,38,39,40}$<0.0001).

DRIP confirmed that the circ_A locus binds to DNA in term placenta. The DRIP-qPCR signal for circ_A was significantly larger in term placenta (0.173 vs 0.025; p=0.0073) than in enzyme-treated internal controls.

Depletion of circ_A by circ_A-specific siRNA in HEK293T cells significantly reduced DNA damage compared to control (for all siRNA concentrations, p>0.0001).

Conclusion

Stillbirth placentae show signs of accelerated ageing. Levels of circ_A prematurely accumulate in stillbirth placentae at a rate consistent with older gestation tissue. Importantly, circ_A binds to DNA and can cause DNA breaks in placenta. Therefore, circ_A plays a role in placental ageing and associates with stillbirth, likely by causing DNA damage.

P1.34

Development of a core outcome set for studies on placenta accreta spectrum disorder (COPAS): design and execution of a Delphi survey

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Objectives

There is little consistency in how outcomes are reported and measured across screening and treatment studies of placenta accreta spectrum (PAS) disorder. The rarity and varied reporting of outcomes in the literature have hindered meaningful synthesis and meta-analysis, essential for guiding practical applications and future research. Our objective is to develop a core outcome set for studies on placenta accreta spectrum disorder (COPAS).

Methods

The development of a COS involves five steps with this study focusing on the third step, an online Delphi survey. A list of 39 outcomes generated through a systematic review (Step-1) and interviews with health care providers (HCPs) and health service users (HSUs) (Step-2) condensed by removing redundancies, was entered into Surveylet (Calibrum, Inc., Utah). To ensure global participation, a multi-modal recruitment strategy utilized social media platforms, personal contacts, direct emails to HSUs and HCPs, and engagement with international PAS organizations.

Results

Recruitment efforts are ongoing; however, based on precedents set by similar COS initiatives, it is anticipated that a minimum of 100 HCPs and HSUs will complete this two-round Delphi survey. Consensus classification will be defined as 'consensus in' for inclusion in COPAS if >70% scoring 7–9 and <15% scoring 1–3; 'consensus out' for exclusion will be defined as >70% scoring 1–3 and <15% scoring 7–9; and 'no consensus' will be defined as those that do not meet either threshold for critical or limited importance outcomes. Outcomes labeled 'no consensus' will be carried forward to the next stage of COPAS development.

Conclusion

The development of a COS for studies focused on PAS is currently in progress. Once completed, COPAS will enable harmonization of outcome reporting and measurement, facilitating appropriate meta-analyses and the drawing of meaningful conclusions to inform clinical practice and health policy.

P1.35

Identifying patient-relevant outcomes for placenta accreta spectrum (PAS) disorder: a qualitative study to guide the development of a core outcome set

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Objectives

Patient perspectives on critical clinical outcomes related to placenta accreta spectrum (PAS) disorder are often overlooked in research trials. Our objective was to identify patient-reported outcomes (PROs) through interviews with individuals who have experienced PAS disorder and elucidate outcomes most important to them, to guide the development of a core outcome set for PAS disorder (COPAS).

Methods

A qualitative study using purposive sampling was conducted with patients who had previously experienced PAS disorder in pregnancy. Semi-structured interviews were conducted over the phone, audio-recorded, and transcribed verbatim. A descriptive interpretive data analysis was conducted using both inductive and deductive approaches.

Results

Between September 2020 and October 2021, 17 individuals who had experienced PAS disorder in a prior pregnancy were interviewed. Participants, aged 26-43, were from the United States (n=14) and Canada (n=3), identifying predominantly as White (88%), with smaller proportions as Hispanic (6%) and Indian (6%). From these interviews, 263 responses were obtained, which underwent item reduction, leading to the identification of five unique outcomes previously not reported in the literature. These included: 1) loss of the "birth experience" due to surgery starting under general anesthesia, 2) delay in initiation of breastfeeding and/or inability to breastfeed, 3) impact on relationships (newborn baby, partner, or other children), 4) Long-term adverse physical implications from surgery including longstanding pelvic floor pain, or sexual dysfunction, 5) psychological impact from the PAS or intervention (hysterectomy).

Conclusion

This study identified patient-centered outcomes rarely addressed in the PAS disorder literature. The integration of the concerns and preferences of patients and their families is vital for guiding future PAS disorder research and effective clinical care as it enhances patient satisfaction and overall outcomes. The findings from this study by informing the development of COPAS, will ensure that PROs are considered in future clinical research and care.

P1.36

Human preeclamptic placental extracellular vesicles exacerbate long-term cardiovascular disease in a rat model.

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Objectives

Preeclampsia, a disease of human pregnancy, usually presents as new onset hypertension after 20 weeks' gestation accompanied by signs of maternal organ damage or fetal compromise. It is not known what causes preeclampsia but a toxin released from the placenta triggers the condition. Removal of the placenta leads to rapid resolution of the signs of preeclampsia. However, women have a 2-fold increased risk of early cardiovascular mortality and morbidity after a pregnancy complicated by preeclampsia. The cause of this increased risk is unknown but it is possible that the toxin that triggers preeclampsia is also responsible for the increased cardiovascular disease risk.

Placental extracellular vesicles are lipid-enclosed packages of cellular contents derived mainly from the syncytiotrophoblast that are known to affect the function of maternal cells/organs.

Methods

Placental macro, micro and nano EVs were enriched by differential centrifugation of conditioned media from human term placental explant cultures from pregnancies complicated by early- (EOPE, n=9) or late-onset preeclampsia (LOPE n=10) or normotensive pregnancies (n=10). The EVs were combined in a physiological ratio and administered i.v. to 14 week-old spontaneously hypertensive female rats (SHR). Systolic blood pressure (SBP) and cardiovascular function were monitored for 12 months after administration via tail-cuff and high-frequency ultrasound.

Results

Injections of either EOPE or LOPE EVs significantly elevated SBP (normalised to baseline) in SHRs compared to EVs from normal pregnancies. This effect persisted for 9 months ($p=0.03$, 42.52±22.76 vs 20.28±20.83 mmHg) for LOPE EVs and 12-months for the EOPE EVs ($p=0.003$, 62.18±20.99, vs 35.26±14.51 mmHg).

Conclusion

Placental EVs from either EOPE or LOPE induced long term changes in the maternal cardiovascular system and exacerbated the cardiovascular damage naturally occurring in SHRs. This work suggests that preeclamptic placental EVs may be a placental toxin that can induce the long-term sequalae experienced after a preeclamptic pregnancy.

P1.37

Associations between hypercaloric diet and caffeine intake and their implications on pregnancy outcomes in a murine model

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Objectives

The objective was to evaluate the effects of chronic maternal consumption of a hypercaloric diet, associated with caffeine intake during pregnancy on placental morphology and fetal biometrics.

Methods

Were used (32) female Swiss mice allocated into four experimental groups: normocaloric diet, without caffeine administration (CC); normocaloric diet and supplementation of 120mg/kg/day of caffeine (C120); hypercaloric diet without caffeine (TC); hypercaloric diet and supplementation of 120mg/kg/day of caffeine (T120). The animals received the respective diets for six weeks and the caffeine supplementation was done by gavage for two weeks, before the mating and throughout the gestation. Pregnant females were euthanized in the gestational day 17.5, maternal blood was collected and the placenta and fetus were dissected for biometric and morphological evaluations. Statistical test: two-way ANOVA ($p<0.05$).

Results

The results point to a metabolic syndrome in TC and T120, which besides presenting obesity, showed hyperglycemia, and insulin resistance compered to CC ($P<0.05$). The C120 and T120 had higher levels of triglycerides and total cholesterol compered to CC ($P<0.05$). The TC had higher levels of total cholesterol, HDL and LDL cholesterol compered to CC. Moreover, TC, C120 and T120 had reduced fetal, liver and placenta weight compered to CC and C120 and T120 had reduced fetal and placenta length ($P<0.05$). Only T120 had increased brain/liver ratio compered to CC ($P<0.05$). These findings were more severe in female fetuses compered to males ($P<0.01$). The placentas from T120, TC and T120 had structural changes in the maternal-fetal interface, with increase in the height of the interhemal membrane compered to CC ($P<0.05$).

Conclusion

Taken together, the findings of the present study demonstrate that the metabolic alterations resulting either from the consumption of a hypercaloric diet, or caffeine, or the association of both, promote placental morphological alterations compromising fetal development, especially in females, predisposing these individuals to future metabolic complications.

P1.38

Effect of high-fat diet and viral infection during pregnancy on placental morphometry, cell turnover, lipid peroxidation, & fetoplacental growth

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Objectives

Obesity and infection have independent effects on the placenta, yet little is known about their cumulative impacts on placental oxidative stress and fetoplacental development. We aimed to assess placental morphometry, cell turnover, lipid peroxidation, and fetoplacental anthropometry, in response to maternal obesity and infection during pregnancy. We hypothesised that co-exposure would have greatest impact on fetoplacental outcomes.

Methods

Female C57BL/6J mice were exposed to 60% high fat diet (HF) or control diet (CON) six weeks before and throughout pregnancy and injected with Poly(I:C) (viral mimic), or saline vehicle 24hr before sample collection (CON-VEH, CON-POLY, HF-VEH, HF-POLY; n=5-8/group) at gestational days (GD)12.5, 15.5 and 18.5. Placental expression and localization of lipid peroxidation (4-hydroxynonenal [4-HNE]), cell proliferation (Ki67) and cell death (Caspase-3) markers were assessed (immunohistochemistry). Cell turnover (Ki67/Caspase3) was determined. Placental labyrinth (LZ) and junctional (JZ) zone areas and protein expression were quantified (ImageJ,1.54d). Effect of diet and infection on fetoplacental outcomes were analysed by two-way ANOVA (*$p<0.05$).

Results

At GD12.5, Poly(I:C) reduced placental weight, but not fetal weight, vs. VEH ($p<0.05$). HF diet did not affect fetoplacental anthropometry. At GD15.5, infection and HF diet decreased placental, but not fetal, weight vs. CON and VEH ($p<0.05$); neither exposure altered JZ and LZ areas. Other GD12.5/15.5 analyses are ongoing. At GD18.5, Poly(I:C) reduced placental and fetal weights vs. VEH ($p<0.05$), but did not affect placental cell proliferation, death, turnover, or lipid peroxidation. In contrast, HF diet did not affect fetoplacental weights, but was associated with increased JZ and LZ areas and LZ cell death vs. CON ($p<0.05$), with no effects on cell proliferation, turnover, or lipid peroxidation.

Conclusion

Acute infection and HF diet differentially, and independently, impact fetoplacental anthropometry, morphometry and cell turnover across gestation. Preliminary findings suggest early placental (mal)adaptations may have later adverse implications for fetal development and health.

P1.39

Physiological function of beta 2 glycoprotein I in placenta

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Objectives

Although β2-glycoprotein I (β2GPI), which is predominantly synthesized in hepatocytes, is an abundantly circulating protein, why is its expression observed in the placenta during pregnancy? This study investigated the physiological function of β2GPI in the placenta, with a focus on the post-translational modification of β2GPI.

Methods

The oxidation of β2GPI was assessed in HTR-8/SVneo trophoblast, primary trophoblast culture, HepG2 hepatocyte cells. To evaluate the effect of endogenous β2GPI, *APOH* gene expression plasmid DNA was transferred into trophoblast and hepatocyte cells. To investigate whether oxidation of β2GPI has different effects on trophoblast cells, cell migration and secretion of soluble fms-like tyrosine kinase-1 (sFlt-1) were assessed after the addition of native or TRX-1 treated recombinant β2GPI. The total-β2GPI and reduced-β2GPI levels were measured in peripheral blood samples collected from pre-eclampsia patients and control women with informed consent.

Results

Endogenous β2GPI secretion in trophoblast cells were predominantly in the reduced-form, whereas in HepG2 cells, it was mostly in the oxidized form. Progesterone increased total-β2GPI secretion in trophoblast cells, while total-β2GPI secretion was inhibited by progesterone in HepG2 cells. The reduced-form of β2GPI in both trophoblast and hepatocyte cells were increased by progesterone. The oxidized-β2GPI significantly inhibited trophoblast cell migration and increased placental sFlt-1. The excess sFlt-1 significantly increased the secretion of oxidized-β2GPI in HepG2 cells. Compared with the control group, women with pre-eclampsia had a significant increase in circulating oxidized-β2GPI.

Conclusion

Placental β2GPI (reduced form of β2GPI) may regulate the placenta to avoid an excess of placental sFlt-1. On the other hand, increased oxidative β2GPI under oxidative stress may lead to an excess of placental sFlt-1, which may contribute to the pathogenesis of pre-eclampsia. Under oxidative stress, excessive oxidation of β2GPI and/or excessive placental sFlt-1 may trigger a negative spiral between trophoblast and liver cells.

P1.40

Title: Cervical Clamping Does Affect Ovine Uterine Perfusion: A Laser Speckle Contrast Imaging Study

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Objectives

Uterine artery adaptations during ovine pregnancy have been described, increasing blood flow to the gravid uterus. It is unknown to what extent collateralization from cervical vessels in the ovine model play a role in uterine perfusion. Furthermore, laser speckle contrast imaging (LSCI) has not been used to assess gravid uterine perfusion. We hypothesized that clamping of the cervix and associated vessels would not significantly decrease uterine perfusion as measured by LSCI, but clamping of a unilateral uterine artery would measurably decrease uterine perfusion.

Methods

A gravid ewe underwent general anesthesia. A low midline Pfannenstiel incision was performed, and the uterus delivered. The uterine arteries bilaterally were isolated to allow occlusion. Uterine perfusion was measured with the RFLSI-ZW LSCI System, which depicts microcirculation. Perfusion was measured after: no intervention (baseline), occlusion of cervical vessels, occlusion of the left uterine artery, and occlusion of the right uterine artery.

Results

The RFLSI-ZW Laser Speckle Contrast Imaging System was used to depict uterine perfusion (Figure 1). Uterine perfusion saliently and reproducibly decreased after cervical clamping, as measured by LSCI, by approximately 100-300 perfusion units (Figure 2). Perfusion was consistently restored after release of the clamp. By comparison, uterine perfusion decreased after left or right uterine artery clamping by approximately 100 perfusion units.

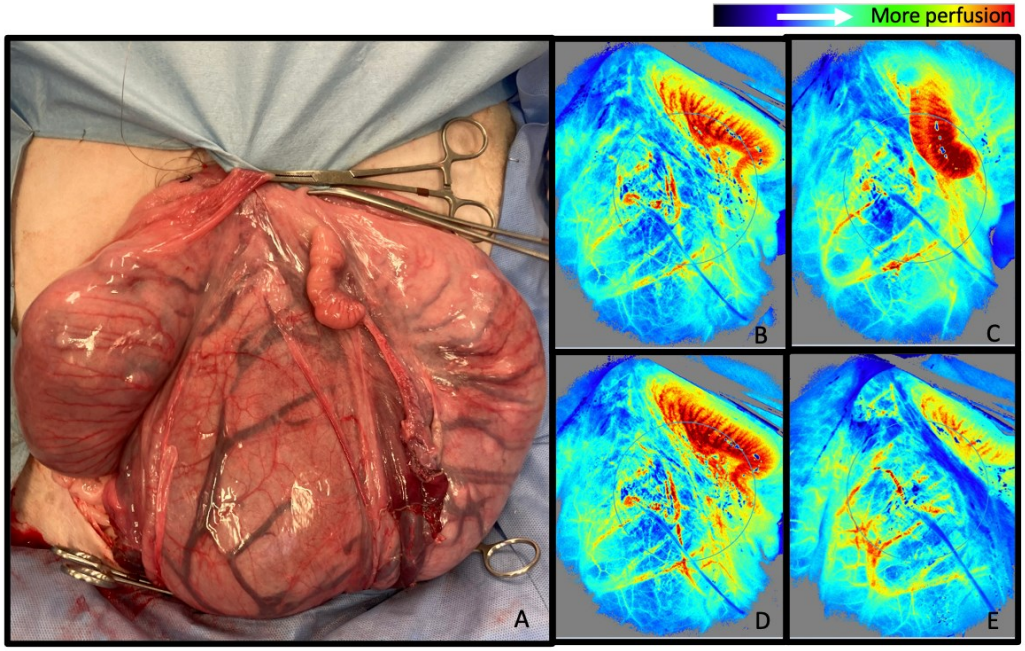

Figure 1. Laser Speckle Contrast Imaging of the gravid uterus. Uterine position setup (A), perfusion images: at rest (B), after cervical occlusion (C), left uterine artery occlusion (D), and after right uterine artery occlusion (E), with scale: from most perfused (red) to least perfused (dark blue)

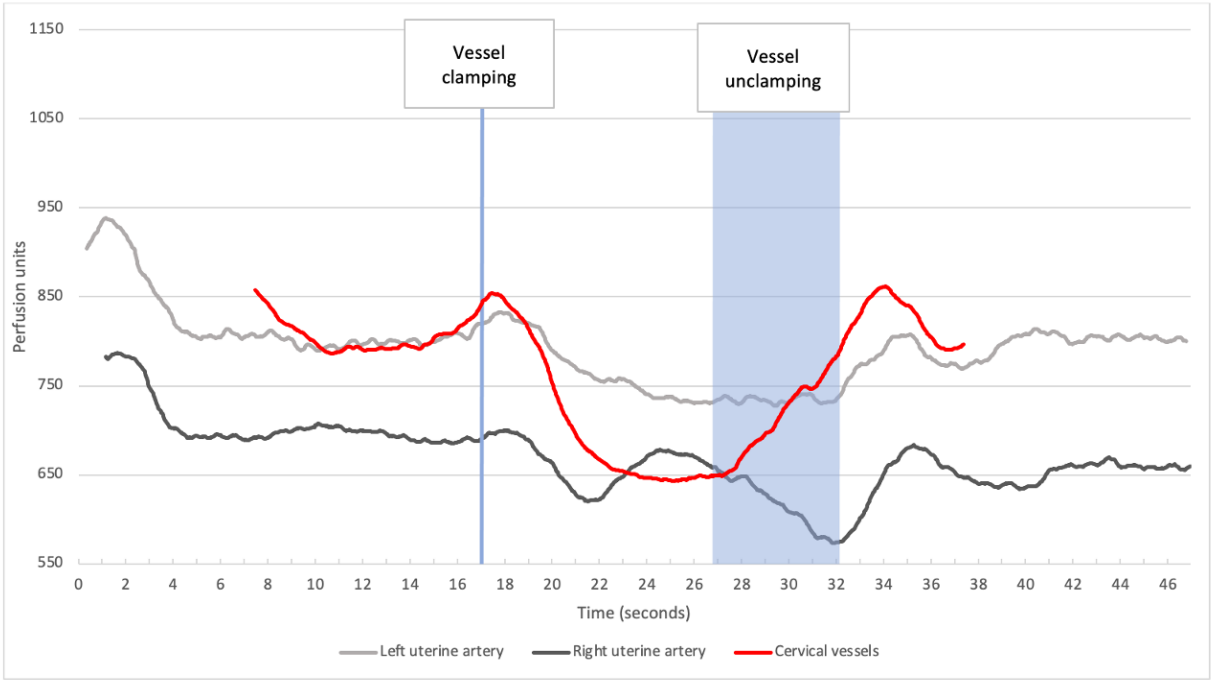

Figure 2. Laser Speckle Contrast Imaging perfusion after cervical occlusion and unilateral uterine artery occlusion in gravid ewe.

Conclusion

The RFLSI-ZW Laser Speckle Contrast Imaging System can be used to measure and depict gravid uterine microcirculation. Occlusion of the cervix and associated vessels does measurably decrease uterine perfusion, and clamping of a unilateral uterine artery measurably decreases uterine perfusion by a lesser extent.

P1.41

Monitoring of maternal-fetal oxygen transfer in ex vivo full term human placenta from normal and growth restricted pregnancies.

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Objectives

Extra-corporeal materno-fetal dual perfusion has been used to study the physiology and transfer of nutrients and gasses across the human placenta, both in normal and complicated pregnancies. The aim of this study was to investigate the oxygen gradient and peak efficiency of oxygen transfer from the spiral arteries to the fetal vasculature in normal (NP) and fetal growth restricted (FGR) pregnancies.

Methods

Placentae (37+ weeks) from normal and FGR pregnancies were retrieved after elective C-section, and informed consent. Single cotyledons, central or peripheral were perfused within 10min of delivery to reverse ischaemia using a well-established method with M199, dextran and albumin at 37 C. Maternal circuit (20 ml/min) was gassed with 95% O_2 and fetal circuit (5ml/min) with 95% N_2. Pressure was sustained at physiological levels throughout the procedure. Using fibre-optic probes, oxygen was monitored for 30 min at maternal inflow, fetal inflow, and fetal outflow sites.

Results

Maternal inflow plateaued at 58 $\pm$ 5% O_2. Oxygen levels at fetal outflow, before establishment of maternal circulation were 1.1 $\pm$ 0.9% in NP v. 4.6% O_2 in FGR. After maternal cannulation oxygen reached 11.1 $\pm$ 1% in NP v. 10.2% O_2 in FGR. Mean time of peak efficiency transfer was 1.6 $\pm$ 0.9min for NP v. 7min for the FGR study group.

Conclusion

Early data imply that chorionic villi in placentae from FGR pregnancies are slower in their ability to pick up oxygen from the IVS, compared to the normal study group. Our future studies will address the physiological and pathological mechanisms behind this phenomenon, focusing on both the impaired maternal venous return and the increased diffusion distance between fetal blood and maternal blood in the IVS.

P1.42

Human-based placenta-embryo chip for developmental toxicity assessment of nanoparticles and drugs

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Objectives

There is an unmet need for innovative human *in vitro* models to assess the developmental toxicity of emerging drugs, nanoparticles and environmental pollutants. Current *in vitro* models mostly focus on direct embryotoxic effects of particles and drugs, neglecting placental transfer and metabolism and a possible indirect effect of particles disrupting placental signaling that is important for embryo-fetal development. Therefore, we aim to establish a human-based placenta-embryo chip allowing for cultivation of a placental barrier model and an embryoid body (EB) in the same device

Methods

The pump-free microfluidic platform features a maternal and embryonic side, separated by a porous membrane. The hanging-drop configuration allows for cultivation of EBs in close vicinity to the placental barrier, enabling intercommunication between the tissues and avoiding losses of short-lived signaling molecules. To achieve human-relevant results, the placenta model consists of a co-culture of primary cytotrophoblast cells (CTBs) derived from human term placenta and human placental vascular endothelial cells (HPVECs), while the embryo model is derived from human induced pluripotent stem cells (hiPSCs).

Results

We achieved the formation of a tight and functional co-culture placental barrier with a spontaneously syncytialized trophoblast layer as demonstrated by immunohistochemical stainings, hCG production and size-dependent exclusion of permeability markers. To verify the placenta-chip model, translocation of different nanoparticles (NPs) and drugs was compared to *ex-vivo* placenta perfusion studies. Furthermore, hiPSC-derived EBs were confirmed to maintain viability and capacity for germ-layer differentiation on chip under dynamic exposure conditions. The placenta-EB co-cultures remained functional for at least three days, enabling extended studies on direct and indirect developmental toxicity mechanisms.

Conclusion

Developing a fully human-based placenta-embryo model will provide a powerful tool to gain early pre-clinical data for drug and NP hazard assessment and the development of safe (nano)therapies for pregnancy while reducing the need for extensive animal testing.

P1.43

Umbilical miRNAs Stratify Risk of Early Childhood Obesity Among Offspring of Mothers with Pre-Pregnancy Overweight/Obesity

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Objectives

Although maternal overweight/obesity increases risk of offspring obesity, identifying offspring at higher risk remains complex. Small noncoding microRNAs (miRNAs), some of which are distinct to the placenta, negatively regulate protein-coding gene translation and are studied for their role in mediating diseases. Our objective was to assess whether miRNAs in umbilical cord blood could stratify risk of childhood overweight/obesity among offspring of women with overweight/obesity.

Methods

This analysis included offspring of women with overweight/obesity (pre-pregnancy BMI kg/m^2≥25) from the Gen3G pre-birth cohort with cord blood miRNA expression data and BMI calculated at 5 years. We used miRNA sequencing data from 112 cord blood samples collected at delivery. We assessed associations between cord blood miRNA levels and early childhood overweight/obesity status (overweight/obesity: BMIz-score >1.00, n=35 vs. not overweight/obese: BMIz-score <0.65, n=77) using DESeq2 R package, adjusting for sequencing run, lane, hemolysis, gestational age, and mode of delivery.

Results

At enrollment, women were on average (mean [SD]) 28 [4] years of age, had a BMI of 30 [5], 13% developed gestational diabetes, and 22% had a cesarean delivery. The differential expression of 16 miRNAs were associated with later childhood overweight/obesity (*P*-value<0.05). Three miRNAs of the placenta-enriched miR-371 cluster (miR-372-3p, miR-373-3p, miR-371a-5p), and two previously associated with catch-up growth (miR-501-3p) and hyperglycemia (miR-96-5p) were all significantly lower in cord blood among the offspring with overweight/obesity at 5 years of age.

Conclusion

Among offspring of women with overweight/obesity, we identified miRNAs in umbilical cord blood that were differentially expressed in offspring who were overweight/obese at 5 years of age. Some of these miRNAs are highly expressed in trophoblast cells and are related to early-life growth or hyperglycemia in

adults. These findings are suggestive of a lasting influence of the placenta and intrauterine environment on later cardiometabolic health

P1.44

Cargo changes in placental extracellular vesicles due to antiphospholipid antibody treatment may reflect differential functionality of different vesicle subtypes

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Objectives

Antiphospholipid antibodies (aPL) are autoantibodies that target the syncytiotrophoblast. Presence of aPL during pregnancy causes an increased risk of recurrent miscarriage, preeclampsia, preterm birth, stillbirth and fetal growth restriction. It is not clear how aPL contribute to these complications but aPL internalisation causes production and accumulation of 'danger signals' within the syncytiotrophoblast. Some of these 'danger signals' are packaged into extracellular vesicles (EVs) extruded into maternal blood. This study explores the extent of differential protein packaging into EVs after aPL treatment of placentae, providing insight into the functional effects these EVs may have on maternal physiology/pathology.

Methods

Term placental explants were cultured in Netwells with 50 µg/mL of aPL (n=5) or control IgG (n=5) in advanced DMEM/F12 with 2% FBS, 1% penicillin/streptomycin overnight. Media were centrifuged at 2000 xg for 5 minutes to remove debris, the supernatants centrifuged at 20,000 xg for 1 hour at 4°C, followed by a 100,000 xg spin for 1 hour at 4°C to obtain large- and small-EVs, respectively. EVs were enriched through a qEV legacy column. EV-protein profiles were compared by quantitative proteomics using SWATH-mass spectrometry followed by enrichment analyses of differentially abundant proteins using DAVID.

Results

aPL treatment of placentae led to extrusion of large-EVs with increased transcription factors and protein translation/splicing machinery. aPL treatment of placentae led to production of small-EVs with a larger number of increased protein production/splicing machinery and proteasome components, as well as decreased DNA damage sensing/DNA repair machinery and cytoskeletal components.

Conclusion

The differential changes in protein packaging into small- and large-EVs from aPL-treated placentae confirms that these EV subtypes carry cargos that are altered in placental pathology. Therefore, each EV subtype may have different effects on maternal physiology. Studying the effects of a single EV subtype on maternal physiology may have misleading results.

P1.45

Exploring steroid dynamics in pregnancy - Insights from the maternal-placental-fetal interface and placental models

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Objectives

The synthesis and secretion of steroid hormones play a crucial role in supporting pregnancy and fetal development. Maternal and fetal steroidogenic tissues, along with the placenta, collectively influence the steroid levels in both maternal and fetal circulation. There is a gap in understanding the paired analysis of maternal blood, placental tissue, and fetal blood, particularly concerning the distribution of novel steroids. We aim to investigate the levels and distribution of steroids across these three compartments in healthy-term pregnancies, focusing on “classical” and novel steroid species. Additionally, we explore how ex vivo and in vitro placental models contribute to understanding steroid secretion and metabolism in human fetal development.

Methods

We collected placental tissue, maternal, and fetal serum samples from healthy-term pregnancies. Additionally, we utilized ex vivo and in vitro models, including human placenta perfusion, placental explants, primary trophoblast cells, and the BeWo choriocarcinoma cell line. Liquid chromatography high-resolution mass spectrometry quantified 51 steroid hormone species across all samples and models.

Results

Of the 51 steroids measured, 33 were co-detected in placental tissue and maternal/fetal serum. Analysis revealed differential detection of “classical” and novel C11-oxy C21 steroids in fetal versus maternal serum. Predominant release of progesterone hormones, including novel C11-oxy C21 steroids, was observed across all models studied. However, “classical” and C11-oxy androgen release was absent at basal levels in ex vivo/in vitro models compared to placental tissue. Importantly, perfusion studies demonstrated differential steroid secretion between maternal and fetal circulation.

Conclusion

Our study highlights the intricate dynamics of steroid distribution within the placenta, maternal, and fetal blood, shedding light on the crucial role of these compartments in steroid hormone regulation during pregnancy. Additionally, characterizing ex vivo and in vitro placental models provides valuable insights into steroid secretion mechanisms, contributing to our understanding of placental physiology and its impact on fetal growth and development.

P1.46

Buprenorphine mediates placental efficiency and perinatal weight gain in a mouse model of prenatal opioid exposure

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Objectives

Exposure of the maternal-fetal dyad to opioids has long-term health consequences. Better understanding of how opioids impact the pregnant mother, fetus, placenta and decidua can help devise targeted interventions. Animal models that faithfully capture various aspects of prenatal opioid exposure are needed to provide direct molecular insights and test therapeutic interventions. Here we bridge that gap with a novel mouse model that enables examination of maternal opioid consumption, maternal-fetal interactions and long-term neurocognitive outcomes for the fetus.

Methods

The backbone of our model is an oral, home-cage apparatus for second-by-second monitoring of volitional opioid consumption. This system established high, stable levels of opioid consumption for 2-3 weeks prior to mating (2.5 - 5.0 mg/day oxycodone). Experimental groups were an oxycodone group (OXY: n=6) and a group switched to buprenorphine at gestational day 12 to model partial agonist intervention during mid-gestation (BUP: n=8). A control gelatin group (VEH: n=4) was included. We monitored placental/fetal weight, litter size, developmental milestones and neurocognitive measures. Parametric (ANOVA) and non-parametric (chi^2) were performed.

Results

Multiple phenotypes were altered in the OXY and BUP groups relative to VEH including preweaning survival, the righting reflex milestone as well as elevated EtOH intake. However, some OXY phenotypes were abrogated by BUP including a 10% decrease in placental efficiency and accelerated pre-weaning weight gain.

Conclusion

Our studies extend our knowledge of the impact of prenatal opioid exposure using a novel mouse model that captures aspects of the human condition including volitional consumption. Importantly, select OXY-altered outcomes were partly ameliorated by BUP intervention thus demonstrating our model's predictive validity. With preliminary correlates of postnatal outcomes to placental efficiency, ongoing work will explore how inflammation, direct opioid action and vasculature impact the maternal-fetal interface to drive acute and long-term sequelae of prenatal opioid exposure.

P1.47

Characterisation of secreted lipids by human term placental trophoblast *in vitro*

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Objectives

The placenta produces a wide range of bioactive lipid signalling molecules such as lysophospholipids, which can be released into the maternal circulation during pregnancy. Such circulating lipid signals have been implicated in diabetes and non-alcoholic fatty liver disease in the non-pregnant state. In pregnancy, the precise roles of these lipid signals are unclear. We hypothesise that these lipids mediate critical communication between the placenta and maternal tissues to facilitate optimal adaptation of the maternal physiology to support the ongoing pregnancy. Our study aimed to characterise the secreted lipids produced by placental trophoblasts.

Methods

Five placentas were collected from women undergoing elective caesarean section at term with written informed consent. Following enzymatic digestion and gradient separation, isolated placental trophoblast cells were cultured in complete growth medium containing fetal bovine serum. After 66 hours, cells were washed and further cultured in serum-free medium containing 1.5% fatty-acid-free bovine serum albumin for another 24 hours. Conditioned medium was then collected and stored at -80°C prior to lipid extraction. Extracted lipids were quantified by liquid chromatography mass spectrometry.

Results

We were able to reliably measure the release of 46 lipid species from across 7 lipid classes by trophoblast cells above a blank media control. Most lipids belonged to classes of phospholipids; there were 18 lysophosphatidylcholines (LPC), 8 lysophosphatidylethanolamines, 1 lysophosphatidylserine, 10 phosphatidylcholines, 1 phosphatidylinositol and 2 sphingomyelins. Additionally, we identified the release of 6 acylcarnitines. The top 3 most abundant lipids in absolute amounts were LPC 16:0, LPC 20:4 and LPC 18:1, which are all long-chain fatty acid LPCs.

Conclusion

Placental trophoblast cells release a diverse variety of bioactive lipids. The specific effects of these lipids on maternal tissues and how they may impact maternal metabolic health remain to be examined.

P1.48

Transcriptomic comparison of in vitro models of the human placenta

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Objectives

Modeling the human placenta *in vitro* is necessary due to limited access and amenability of human placental tissue to certain experimental methods as well as distinct anatomical and physiological differences of animal placentas compared to humans. Selecting an in vitro model of the human placenta is challenging due to representation of different trophoblast cell types and limited comparative studies that define key characteristics of these models. The aim of this research was to create a comprehensive transcriptomic comparison of in vitro models of the human placenta compared to bulk placental tissue.

Methods

We performed differential gene expression analysis on publicly available RNA sequencing data from 6 in vitro models of the human placenta (HTR-8/SVneo, BeWo, JEG-3, JAR, Primary Trophoblasts, Villous Explants) compared to CANDLE and GAPPS bulk placental tissue (N=1083) or cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast primary cells derived through cell sorting of placental tissue.

Results

All placental in vitro models had a substantial number of differentially expressed genes (DEGs, FDR<0.01) compared to the CANDLE and GAPPS placentas (Average DEGs=10,873) and the cell-sorted primary placental tissue (Average DEGs=5,346), indicating that there are vast differences in gene expression compared to bulk and cell-type specific human placental tissue. Hierarchical clustering identified 53 gene clusters with distinct expression profiles across placental models with 22 clusters enriched for KEGG pathways, 7 clusters enriched for high-expression placental genes, and 7 clusters enriched for absorption, distribution, metabolism, and excretion genes.

Conclusion

Overall, no single in vitro model of the placenta emerged as the strongest model of the human placenta and our results suggest it is important for model selection to be based on specific biological research questions. To empower future investigations, we have compiled the results of this analysis into an accessible web application to aid in selection of in vitro placental models.

P1.49

Associations between Maternal Plasma Concentrations of Placental Corticotrophin Releasing Hormone and the Placental Transcriptome

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Objectives

The placenta produces corticotrophin releasing hormone (pCRH), which rises exponentially in maternal plasma across pregnancy. pCRH plays a functional role in fetal development, labor initiation, and the regulation of gestational length. The objective of this study was to understand how maternal plasma pCRH during pregnancy reflects placental physiology during parturition by relating maternal plasma CRH during pregnancy to the placental transcriptome at birth and comparing to transcriptomic signatures of gestational length.

Methods

Maternal plasma pCRH concentrations were measured via radioimmunoassay at 2 timepoints and the placental transcriptome was quantified via RNA sequencing in 516 pregnant participants enrolled in the Conditions Affecting Neurocognitive Development and Learning in Early childhood (CANDLE) cohort. pCRH concentrations at delivery were imputed. Robust linear models were fitted to estimate associations between pCRH and placental gene expression at birth, adjusting for confounding variables. KEGG Pathway analysis was performed using rotational gene set testing.

Results

pCRH concentrations at the mid-pregnancy visit (median visit 22.9 weeks) were associated with placental expression of 8 differentially expressed genes (DEGs). pCRH concentrations in late pregnancy (median visit 31.9 weeks) were associated with 283 DEGs; and pCRH concentrations at delivery (imputed) were associated with 726 DEGs, with many DEGs overlapping between timepoints. 35 DEGs were significantly associated with pCRH at more than 1 timepoint and with gestational length and significantly mediated the relationship between pCRH and gestational length. Placental expression of genes within the NOTCH signaling pathway was associated with both pCRH and gestational length.

Conclusion

Overall, this study reveals potential mechanisms by which pCRH acts as the “Placental Clock” of pregnancy, including novel mechanisms by which pCRH may reflect placental physiological changes required for parturition, such as regulation of lipid transport and metabolism. This study also highlights mechanisms by which pCRH regulate gestational length, including via NOTCH signaling.

P1.50

Gene expression changes in basal plate cytotrophoblast compared to villous core cytotrophoblast are consistent with progression in the epithelial-mesenchymal transition

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Objectives

As part of a program studying the mechanism of cytotrophoblast (CTB) differentiation to extravillous trophoblast (EVT), we investigated the progression of these cells through the epithelial-mesenchymal transition (EMT). We performed RNA-sequencing on cells derived from the villous core and basal plate of the term human placenta. We hypothesized we would see a shift across the EMT spectrum as cells progressed through the differentiation process, from villous core to basal plate, as an epithelial phenotype shifts towards the mesenchymal.

Methods

Villous CTB (vCTB) and basal plate CTB (bCTB) and EVT (bEVT) were isolated from normal term placentae to >95% purity (n=4, 9, 9) and RNA was extracted and sequenced. Statistical testing for differential transcript abundance was performed using DESeq2, with FDR<0.5 as the threshold.

Results

PCA and clustering analysis of these three cell types using the full dataset (~23K transcripts) revealed bEVT clearly separated from both bCTB and vCTB, the latter two being intermixed. Focusing on a curated set of EMT transcripts, we found differential expression in 25 genes between vCTB and bCTB, compared to 395 between bCTB and bEVT. Examining the 25 common genes, there is a clear pattern showing incremental change towards the mesenchymal going from vCTB to bCTB, with much larger changes between bCTB and bEVT. This is echoed in the Hallmark GSEA analysis which shows the EMT geneset as the top ranking process (2.5-fold enrichment), similar to that shown for the bCTB-bEVT comparison. Mesenchymal genes such as *ITGA1*, *TIMP1* and *VIM* are upregulated whereas epithelial genes are relatively unchanged. Notable are the changes in *ZEB1* (5-fold), an EMT master regulator, and *MKI67* (0.5-fold), a proliferation marker.

Conclusion

Compared to villous core, CTB in the basal plate show a shift on the EMT spectrum towards the mesenchymal, confirming the spatial progression of the EMT in the differentiation process.

P1.51

Sex differences in placental gene expression and intraplacental variation in normotensive versus hypertensive pregnancies

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Objectives

Many studies have reported fetal sex biases in the incidence of pregnancy complications including gestational hypertension and preeclampsia, but the mechanisms underlying the relationship between fetal sex and pregnancy complications are poorly understood. Having previously demonstrated sex differences in placental gene expression in uncomplicated pregnancies, we sought to determine if gestational hypertension affects placental gene expression differently based on fetal sex.

Methods

We performed sex-informed differential gene expression analysis of total RNA from placental tissue from full-term deliveries of ten hypertensive and ten normotensive pregnancies, each with half carrying a fetus assigned female at birth (XX) and half male at birth (XY) (limma-voom::DREAM workflow). For each placenta, we sampled four locations across the placental disc to assay for intraplacental variation in gene expression. We used single cell deconvolution to assay for differences in cell type proportion between conditions.

Results

We saw high intraplacental variation in genes involved in extracellular matrix organization, hormone secretion, and the immune response. We found a distinct profile of placental genes differentially expressed between normotensive and hypertensive pregnancies for each fetal sex. Differentially expressed genes from placenta in male-bearing pregnancies were functionally enriched for metabolism and cellular response to stress, while those from female-bearing were enriched for invasion, angiogenesis, and immune function. Biomarkers of preeclampsia such as LEP, FLT1, and FSTL3 were only differentially expressed in placenta from male-bearing pregnancies. We found sex-specific differences in cell type proportions in hypertensive pregnancies, suggesting potential mechanisms by which sex differences in placenta biology could lead to different health outcomes.

Conclusion

We observed that placental gene expression changes from normotensive to hypertensive pregnancies are quite different between sexes. Our study highlights the importance of increased sampling of the placenta when studying pregnancy complications as genetic sex and variation across the placental disc could have a large effect on gene expression.

P1.52

Profiling of the 2022 placental transcriptome after maternal COVID-19 disease

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Objectives

SARS-CoV-2 infection during pregnancy has been directly related to adverse pregnancy outcomes and alterations in the placenta during initial stages of the pandemic (2020). Although maternal COVID-19 disease is no longer considered an obstretic/pediatric concern in healthy pregnancies after vaccination, it remains poorly understood whether it still affects placental health. To address this issue, we compared the transcriptional landscape in the placentas delivered in 2022 by mothers who suffered an episode of COVID-19 disease during pregnancy with pre-COVID-19 placentas from healthy pregnancies.

Methods

Placental RNA was purified from placental samples taken either in 2022 from SARS-CoV-2 positive mothers (termed COVID 2022) (n=21); or from healthy pregnancies predating the pandemic (termed preCOVID-19). We performed RNA-seq, identified differentially expressed genes and examined to which biological, biochemical and cellular pathways they belong. The differentially expressed genes (DEG) identified were used to carry out gene set enrichment analysis using Gene Set Enrichment Analysis (GSEA).

Results

We identified 71 differentially expressed genes (DEG) (defined by p-value ≤0.05 and fold change (FC) of ≤-1 or ≥1). The alterations identified in placentas delivered in 2022 by mothers who suffered an episode of COVID-19 disease can be mainly attributed to pathways related to organogenesis, extracellular matrix organization and oxygen transport. Several of the DEGs representative of those pathways have been previously reported as dysregulated in placental disorders related to hypertensive disorders of pregnancy including preeclampsia.

Conclusion

The alterations in gene expression revealed in this study, may be indicative of differences in the pathophysiology of placentas from pregnant women infected by SARS-CoV-2 in 2022 compared to placentas delivered in uneventful pregnancies, suggesting that a close follow up of offspring may be warranted.

P1.53

Ancestry and Fetal Sex Differences in Hofbauer Cell Gene Expression and Cell Communications in Mid-Gestation Human Placenta

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Objectives

In the U.S., Black individuals are more likely to experience adverse pregnancy outcomes driven by placental dysfunction than White individuals. Our previous study suggested that mid-gestation placentas from Black women were inflamed, which is associated with multiple pregnancy complications. The aim of this study was to investigate changes in gene expression and cell-cell communications underlying these ancestry differences at single cell level, particularly in Hofbauer cells. In addition, as there are sex differences in fetal and neonatal outcomes, we also compared males and females.

Methods

We performed single nuclei RNA-seq analysis with 10x Genomics platform in male and female placentas from Black and White pregnant individuals (n=5 per group) from healthy second trimester pregnancies at 18-22 weeks (no maternal disease, no congenital anomalies). Cell-cell interaction (ligand-receptor pair) was analyzed using Cellchat.

Results

The transcriptome profiles were obtained from 101,001 cells, including 11,659 Hofbauer (HB) cells. Multiple ancestry- and sex-associated differentially expressed genes (q-value<0.05, fold change>1.5x) were identified in HB cells with profound ancestry differences between Black and White females and profound sex differences between Black females and males, including genes critical for macrophage function, such as *NAMPT, PLAUR*, *NR4A2*, *GALNT14*, and *GPNMB*. Signaling pathways significantly altered included regulation of inflammation, oxidative stress, metabolism, and autophagy. The enrichment of interaction signaling networks between HB cells and other cell types, such as trophoblast and fibroblast, also showed ancestry and sex differences. IGF1 signaling network was enriched in Black male and White female placentas. In contrast, TGFA-EGFR, HBEGF-EGFR, and COL4A2 signaling networks were enriched in Black female placentas.

Conclusion

Our results demonstrate significant and biologically relevant ancestry and sex differences in gene expression in HB cells as well as their communications with other cell types in placenta. The differences may underlie the ancestry and sex disparities in placental function and pregnancy outcomes.

P1.54

Associations Between Prenatal Vitamin D and Placental Gene Expression

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Objectives

Vitamin D is a hormone regulating gene transcription. In the placenta, a key functional tissue in pregnancy, vitamin D has been linked to immune and vascular function. To date, studies of vitamin D and human placental gene expression have focused on a limited number of genes. Genome-wide RNA sequencing can provide a fuller representation of the placental effects of vitamin D. We investigated the association between prenatal vitamin D levels and placental gene expression in a large, prospective birth cohort.

Methods

Participants were recruited in Shelby County, Tennessee in the Conditions Affecting Neurocognitive Development and Learning in Early childhood (CANDLE) study. Vitamin D was measured as plasma 25-hydroxyvitatmin D (25-OH-D) at enrollment (16-28 weeks' gestation) and delivery. Placenta samples were collected at birth, and RNA was isolated and sequenced. We identified differentially expressed genes (DEGs) (FDR<0.1) using adjusted linear regression analyses and conducted weighted gene coexpression network analysis (WGCNA).

Results

This analysis included 774 participants with vitamin D and transcriptomic data. The median vitamin D level of participants was 21.8 ng/mL 25-OH-D at enrollment (IQR: 15.4-26.5 ng/mL) and 23.6 ng/mL at delivery (IQR: 16.8-29.1 ng/mL). Placental expression of 25 DEGs was associated with 25-OH-D at enrollment, but no DEGs were associated with 25-OH-D at delivery. DEGs were related to energy metabolism, cytoskeletal function, and RNA transcription. Using WGCNA, we also identified 2 gene modules whose expression was associated with 25-OH-D at enrollment. These modules were enriched for genes related to mitochondrial and cytoskeletal function, and were regulated by transcription factors including *ARNT2*, *FOSL2*, *JUND*, and *NFKB1*.

Conclusion

Our results indicate that 25-OH-D during mid-pregnancy, but not at delivery, is associated with placental gene expression. Future research is needed to investigate a potential programming role of vitamin D on placental mitochondrial metabolism, intracellular transport, and transcriptional regulation during pregnancy.

P1.55

Maternal gestational protein restriction affects placental and fetal hepatic ammonia metabolism in intrauterine growth restricted rats

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Objectives

Clinical metabolomics enables the characterization of diseases beyond genomic and proteomic levels, thus enhancing the etiopathological comprehension. Human pregnancies afflicted with intrauterine growth restriction (IUGR) exhibit a distinct metabolic profile. In this study, we investigated the similarity of these metabolic changes in a rat model of IUGR.

Methods

Pregnant Wistar dams were fed a low protein (8%, LP; IUGR) or an isocaloric normal protein diet (17%, NP; controls). Maternal serum, placenta and fetal liver was subjected to reverse-phase liquid chromatography mass spectrometry (LC-MS) (Agilent Eclipse XDB-C18 column) coupled with an ESI-MS/MS-system (API6500+, Sciex; Agilent HPLC1260 series) using the AbsoluteIDQ p180 kit (Biocrates). Data analysis was performed using MetaboAnalyst v5.0.

Results

Pathway enrichment analysis revealed changes in hepato-placental ammonia metabolism in LP rats, along with the engagement of maternal metabolic pathways associated with epigenetic programming and energy regulation. Logistic regression, combined with receiver-operating characteristic (ROC) curve analysis, pinpointed the tryptophan/kynurenine ratio in fetal liver and the phenylalanine/tryptophan ratio in the placenta as significant discriminators between groups.

Conclusion

LC-MS/MS fingerprinting has emerged as a promising method for probing metabolic shifts. In our rat model of intrauterine growth restriction (IUGR) induced by low protein intake, we noted metabolic changes in multiple fetal compartments and maternal blood. These changes closely mirror metabolic alterations seen in human pregnancies affected by this condition. Our results emphasize the need for additional investigation into the underlying mechanisms and potential interventions.

P1.56

Placental Gene Expression Associated with Early Childhood Growth Trajectories and Obesity Risk

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Objectives

The placenta is critical for fetal development and has a long-term impact on child development after birth. We aimed to investigate associations between placental gene expression and early childhood growth outcomes.

Methods

This study included 794 children from the Conditions Affecting Neurocognitive Development and Learning in Early Childhood (CANDLE) pregnancy cohort study. Placental samples were collected at delivery, and placental transcriptome data were obtained using paired-end RNA sequencing. Three body mass index (BMI) trajectories from birth to 4 years (rising-high-BMI, moderate-BMI, and low-BMI trajectories) and overweight/obesity at 4 years were the growth outcomes of interest in this study. Differentially expressed genes (DEGs) associated with outcomes were identified using linear models implemented in DESeq2 with adjustment for confounding factors. The causal relationship between placental DEGs and children's growth outcomes was further inferred using the Mendelian Randomization (MR) approach.

Results

We identified 22 and 23 DEGs associated with early childhood BMI trajectory and overweight/obesity outcomes, respectively, with false discovery rates (FDRs) < 0.05. The pathway enrichment analysis identified 26 biological pathways, most of which are either processes of or disorders related to the immune system (e.g., the pathways of antigen processing and presentation and asthma). The MR analysis suggested that one (*SSX2B*) and eight (*HSPA1A*, *DNAJB1*, *HSPA1B*, *MYH6*, *NFIL3*, *DSP*, *CRKL*, and *UGP2*) placental DEGs might be causally associated with the BMI trajectories and overweight/obesity outcomes (FDRs < 0.05), respectively.

Conclusion

Our study is among the first efforts to systematically identify placental gene expression associated with early childhood growth outcomes. Consistent with recent reports, our findings further support that the immune system gene expression of the placenta influences child growth and obesity development. Further investigations to clarify the mechanisms underlying these associations are warranted.

P1.57

Excessive hypercholesterolemia in pregnancy induces sex-specific differences in the placental unfolded protein response

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Objectives

Placental dysfunction in preeclampsia may be linked to placental endoplasmic reticulum (ER) stress. Outside of pregnancy, hypercholesterolemia induces ER stress, which activates the unfolded protein response (UPR) to restore protein homeostasis. During pregnancy, *excessive* maternal hypercholesterolemia is associated with preeclampsia, and creates a preeclampsia-like phenotype that includes reduced placental efficiency. However, whether excessive hypercholesterolemia in pregnancy activates the placental UPR, and if there are any sex-specific differences in this process, is not known. Our objective was to determine the impact of excessive hypercholesterolemia in pregnancy on the placental UPR.

Methods

Sprague Dawley rats were fed a control diet (CD) or high cholesterol diet (HCD) from gestational day (GD) 6 to 20 (term=22 days; n=5/group). On GD20, male and female placentas were collected, and protein expression of the three branches of the UPR (ATF6, eIF2α [the downstream target of PERK], and IRE1α), GRP78 (master negative regulator of the UPR), and the NLRP3 inflammasome (marker of uncompensated ER stress) were assessed by Western blotting. Data were analyzed by Student's t-test (significance: $p<0.05$).

Results

In female placentas, no differences were observed between the CD and HCD groups for all proteins analyzed. In male placentas, protein expression levels of GRP78, p-eIF2α, and cleaved ATF6 were similar between the CD and HCD groups. However, p-IRE1α protein expression levels decreased (99.99±0.85 *vs.* 93.12±2.32, p=0.02), while the NLRP3 inflammasome protein expression levels increased (100.4±15.32 *vs.* 180.5±22.88, p=0.02) in the HCD group compared to CD.

Conclusion

Exposure to excessive hypercholesterolemia in pregnancy increased NLRP3 inflammasome expression in the male placentas, but not in the females, which may be attributed to reduced p-IRE1α levels (marker of prolonged, high ER stress exposure). These data indicate that there are sex-specific differences in the activation of the placental NLRP3 inflammasome, potentially via the UPR, in pregnancies complicated by excessive *pregnancy-specific* hypercholesterolemia.

P1.58

Unravelling the Protective Role of Placental ACE2 During Chronic Hypoxia/Reoxygenation

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Objectives

Fetal growth restriction (FGR), a major pregnancy complication, increases infant morbidity and mortality risk. FGR is often characterised by placental oxidative stress due to deficient placentation and exposure to intermittent hypoxia and reoxygenation (H/R). FGR placentae exhibit reduced expression of the antioxidant angiotensin-converting enzyme 2 (ACE2), likely reducing placental antioxidant capacity and exacerbating oxidative stress. We aimed to induce oxidative stress using an *in vitro* model of chronic H/R and activate or replace ACE2 to determine its role in driving placental antioxidant capacity in FGR.

Methods

Healthy term placental villous explants (n=8/group) were cultured for 24hrs under normoxic conditions (8% O_2) or were treated with either a) media alone, b) diminazene aceturate (DIZE) or c) recombinant human (rh)ACE2 to activate or replace ACE2, respectively, and exposed to H/R conditions (alternating 6hr periods of 1% and 8% O2). Subsequently, ACE2 expression and release and oxidative and antioxidant markers were assessed via qPCR, immunoblot, ELISA and activity assays.

Results

In response to H/R, *ACE2* mRNA was unchanged, however levels of membrane bound ACE2 protein and soluble ACE2 in the supernatant were reduced ($p<0.01$). H/R increased the mRNA expression of oxidative markers *NOX5* and XDH, and increased the antioxidants SOD1, *GSR*, Nrf2, *HO-1* and NQO1 ($p<0.01$). The antioxidant activity of both SOD and CAT were reduced in response to H/R ($p<0.01$). Treatment with DIZE or rhACE2 was able to partially mitigate oxidative stress by reducing *NOX5* mRNA expression and increasing both SOD and CAT activity ($p<0.01$).

Conclusion

This data shows for the first time that H/R reduces ACE2 protein levels and soluble ACE2 release from the placenta. Furthermore, therapeutic activation or supplementation of placental ACE2 mitigated the H/R induced increase in oxidative stress marker NOX5 and increased antioxidant capacity. This demonstrates the potential for ACE2-stimulating therapies to be used for the prevention or treatment of FGR.

P1.59

Exploring the Importance of Estrogen Receptor α in Mast Cells Throughout Pregnancy: Insights from a Mouse Model

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Objectives

Mast cells (MCs) belong to the complex cell network that ensures a successful progression of pregnancy. MCs' depletion in pregnant mice leads to several complications, such as fetal growth restriction and impaired spiral artery remodeling. However, despite their evident importance, the specific mechanisms through which MCs, particularly their receptors, take part to pregnancy processes remain unclear. Here, we aim to understand whether the presence of estrogen receptor α (ERα) in MCs contributes to their essential role in pregnancy. For this purpose, ERα was knocked-out specifically in mast cell protease 5 (Mcpt5)-expressing MCs (Mcpt5.cre^{+}/ERα$^{fl/fl}$).

Methods

Knock-out (KO; Mcpt5.cre^{+}/ERα$^{fl/fl}$) or control (Mcpt5.cre^{-}/ERα$^{fl/fl}$) females were paired with BALB/c male mice. The day of vaginal plug detection was set as gestational day (gd) 0. Mice were randomly assigned to three groups having different sacrifice time points: gd5, gd10, gd18. Pregnancy was then monitored with the VEVO 2100 high-frequency ultrasound system, that allowed to analyze placental, fetal and blood flow parameters *in vivo.* At gd18, placental and offspring's weights were measured following the dams' sacrifice.

Results

The ultrasound data analysis showed significantly smaller placental areas and thicknesses at gd14 in the KO group. The weight of the placentas belonging to the KO dams' fetuses at gd18 was likewise significantly lower compared to the control group. Additionally, the KO condition led to a higher feto-placental index, which might be a sign of placental inefficiency. Furthermore, we found out that in the KO group 2.2% of the litter was growth restricted, while the 8.8% was small for gestational age.

Conclusion

Our mouse model highlighted the importance of ERα signaling in MCs during mice gestation. The lack of this receptor led to alterations in placenta development and fetal growth irregularities. These findings represent an initial step towards unraveling the mechanisms underlying MCs pivotal role in pregnancy.

P1.60

Role and Mechanism of Elabela/Apelin-APJ in Fetal Growth Restriction

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Objectives

To investigate pathophysiological significance of Elabela/Apelin in enhancing the proliferation, migration, and invasion of human trophoblasts, as well as its correlation with fetal growth within the uterus.

Methods

Expression of ELA in maternal serum and its potential effect on fetal growth were quantified with ELISA. Human placentas were collected from pregnancies with normal growth and fetal growth restriction (FGR). APLN and APJ expression in human fetal disc tissue and HTR-8/SVneo trophoblast cells were detected using real-time quantitative fluorescent PCR (qRT-PCR) and Western blot. Additionally, siRNA-mediated ELA knockout was used to investigate ELA/APLN expression patterns and regulation, performed functional analysis of ELA/APLN, including regulatory processes between ELA/APLN and APJ.

Results

The serum expression level of ELA was lower in pregnant women with fetal growth restriction (FGR) compared to normal pregnant women. Quantitative measurements show a significant decrease of ELA, APLN, and APJ in FGR placental tissue. Functionally, knockdown ELA inhibited the proliferation, migration, and invasion of HTR-8/SVneo cells. Additionally, the expression levels of APLN and APJ were down-regulated in HTR-8/SVneo cells and showed a positive correlation with ELA expression. Inhibition of ELA expression effectively suppressed the cellular proliferation, migration, and invasion of HTR-8/SVneo cells by reducing the expression levels of APLN and APJ.

Conclusion

The recently identified ELA/APLN-APJ axis has the potential to serve as a valuable biomarker or therapeutic target for FGR, as decreased levels of ELA are closed to the proliferation, migration, and invasion of trophoblast cells. This study provides important insights into the mechanisms driving the targeted multiplication and movement of HTR-8/SVneo cells. Future research should investigate the precise molecular mechanisms and specific roles of ELA/APLN in other pregnancy-related disorders. This has the potential to develop new tactics for the prevention and treatment of these conditions, ultimately enhancing perinatal outcomes for both mothers and infants.

P1.61

Trophoblastic epithelial-mesenchymal transition with consecutive fibrosis: a cause of reduced placental function in hyperglycemic pregnancies?

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Objectives

In Germany, perinatal mortality among women with type 1 diabetes mellitus ranges from 1.5 to 2 %, which is significantly higher than in metabolically healthy pregnancies. This can result in sudden placental failure near term, leading to intrauterine fetal demise. Standard obstetric monitoring tools prove ineffective in recognizing cases at risk. Histological examinations of placenta samples of type 1 diabetes patients so far do not reveal typical changes associated with placental insufficiency.

Since diabetes can induce fibrosis and functional loss in organs like the liver and kidneys through pathways including Advanced-Glycation-End products (AGEs) and transforming growth factor beta1 (TGFβ1), we explore whether comparable changes occur in affected placentas. Furthermore, we aim to investigate, if trophoblast cells undergo epithelial-mesenchymal transition (EMT) *in vitro.*

Methods

To simulate maternal hyperglycemia *in vitro,* the trophoblastic cell line AC-1M32 is exposed to varying glucose concentrations. EMT-activating growth factor TGFβ1 will be analyzed via ELISA and EMT markers are identified using Western blot.

EMT marker expression and distribution over placental cell types were analyzed in type 1 diabetic patients and controls using Multiplex Immunofluorescence (mIF) staining. IHC is used to determine the amount of the AGE Carboxymethyllysin (CML) in diabetic and control tissues. Elastica-van-Giesson-Staining is planned to estimate fibrosis prevalence in diabetic placentas compared to controls.

Results

We anticipate higher TGFβ1 and CML expression with increased glucose concentrations, elevated SNAIL1 and Vimentin alongside reduced E-cadherin levels indicating EMT in trophoblastic cells. This marker profile suggests augmented stroma and extracellular matrix with decreased functional epithelial tissue. We expect similar findings in mIF staining of placenta sections from hyperglycemic pregnancies, alongside increased CML staining intensity.

Conclusion

Through these experiments, we aim to clarify the impact of hyperglycemia-induced placental fibrosis on diabetic pregnancies. Our findings may provide valuable insights into improving management strategies for pregnant women with diabetes, potentially reducing perinatal mortality rates.

P1.62

Thyroid antibody positivity is associated with impaired fertility, altered placental morphology, and reduced male fetal survival in a rodent model of Autoimmune Thyroiditis

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Objectives

Autoimmune thyroiditis (AIT) is a common cause of thyroid disease, characterised by auto-antibodies targeting proteins of the thyroid gland. Importantly, 1 in 5 women have these thyroid autoantibodies (TAbs) during pregnancy. TAb positivity (TAb+) is associated with increased risk of many pregnancy complications, including miscarriage and gestational diabetes. However, there is limited mechanistic research looking into how TAb+ leads to pregnancy dysfunction. This study aimed to establish an animal model of AIT during pregnancy to investigate the impact of TAb+ on maternal, placental and fetal physiology.

Methods

TAb+ was induced in female Lewis rats by combination of routine porcine thyroglobulin injection (2ug/ml) and daily administration of sodium iodide (0.5% w/v). Impacts to estrous cycling were assessed by vaginal impedance prior to mating. Plasma was collected 1-week prior to mating and at the end of pregnancy to assess TAb and hormone levels. All rats were culled at E20 and tissue collected for further analysis.

Results

TAb+ was associated with disrupted estrous cycling but did not impair pregnancy success. In addition to TAb+, AIT animals had elevated thyroxine in late gestation but no changes to TSH. TAb+ did not impact litter size but did impair male fetus survival. Placentas from TAb+ dams were larger in both zones, with an increase in glycogen content seen in the JZ. Placental expression of syncytialisation and angiogenesis genes were also altered by TAb+.

Conclusion

These findings demonstrate changes to thyroid status, including TAb+, can lead to altered reproductive function, reduced male fetal survival, and may be indicative of placental dysfunction. This study highlights the complex presentation of AIT in pregnancy and provides a solid basis for future research to build upon.

P1.63

Metformin treatment for a mouse model of diabetes during pregnancy reduces placental size and mitochondrial function but does not impact fetal growth.

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Objectives

Diabetes in pregnancy can have detrimental effects on placental mitochondrial function and development, leading to adverse fetal outcomes. Metformin is a popular anti-diabetic medication outside of pregnancy, however exact mechanisms of action are still poorly understood. This understandably causes hesitation around its use during pregnancy, where protecting fetal development is of utmost importance. This study investigated the impact of chronic maternal metformin treatment on placental mitochondrial function, in mice with and without diabetes in pregnancy and evaluated the subsequent fetal outcomes.

Methods

Four-week-old C57BL/6J female mice consumed either a control diet (LFD) or a high fat diet (HFD; 60% calories from fat) for five weeks to induce glucose intolerance. Mice were treated with metformin (300mg/kg/day) or sterile water via oral gavage for two weeks prior to mating and throughout pregnancy. Mice were culled on embryonic day 18.5. To observe chronic effects of metformin treatment, daily dose was not delivered on cull day. Placental mitochondrial respiration was measured using an Oxygraph-2k respirometer (Oroboros Instruments, Austria). Fetal growth, blood glucose and insulin levels were assessed at cull.

Results

Maternal glucose intolerance decreased fetal growth by 8%, though brain weight was maintained. Metformin did not rescue fetal growth. Chronic metformin treatment increased placental weight in HFD mice only, resulting in a decreased fetal-placental ratio, suggestive of reduced placental efficiency. Maternal glucose intolerance and metformin treatment independently decreased placental oxidative phosphorylation capacity through complex I (CI). Metformin treatment decreased mitochondrial spare capacity in both LFD and HFD mice, indicating that the mitochondria are operating near their limit. LEAK, OXPHOS CI + CII, CIV and ETS were not affected by either treatment.

Conclusion

In our mouse model of diabetes during pregnancy, chronic metformin treatment significantly reduced placental mitochondrial function but increased placental growth. Despite these changes, metformin failed to restore fetal growth restriction in our diabetic-like mice.

P1.64

Metabolomic and transcriptomic profiling of the placental endocannabinoid system: a comparative study between term and preterm births

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Objectives

The endocannabinoid system (ECS), a complex array of receptors and signaling molecules, plays a crucial role in regulating a variety of physiological processes, including pregnancy and childbirth. It consists of signaling molecules named endocannabinoids, enzymes for their synthesis and metabolism, and cannabinoid/non-cannabinoid receptors. Studies revealed that the placenta expresses ECS similar to that in the brain, and levels of ECS components may alter during different pregnancy pathologies, including preeclampsia and miscarriage. This study aimed to compare the placental ECS in preterm and term pregnancies and discover potential connections between changes in the ECS and the development of preterm birth.

Methods

We employed qPCR analysis of key ECS genes, including fatty-acid amide hydrolase (*FAAH*), N-acyl phosphatidylethanolamine-specific phospholipase D (*NAPEPLD*), monoacylglycerol lipase (*MGLL*), diacylglycerol lipase beta (*DAGLB*), α/β-hydrolase domain containing 6 (*ABHD6*), and prostaglandin synthase 2 (*PGS2*); cannabinoid receptors CB-1 (*CNR-1*), CB-2 (*CNR-2*); and non-cannabinoid targets, such as transient receptor potential cation channel subfamily V member 1 (*TRPV1*), and peroxisome proliferator-activated receptor gamma (*PPARG*). Moreover, we used targeted metabolomic analysis to quantify and compare endocannabinoid metabolome, including main endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), in preterm and term placentas.

Results

We demonstrate that in preterm samples, *DAGLB, NAPEPLD*, and *ABHD6* levels were significantly downregulated when compared to those in term placenta samples. Additionally, *CNR-1* gene was found to be upregulated in preterm birth placentas. The metabolomic data indicated that the ECS metabolome in preterm birth placentas differed from those in term deliveries: preterm birth was accompanied by higher levels of monoacylglycerols, and minor elevation in N-acylethanolamines.

Conclusion

In this study, we revealed changes in the placental ECS associated with preterm birth, indicating the ECS's involvement in the pathology.

The study was supported by the Grant Agency of Charles University: GAUK 170/50/225010, AZV NW24-07-00129

P1.65

Characterizing placental NLRP3 inflammasome and lipidome dynamics: Insights from glucose, metformin, and LPS exposure in placental explants

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Objectives

Gestational diabetes mellitus (GDM) is one of the most common obstetric complications defined as glucose tolerance first detected during pregnancy. This metabolic disorder is linked with disturbances in lipid metabolism and a state of sterile inflammation mediated by the NLRP3 inflammasome. Recent literature highlights the placenta's central role in this dynamics, given its significant expression of NLRP3 inflammasome pathway components and its function as a site for lipid storage. Moreover, metformin, a hypoglycemic agent used in pregnancy, has recently attracted
interest as a potential inflammasome activator. Thus, this study aimed to characterize the links between placental inflammasome and lipidome upon exposure to the hyperglycemic environment, metformin, and LPS.

Methods

Villous placental explants were isolated from human term placenta. Explants were exposed to glucose, metformin, and LPS in a time- and concentration-dependent manner. qPCR and ELISA were used to evaluate the NLRP3 inflammasome gene expression and cytokine release, respectively. Lipidomic analysis was performed using SFC/MS.

Results

We show a strong and modest effect of LPS and high glucose, respectively, on the placental NLRP3 pathway. This is in line with the evidence that links hyperglycemia with a low-grade inflammatory state. Moreover, our investigation also unveiled the regulatory effects of metformin on the NLRP3 inflammasome in placental explants. Lastly, we identified significant alterations in lipid profiles within both tissue and media following exposure to glucose, metformin, and LPS.

Conclusion

The findings of our study emphasize the intricate modulation of inflammatory responses, notably through the NLRP3 inflammasome pathway, as well as alterations in the lipidome profile within placental explants. These insights highlight the multifaceted nature of GDM pathophysiology and suggest a further investigation into the mechanistic nature of these outcomes.

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P1.66

Senescence markers in the placenta in preeclampsia and fetal growth restriction

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Objectives

The placenta develops, matures and ages over the course of 40 weeks of pregnancy leading to an increased expression of senescence markers in the placenta as the pregnancy progresses. Accelerated aging of the placenta represents a pathological condition and is a hallmark of pregnancy complications associated with placental insufficiency and oxidative stress such as fetal growth restriction (FGR) and preeclampsia (PE). The aim of the project is to develop an understanding of the physiologically occurring placental aging by examining markers for senescence and proteins involved in the oxidative stress processes.

Methods

We use formalin-fixed, paraffin-embedded placenta samples from healthy term births and placentas with PE and or FGR (n=5 each). Using multiplex immunofluorescence (mIF), the expression of the senescence markers p21 and p16, markers for occurrence of oxidative stress (SOD-2) in the different cell populations present in the placenta were investigated. The following markers were used to define cell populations: vimentin (stroma), CD31 (endothelium),e-cadherin (cytotrophoblast) and β-hCG (syncytiotrophoblast). mIF allows the simultaneous detection of up to six antigens. With the help of tyramide signal amplification (TSA), the application of different primary antibodies of the same species is possible.

Results

Our results demonstrate differences in the tissue distribution of the investigated marker between healthy and pathological placentas. In addition, pathological placentas show a reduced expression of the antioxidant SOD-2 in the stroma and an up-regulation of senescence markers in the functional trophoblast.

Conclusion

Our results confirm that mIF is methodologically suitable to investigate the expression of senescence markers in a cell population-specific manner in placenta samples. We show first results of mIF studies on expression of senescence markers and proteins involved in oxidative stress in different pregnancy complications.

P1.67

Pregnancies complicated by gestational diabetes mellitus: Placental histomorphological features and immunohistochemical markers of senescence

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Objectives

Gestational diabetes mellitus (GDM) is acknowledged as one of the great obstetrical syndromes, and associated with functional and structural changes of the placenta. Accelerated placental senescence has been suggested as a contributing factor to placenta dysfunction related syndromes, such as preeclampsia. However, in GDM, this is understudied. Our aim was to examine the histomorphological features and immunohistochemical markers of senescence in placentas from women with GDM compared to a euglycemic control group.

Methods

We included 40 placentas (GDM: n=20 and gestational age-matched euglycemic controls: n=20). Exclusion criteria included hypertension, smoking and use of antiplatelet/-coagulation medication. Histomorphological assessment of Hematoxylin Eosin stained placentas (n=37) was performed by two perinatal pathologists, using the standardized Amsterdam Workshop Consensus Criteria, blinded for clinical characteristics (except for gestational age). Immunohistochemical antibodies to p21 and 8-hydroxy-2-deoxyguanosine (8-OHdG) were used as senescence markers and analyzed in trophoblast nuclei (n= 40 placentas).

Results

The two groups were similar in mean maternal age, parity, neonatal birth weight and gestational age at time of delivery (39.1 weeks). The most common histopathological finding was delayed maturation, present in 84% of the GDM placentas and 68% in the control group (p=0.25). Increased syncytial knotting for gestational age was seen in 26% and 32% respectively (p=0.72). The interrater agreement for the histomorphological criteria was on average 74%. Positivity for p21 was present in 75% of women with GDM and 100% of the euglycemic controls (p=0.047). 8-OHdG was positive in 30% and 35% respectively (p=0.74).

Conclusion

There was no significant difference in the histopathological findings in placentas from women with GDM compared to euglycemic controls. For two immunohistochemical markers of cellular senescence, increased expression was seen in euglycemic controls. Our findings may suggest that the effect of GDM on the placenta is mediated through other pathways than cellular senescence.

P1.68

Placental ceramide content is decreased in maternal obesity and GDM, independent of fetal sex.

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Objectives

Ceramides inhibit insulin signaling in many tissues, including the placenta. We have previously shown that adiponectin inhibits insulin signaling and insulin-stimulated amino acid transport by increasing ceramide synthesis. This has been proposed to be a mechanism by which maternal adiponectin controls placental function, including nutrient transfer to the fetus, and fetal growth. In pregnancies complicated by obesity or gestational diabetes (GDM) maternal plasma adiponectin is typically decreased. Moreover, maternal adiponectin levels are inversely correlated with birth weight. The aim of this study was to determine placental ceramide content in maternal obesity with or without GDM.

Methods

Placental sphingolipid content was assessed by LC/MSMS in female and male placentas collected at term from normal weight women (n=10), obese women (n=10), and obese women with GDM (n=10). The average placental weight was 579.4 ± 161.9, 661.7 ± 117 and 698.4 ± 136.4 grams for the normal weight, obese and obese with GDM group respectively. The average birth weight was 3.3 ± 0.199, 3.46 ± 0.27 and 3.66 ± 0.47 kilograms for the normal weight, obese and obese with GDM group respectively. The results were compared using one or two-way ANOVA and expressed as the mean ± SD.

Results

Total ceramides were decreased in placentas from obese and obese women with GDM ($p<0.02$). In both obese and obese women with GDM, ceramides 16:0 were decreased only in male placentas ($p<0.05$), while ceramides 22:0 ($p<0.02$) and 24:0 ($p<0.04$) were lower in female placentas only.

Conclusion

Our data suggest that obesity and GDM decreases placental ceramide content, independent of fetal sex. These changes may be due to lower circulating maternal adiponectin, which has been shown to decrease placental ceramide synthesis leading to activation of insulin and mTOR signaling. The lower placental ceramide content in obesity and GDM may contribute to fetal overgrowth in these pregnancy complications through the activation of placental nutrient transport by mTOR.

P1.69

Contrasting Maternal Immune Contributions in Hypertensive Disorders of Pregnancy

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Objectives

Hypertensive disorders of pregnancy (HDP) affect up to 16% of all pregnancies worldwide. HDP includes gestational hypertension (gHTN) and preeclampsia (PE), characterized by new onset hypertension after 20 weeks of gestation, in addition to end organ damage seen in PE. While it has been shown that PE originates from the placenta, its involvement in gHTN is unclear, and the circulating immune changes associated with HDP are poorly understood.

Methods

Blood was collected from 145 pregnant people (uncomplicated pregnancies – control, N=95; PE, N=19; gHTN, N=31); Mayo Clinic, MN, USA). Peripheral blood mononuclear cells (PBMCs) and plasma were isolated for analysis of the circulating inflammatory profiles (multiplex) and cytometry by time of flight (CyTOF) to characterize immune cell subsets. We also performed co-culture between PBMCs and endothelial cells to characterize their impact on the vascular endothelium. Histopathological analysis of the placentas were performed to correlate with the circulating immune changes.

Results

We observed increased placental lesions in both PE and gHTN vs control. Within the maternal circulation, multiplex cytokine/chemokine analysis revealed increased inflammatory mediators in both PE and gHTN. Interestingly, only two of these mediators were increased in both pathologies– IL-6 and CXCL-10. IL-6 was significantly more abundant in PE compared to gHTN. The rest of the altered mediators were unique to each disorder. We analyzed subtypes of immune cells by CyTOF and observed decreased proportions of CD4-T cells in PE and gHTN vs control. Cocultures showed evidence of endothelial activation/dysfunction in the presence of PBMCs from PE patients only.

Conclusion

While the systemic similarities between PE and gHTN may suggest similar placental origins, there remain distinct phenotypic differences. Further work will aim to trace back the steps from gHTN and PE to better understand the making of these disorders and address the contribution of placental dysfunction in these disorders.

P1.70

Chronic gestational inflammation: Comparisons of obesity-mediated and preeclampsia-mediated placental inflammation using transcriptomic and histological methods

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Objectives

Several obstetrical complications, including maternal obesity (MO) and preeclampsia (PE), are associated with states of chronic gestational inflammation and poor pregnancy outcomes. However, commonalities and/or differences from these divergent chronic inflammatory insults on placental development and function remain elusive. Preliminary work by our group suggests placental NAD+ depletion and subsequent mitochondrial dysfunction may be common pathophysiology features. Using rodent MO- and PE-mediated gestational inflammation models, our aim was exploring common and differing mechanisms through which placental function may become compromised, targeting NAD+ signalling pathways and mitochondrial health.

Methods

Rodent models of immune-driven PE and MO were established. Dams were euthanized at embryonic day 18.5, with placentas collected and processed for detailed histomorphometry and transcriptomic analyses. Placenta transcriptomics data underwent differential gene expression analysis using DESeq2. Ranked gene set enrichment was conducted using fold change values through GO Annotation Database. Similarly, pathway enrichment was performed through KEGG database (FDR<=0.10).

Results

Fetal growth restriction was observed in both chronic gestational inflammation models. Both MO- and PE-mediated inflammation was associated with reduced placental labyrinth exchange areas. MO-mediated inflammation additionally demonstrated increased junctional zone areas. Transcriptomic analysis revealed enriched inflammatory signalling pathways in both models; the PE model demonstrated profound TNF-Alpha signalling activation while the MO model demonstrated activated IL-1β, IFN-β and TNF signalling, and leukocyte chemotaxis. Likewise, both models demonstrated transcriptomic evidence of mitochondrial dysfunction including downregulated mitochondrial ATP synthesis, oxidative phosphorylation and NADH synthesis pathways.

Conclusion

Chronic gestational inflammation is associated with disrupted placental structure and gene expression, culminating in altered fetal growth. While our PE and MO models displayed clinically relevant inflammatory phenotypes, the pathological mechanisms underlying the established placental dysfunction were distinct. Further studies are needed to clarify the potential role of inflammation-mediated mitochondrial dysfunction in establishing inflammatory placental pathologies to inform design of better therapeutic management of these pregnant population.

P1.71

Ironing out the mystery: Exploring Cellular Iron Transport and Iron-dependent Intracellular Pathways in Fetal Growth Restriction

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Objectives

Fetal growth restriction (FGR) is a complex fetal pathology, commonly associated with altered placental structure and function. Consequently, fetal nutrition and oxygen supply to the fetus is compromised, impairing growth and development. Iron is crucial for oxygen transport to the fetus and participates in many intracellular functions, including mitochondrial heme synthesis and erythrocyte biogenesis within the placenta. However, little is known about these pathways in FGR. This project seeks to characterise iron transport, mitochondrial heme synthesis and erythrocyte biogenesis pathways in FGR placentae.

Methods

Placental tissues from healthy (n=19) and FGR (n=18) pregnancies delivered at 37-40 weeks gestation were collected. RT-qPCR was performed to determine gene expression profiles of iron transport, mitochondrial heme synthesis and erythrocyte biogenesis pathways.

Results

FGR placentae displayed significantly upregulated genes associated with iron transport, TFRC (p=0.006) and SLC11A2 (p=0.03) compared with healthy placentae. However, SLC40A1 expression was similar in FGR and healthy placentae, indicating increased requirements for placental iron use. There was increased expression of mitochondrial heme synthesis pathway genes, CPOX (p=0.002) and FECH (p=0.003), in FGR placentae compared with healthy controls, suggesting increased heme generation. Investigation into erythrocyte biogenesis showed no significant alterations in HEMGN mRNA expression, implying that progenitor cells remain unaffected in FGR. However, HBB mRNA expression (p=0.04) was significantly increased in FGR placentae. Conversely, a structural component of the erythrocyte membrane, EPB41, was significantly decreased in FGR placentae (p=0.027), which may indicate potential alterations in membrane integrity in erythrocytes within FGR placentae, despite increased hemoglobin synthesis.

Conclusion

Our study revealed altered iron transport, mitochondrial heme synthesis and erythrocyte biogenesis pathways in FGR placentae. Specifically, FGR placentae may be increasing iron retention within the placenta for placental utilization. Despite upregulated heme and haemoglobin synthesis, compromised erythrocyte structure may contribute to inadequate fetal oxygen delivery in FGR, leading to reduced fetal growth.

P1.72

Purinergic signalling in the human placenta and functional analysis of purinoceptor P2Y6 in placental development

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Objectives

The purinergic signalling pathway has been highly conserved throughout evolution. Its dysregulation underlies multiple pathologies in various tissues. Surprisingly, its expression and role in the placenta remains largely unknown. This study aimed to characterise the placental purinome throughout gestation and in pregnancy complications where placental dysfunction is central. While also investigating the potential functional role of purinoceptor P2Y6 in placental expansion, vasculogenesis and angiogenesis.

Methods

Primary human placental tissue was collected from first (7-10 weeks; n=11), second (24-28 weeks; n=9), and third trimester (term 38-39 weeks; n=11) normotensive pregnancies. Placental tissue was collected from preterm pathological pregnancies at <34 weeks' gestation from pregnancies complicated by preeclampsia (n=63), fetal growth restriction (FGR; n=14), and gestation-matched normotensive controls (n=15). Expression of purinoceptors was assessed (RT-qPCR). Angiogenesis was measured in mouse aortic ring outgrowths and HUVEC tube formation assays with or without P2Y6 receptor inhibition via antagonist MRS2578.

Results

mRNA expression was detected for all 19 purinoceptors, with differential expression of many purinoceptors evident throughout gestation and with additional differential regulation in pathology. Notably, expression of P2Y6 increased significantly throughout gestation from first trimester but decreased significantly in preterm preeclampsia. P2Y6 inhibition in aortic ring outgrowths and HUVEC tube formation assays with P2Y6 receptor antagonist MRS2578 demonstrated a significant decrease in de novo vessel formation and angiogenic sprouting.

Conclusion

These data identify and characterise, for the first time, the entire purinome in the human placenta throughout gestation, and its dysregulation in placental pathologies. Furthermore, these data reveal a novel functional role for P2Y6 in placental de novo vessel sprouting and angiogenesis, which has important implications for placentation and vascularisation. Additional studies are currently underway to elucidate further crucial functional roles that P2Y6 plays in placental development.

P1.73

Investigation of Japanese traditional medicines, Kampo, for the treatment of preeclampsia

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Objectives

Preeclampsia is a devastating complication of pregnancy, featuring profound injury to the maternal vascular system, major-organs, and utero-placental unit. Despite significant morbidity and mortality, a treatment for preeclampsia currently remains an unmet need in clinical care. Here we assess several Japanese traditional medicines with precisely defined herbal formulations, known as Kampo, for their ability to mitigate preeclampsia pathophysiology in primary human cells and tissues.

Methods

Isolated primary human cytotrophoblasts, umbilical vein endothelial cells (HUVECs), uterine microvascular endothelial cells (UtMVs), and placental tissue explants were treated with Kampo formulations #17 (Goreisan; 5 – 10 mg/ml), #23 (Tokishakuyakusan; 2.5 – 5 mg/ml), #43 (Rikkunshito; 5 – 10 mg/ml), #68 (Shakuyakukanzoto; 1.25 – 2.5 mg/ml), #86 (Tokiinshi; 1.25 – 2.5 mg/ml), and #102 (Tokito; 1.25 – 2.5 mg/ml), carefully selected for their beneficial properties. TNFα was used to induce endothelial dysfunction in both HUVECs and UtMVs. Cell viability was assessed by MTS assay. Secretion of anti-angiogenic soluble fms-like tyrosine kinase (sFLT)1 and pro-angiogenic placental growth factor (PlGF) were assessed by ELISA. Expression of anti-oxidant heme oxygenase *(HO)1*, pro-inflammatory cytokine interleukin 1b, and markers of vascular dysfunction endothelin 1 and vascular cell adhesion molecule (*VCAM*) were assessed by qPCR.

Results

Kampo formulations had a positive effect on cellular viability. In primary cytotrophoblasts, Kampo formulations modestly increased secretion of sFLT1, while in placental explant tissue, Kampo formulations significantly decreased sFLT1 secretion. PlGF secretion increased up to 8-fold and *HO1* expression increased up to 3.5-fold in cytotrophoblast cells with Kampo treatment. Endothelial dysfunction (both HUVEC and UtMV) was at least somewhat mitigated, with Kampo formulations significantly decreasing expression of *VCAM*.

Conclusion

In our *in vitro* models utilising primary human cells and tissues, Japanese traditional medicines, Kampo, show potential as a novel therapeutic approach in the treatment of preeclampsia and warrant further investigation.

P1.74

ARFGAP1 expression is reduced in pregnancies complicated with preeclampsia

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Objectives

Actin cytoskeleton undergoes structural changes during cell proliferation and migration, events essential in the initial stages of mammalian pregnancy. ARFGAP1, a GTPase activating protein (GAP) for Arf1 is found on Golgi membranes and is known to have impact on the actin cytoskeleton and its reorganization. Hence, it may also affect the remodeling of cytoskeleton in trophoblast cells, regulating their migration and invasion into the uterine endometrium during implantation. Aberrant trophoblast cell migration and invasion can lead to pregnancy complications such as preeclampsia. Thus, the present study was designed to determine the expression of ARFGAP1 in early and late onset preeclampsia patients as the process of trophoblast migration is presumed to be more compromised in the early onset preeclampsia.

Methods

ARFGAP1 expression was assessed by immunohistochemistry and western blot in placentae from early and late onset (EOPE and LOPE, n=10 each) preeclamptic and their gestational age-matched normotensive, non-proteinuric control (n=20) pregnancies. Serum ARFGAP1 level was assessed by sandwich ELISA in EOPE (n=20), LOPE (n=20) and gestational age-matched normotensive, non-proteinuric control (n=40) women.

Results

In EOPE, significantly reduced ARFGAP1 expression was seen as compared to LOPE and their gestational age-matched controls in both placentae and sera. Additionally, though the ARFGAP1 expression was lower in LOPE women as compared to their age-matched controls, however it was not statistically significant.

Conclusion

Differential expression of ARFGAP1 among EOPE and LOPE pregnant women suggest its diverse contribution and point towards the different etiologies of the two subsets of preeclampsia. Further explorations of the precise mechanisms underlying ARFGAP1 functions could yield valuable insights into placental development offering potential interventions to mitigate pregnancy complications and contributing to its role in unravelling the origins of preeclampsia.

P1.75

ZKSCAN1 expression in placentae of preeclampsia pregnant women: implications in the pathogenesis of preeclampsia

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Objectives

ZKSCAN1 plays regulatory roles in the transcription level of multifold gene and codes for protein known as Zinc Finger with KRAB and SCAN Domains1. Various in vitro and in vivo experiments indicate that ZKSCAN1 may promote cell viability and proliferation by modulating the apoptotic pathways. Additionally, its role in other biological functions like cell migration invasion and angiogenesis have been shown to influence cancer progression. The association of ZKSCAN1 and trophoblast cell invasion has not yet been explored. Successful trophoblast invasion is crucial for remodelling of maternal spiral arteries to maintain sufficient blood flow to the placenta, which in turn ensures adequate delivery of oxygen and nutrients to the foetus. Incomplete or abnormal spiral artery remodelling leads to reduced placental perfusion, which is considered as a hallmark of preeclampsia.

Methods

Placentae were collected from pregnancies complicated with preeclampsia (n=20) and normotensive, non-proteinuric controls (n=20). Gestational age matched preeclamptic as well as control placentae were analyzed for ZKSCAN1 expression by immunohistochemistry.

Results

The ZKSCAN1 expression was seen in the cytoplasm of different cell types of placenta i.e. syncytiotrophoblast, stromal cells and endothelial cells. The expression of ZKSCAN1 was increased significantly in placentae of preeclampsia pregnant women when compared to placentae of matched controls.

Conclusion

The present study revealed elevated expression of ZKSCAN1 in the placentae of women with preeclampsia compared to normotensive pregnant women. This increased expression may be associated with reduced ability of trophoblast cells to invade the maternal endometrium during the early stages of pregnancy. Further investigations of the precise mechanisms underlying ZKSCAN1's functions could yield valuable insights into the placental development. This in turn could offer opportunities for interventions to address pregnancy complications and further understanding of the relationship between pregnancy and the genesis of preeclampsia.

P1.76

Advancements in Placental Contraction Detection: A Deep Learning Approach Using 4D MRI Data

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Objectives

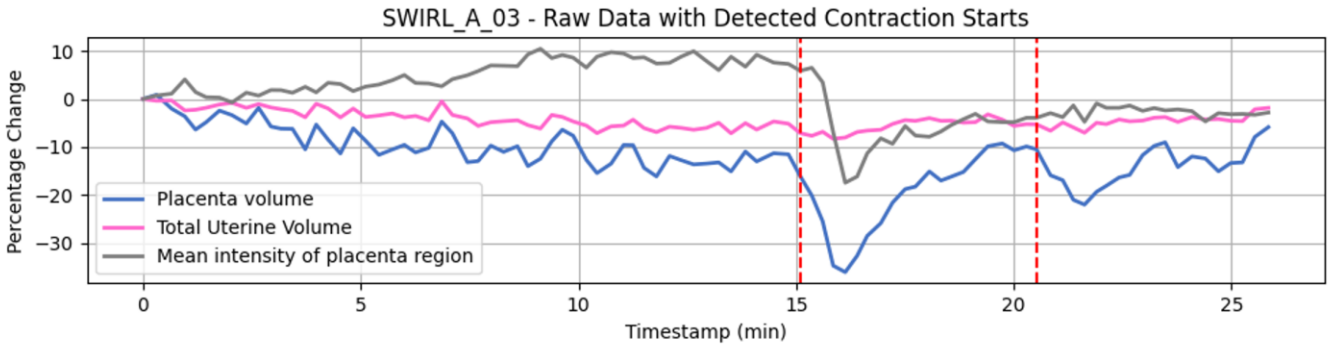
This study aims to predict placental contractions using advanced imaging and machine learning techniques. By leveraging 4D time-series MRI data and deep learning algorithms, we seek to develop an intelligent method to accurately identify and analyze placental contractions from MRI scans, a crucial factor in understanding fetal health and pregnancy outcomes.

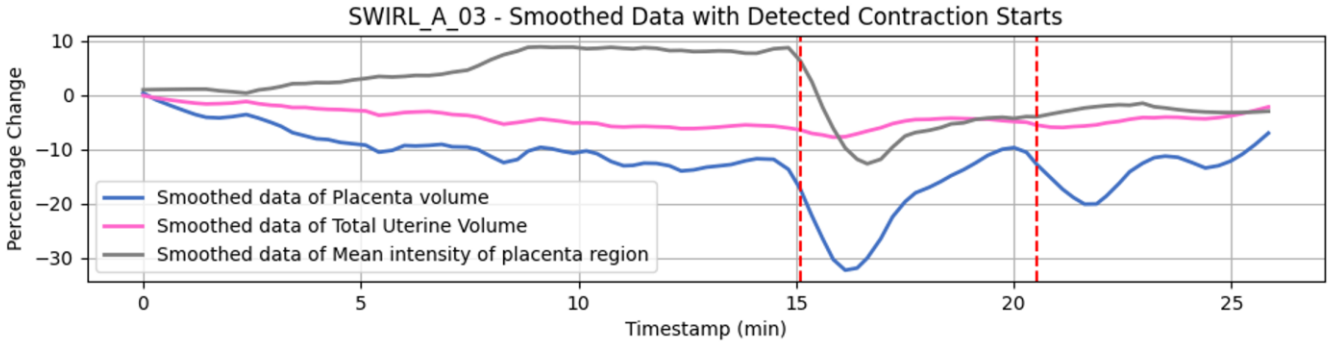
Methods

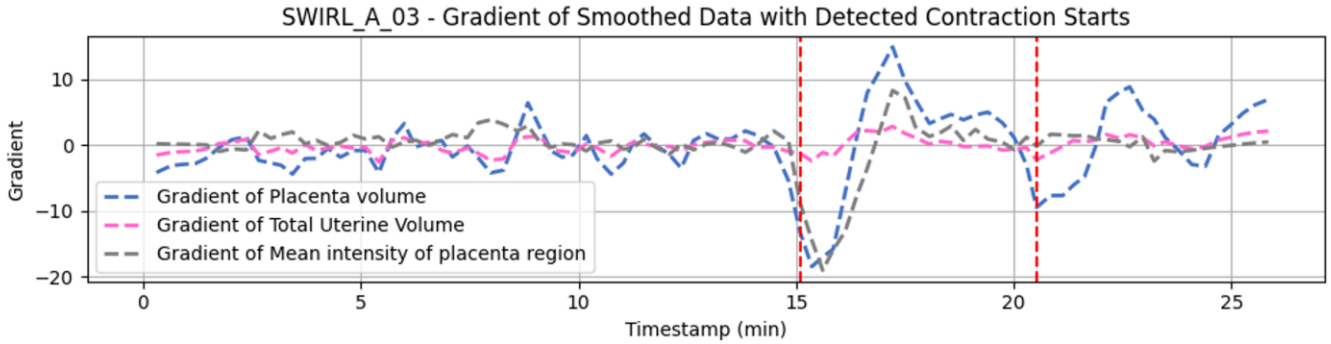
Our study analyzes 4D time-series MRI scans of 26 pregnancies, observed over approximately 30 minutes at a temporal frequency of approximately 9s (respiratory gated), with 44 manually segmented 3D volumes for only 11 pregnancies. We employed a deep learning model (nnU-Net 3D) to generate segmentation masks for the uterus and placenta. To detect contractions, we analyzed the changes in placental and uterine volumes and the mean intensity within the placental region, applying a Savitzky-Golay filter for curve smoothing and gradient analysis for contraction onset detection. Additionally, an unsupervised registration model was trained on all data, monitoring uterine and placental movement and producing another contraction detection result set. This dual approach allowed for comprehensive comparison and validation, enhancing method reliability.

Results

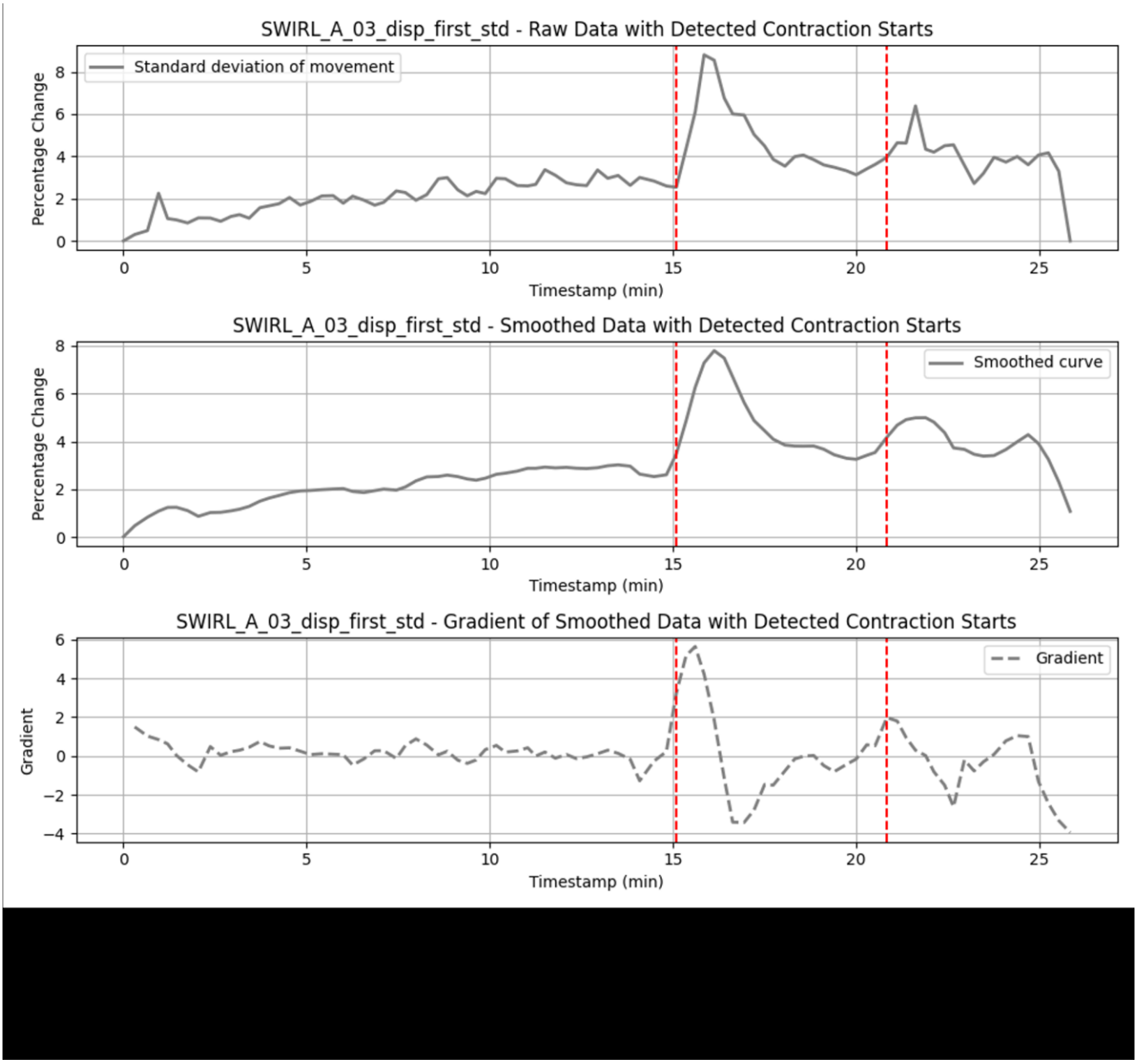
Through segmentation and displacement field analysis, as shown in Figures 1 and 2, respectively, we identified significant fluctuations in placental contractions, consistent with our gradient analysis thresholds. These fluctuations, confirmed by a visual assessment of MRI scans, suggest that our methodologies can provide valuable insights into the timing and occurrence of placental contractions. By comparing outcomes from these different analytical methods, we have begun to establish a basis for reliable detection of placental contractions.


SWIRL_A_03 - Raw Data with Detected Contraction Starts
Percentage Change
Timestamp (min)
Placenta volume
Total Uterine Volume
Mean intensity of placenta region


SWIRL_A_03 - Smoothed Data with Detected Contraction Starts
Percentage Change
Timestamp (min)
Smoothed data of Placenta volume
Smoothed data of Total Uterine Volume
Smoothed data of Mean intensity of placenta region


SWIRL_A_03 - Gradient of Smoothed Data with Detected Contraction Starts
Gradient
Timestamp (min)
Gradient of Placenta volume
Gradient of Total Uterine Volume
Gradient of Mean intensity of placenta region

[redacted]



Conclusion

This study introduces innovative methodologies for the non-invasive detection of placental contractions using MRI time-series scans. Future work will focus on pinpointing specific contraction intervals and developing a comprehensive dataset with validated ground truth, achieved through the corroboration of our detection methods. This advancement aims to improve pregnancy monitoring and fetal health assessment significantly.

P1.77

Fluid flow patterns in the intervillous space of a scaled, 3D-printed, human placental cotyledon as visualized by high-resolution MRI

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Objectives

Previous research has demonstrated only numerically the effects of spiral artery conversion on the uteroplacental blood flow. Our objective was to determine experimentally the flow patterns in the intervillous space (IVS) of a human placental cotyledon as a result of physiological and pathological spiral artery conversions.

Methods

A single cotyledon was measured by Micro-CT with a voxel size of 50 µm. The resulting images were processed to identify the villous tree and to remove unresolved structures. A 3D model of the cotyledon was reconstructed from the images in Blender. Inlets for the spiral artery openings and outlets for the draining veins were designed and added to the model. The morphology of the spiral artery was reconstructed in 3D from serial histological sections and added to the model. Four variants of the model were constructed based on the combination of spiralization and terminal dilation of the artery. This model was scaled up by a factor of 8 for 3D-printing. Finally, a fluid mimicking the properties of human blood was introduced in the artery at physiological velocities. The resulting distribution of velocities of the fluid in the IVS was measured by MRI.

Results

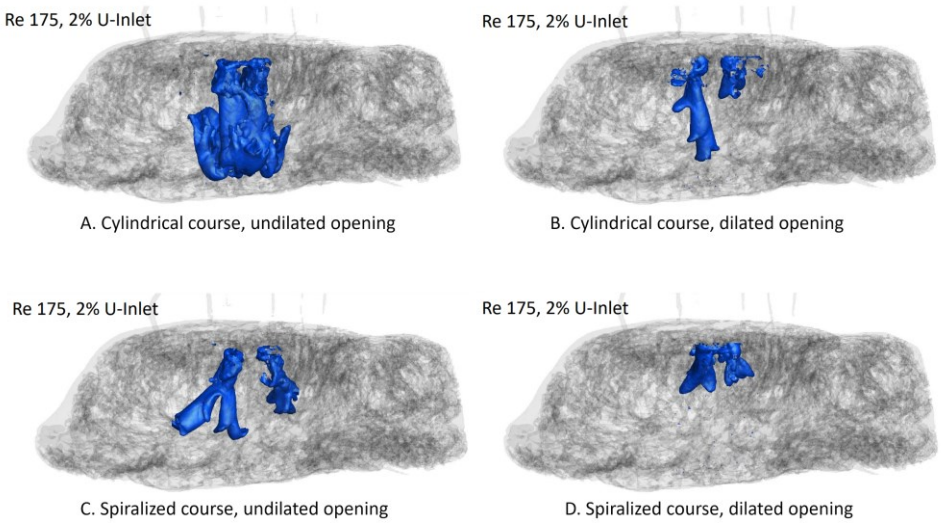

Figure 1: Magnetice Resonance Velocimetry (MRV) measurements of fluid flow pattern in the IVS of a human placental cotyledon.

Fig. 1 shows the fluid flow for the four variants at 2% inlet velocity and Reynolds number 175. The measurements show that high-velocity jets are observed in non-spiralized variants as well as in undilated variants. Only the spiralized, dilated variant showed a gentler flow in the IVS. Furthermore, measurement with a Reynold's number of 80 showed a very low, negligible motion of the fluid.

Conclusion

We show by experimental measurements that terminal dilation and spiralization of arteries has profound effect on the fluid flow in the IVS, wherein the terminal dilation has a far greater effect. Furthermore, the uteroplacental blood flow is strongly associated with the Reynold's number.

P1.78

Investigating Villous Tree Alterations in Gestational Diabetes: Beyond Macrosomia

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Objectives

Poorly managed Gestational Diabetes Mellitus (GDM) may cause macrosomia in both the placenta and newborn, affecting the villous tree structure. Conversely, well-managed GDM doesn't significantly alter placental/newborn gross characteristics compared to normal placentas. This study aims to explore if the microscopic structure of the villous tree reflects these macroscopic normalizations in well-controlled GDM.

Methods

GDM was identified by an abnormal Glucose Tolerance Test in the first trimester. Whole-depth tissue samples were collected by systematic random sampling from control (n=12) and GDM-affected placentas (n=12), following established guidelines. By immunohistochemical staining for perivascular contractile sheath the contractile parts of the villous tree were differentiated from the non-contractile parts. Among others, respective volumes and branching indices were estimated by Stereology. Branching index is an estimate of the spatial density of branching points of the villous trees.

Results

There was no significant difference between the groups in the volumes of villi with or without perivascular myofibroblasts. However, the branching index was significantly reduced in GDM placentas compared to normal ones, across both villi types. No sex differences were observed in this reduction.

Conclusion

This study reveals that well-managed GDM impacts the villous tree's branching patterns, even in the absence of macrosomic effects, indicating GDM's persistent influence on villous architecture throughout the second and third trimesters. Despite known sexual dimorphism in GDM effects, this particular outcome was not sex-dependent.

P1.79

Fluidic cellular in vitro model resembling the maternal-fetal interface

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Objectives

Pregnant women often require medication for pregnancy-related or pre-existing conditions. We aimed to develop a flow-driven *in vitro* model using primary trophoblasts and endothelial cells. This model shall be highly physiological, mimicking the human maternal-fetal barrier, and should have the potential to be applied in monitoring maternal-fetal drug transfer.

Methods

Cytotrophoblasts (CTB) and human umbilical vein endothelial cells (HUVEC) were isolated using trypsin/DNAse/collagenase digestion. Co-cultures of CTB-HUVEC were seeded onto Transwell® inserts and cultured under static conditions. The monolayer formation was assessed by measuring transepithelial-electrical-resistance (TEER) and the permeability of fluorescent dyes, including Lucifer yellow (LY; 50µM) and Inulin (20µM). To evaluate the diffusion properties, caffeine (3.5mM) and antipyrine (0.42mM) transfer were studied over 24h. Additionally, the transport of Rhodamine 123 (10µM), mediated by the ABC transporter P-glycoprotein, was investigated on day 5 in the absence or presence of the P-gp inhibitor Cyclosporine (CsA, 10µM) at flow rates of 50 and 150µL/min.

Results

TEER values increased on day 2 and remained constant until day 6. There was a decrease in LY permeability on day 3, which was stable until day 6. The transfer of Inulin after 24h was 7.5%, falling within the desired range of <1%/h. After 24h, the transfer of caffeine and antipyrine were 41.3% and 27.7%, respectively, with no significant differences observed between the two flow rates. The transport of Rhodamine 123 to the basal compartment did not differ in the presence of CsA; however, an increase in intracellular accumulation was detected when CsA was applied.

Conclusion

The initial results obtained using the fluidic *in vitro* model with primary cells are promising. These findings will be further expanded by performing nutrient and drug transport assays. Our primary cell co-culture model has the potential to serve as a secure preliminary drug screening tool, potentially averting harmful effects on the unborn

P1.80

Intergenerational impact of maternal inflammation on uteroplacental expression of the amino acid transporters SNAT1 and SNAT2

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Objectives

Compared with women born with normal weight, women born with low birth weight have a higher risk of delivering children with low weight at birth. Fetal growth restriction (FGR) is often associated with aberrant maternal inflammation, and our previous research revealed that aberrant inflammation in pregnant rats, induced by administration of lipopolysaccharide (LPS), resulted in FGR in two subsequent generations. Moreover, F2-generation pups of LPS-lineage exhibited decreased expression of glucose transporter-1 (GLUT1) in their uteroplacental units. Because an adequate supply of amino acids is critical for fetal growth, we investigated the intergenerational impact of abnormal maternal inflammation on the uteroplacental expression of amino acid transporters.

Methods

Using immunohistochemistry and HALO® image analysis software, we assessed the uteroplacental expression of sodium-coupled neutral amino acid transporters (SNAT) 1 and 2 across two generations of rats following LPS administration to the F0-generation pregnant mothers. We also analyzed GLUT1 expression in the F1-generation uteroplacental units to further explore potential intergenerational differences in glucose transport.

Results

SNAT1, SNAT2, and GLUT1 were localized to the mesometrial triangle, junctional zone, and labyrinth of uteroplacental samples. SNAT1 immunostaining was decreased in all three regions of the uteroplacental units of F2 pups whose grandmothers were exposed to LPS, while SNAT2 expression was not altered. Uteroplacental expression of SNAT1, SNAT2, and GLUT1 did not differ between LPS- and saline-treated lineages in the F1 generation.

Conclusion

Aberrant maternal inflammation may impair fetal growth cross-generationally through decreased expression of amino acid transporters and other nutrient transporters. Additional mechanisms may also contribute to inflammation-induced FGR.

P1.81

Store-operated Ca2+ entry in the human placenta

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Objectives

As the pregnancy progresses, the requirements of the fetus for minerals such as calcium (Ca2+) increase significantly. Dysregulation in Ca2+ signaling and altered Ca2+ homeostasis have been associated with severe placental pathologies, including gestational diabetes (GDM), intrauterine growth restriction (IUGR), and preeclampsia (PE). However, the underlying mechanisms of intracellular Ca2+ signaling and placental Ca2+ transfer, including the expression of proteins involved in placental Ca2+ regulation and their function in pregnancy complications remain largely unknown.

Methods

This study investigated the expression of Ca2+-release activated Ca2+ (CRAC) channels, composed of Orai1-3 subunits and the ER Ca2+ sensor proteins stromal interaction molecules (STIM1 and 2) in primary human trophoblasts, placental cell lines and tissues derived from healthy and pathological placentae using RT-qPCR. Further, we analyzed store-operated Ca2+ entry (SOCE) in healthy primary trophoblast cells and immortalized trophoblast cells with Fura-2AM-based Ca2+ imaging.

Results

We found that the mRNA expression of STIM and Orai proteins is increased upon syncytium formation. Primary human trophoblast cells showed a high expression of Orai3 and a lower expression of Orai1, suggesting that Orai3 is the predominant isoform. In placental tissue affected by GDM (n=8), IUGR (n=7), and PE (n=6) the expression of Orai1 was equally low compared to normal placenta. In contrast, Orai3 expression was significantly higher in PE and moderately elevated in GDM and IUGR compared to healthy controls. Upon spontaneous syncytialization of primary trophoblast cells Fura-2AM-based SOCE was elevated. Similarly, in BeWo cells we observed changes in SOCE upon forskolin-induced syncytium formation.

Conclusion

In conclusion, our data provide evidence for SOCE in human trophoblast cells. Although the role of CRAC channels in placental tissue is poorly understood, their involvement may have consequences for transplacental Ca2+ transport and potentially contribute to the pathophysiology of placenta-related pregnancy complications.

P1.82

Trophoblast-Specific Overexpression of *Slc7a5* (LAT1) Increases Fetal Amino Acid Concentrations and Fetal Growth in Mice

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Objectives

Placental System L amino acid transporter activity are decreased in IUGR and increased in fetal overgrowth. We have demonstrated that trophoblast-specific Large Neutral Amino Acid Transporter Small Subunit 1 (*Slc7a5*/LAT1) overexpression (OX) in mice increased the transplacental leucine transport and fetal growth. However, the mechanistic link between the increased transfer of leucine and the stimulation of fetal growth is unclear. One possibility is that trophoblast-specific *Slc7a5*/LAT1 results in elevated circulating amino acids, which may stimulate increased secretion of insulin and IGF-1, two critical fetal growth hormones. We hypothesized that trophoblast-specific *Slc7a5* OX increases fetal plasma amino acid levels.

Methods

At Embryonic day (E) 3.5, blastocysts from super-ovulated, time-mated B6D2F1 females were collected and transduced with lentivirus with constructs for *Slc7a5*-OX or scramble (SCR) sequences. Blastocysts transduced with *Slc7a5* OX (containing a mCherry reporter gene) and SCR (GFP reporter gene) were mixed and transferred in both uterine horns of pseudo-pregnant CD-1 recipient dams. Maternal and pooled fetal plasma samples (n=6/each group) were collected to measure amino acid concentrations using HPLC. Statistical significances of differences were determined by a paired t-test. P values < 0.05 were considered significant.

Results

On E18.5, Slc7a5 mRNA expression was 5-fold higher in *Slc7a5* OX) compared to SCR placentas (P=0.001). Maternal amino acid levels were not different between groups. Trophoblast-specific *Slc7a5* OX increased the fetal circulating essential amino acids levels such as leucine (+ 95%, p=0.007), phenylalanine (+84 %, p=0.009), tryptophan (+47%, p=0.02), lysine (+102 %, p=0.006), histidine (+41 %, p=0.04), valine (+62 %, p=0.03), methionine (+85% p=0.02), and isoleucine (+58%, p=0.03) as compared to SCR.

Conclusion

This is the first demonstration that trophoblast-specific overexpression of LAT1 increases the steady-state fetal levels of circulating essential amino acids, which may be mechanistically linked to fetal growth. We postulate that targeting placental LAT1 may represent a novel intervention in abnormal fetal growth.

P1.83

Assessing the expression of placental nutrient transporters following teratogenic valproic acid exposure in CD-1 mice.

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Objectives

Valproic acid (VPA) is a potent human and rodent teratogen that causes numerous adverse pregnancy outcomes. Specifically, VPA can cause fetal growth restriction (FGR), the failure of a fetus to achieve its genetic growth potential. FGR is often the result of placental insufficiency, characterized by insufficient transplacental nutrient transport. Placental nutrient transporters facilitate nutrient uptake from maternal circulation and their downregulation has been linked to placental insufficiency and, consequently, FGR. Multiple studies have shown that VPA can alter placental nutrient transporter expression; however, the direction of expression varies across experimental models. Therefore, this research aims to characterize the effect of VPA on placental nutrient transporter expression using a CD-1 mouse model.

Methods

Pregnant female CD-1 mice were subcutaneously injected with either saline or one of two teratogenic VPA doses (400 mg/kg or 600 mg/kg) on gestation day (GD) 9. The dams were euthanized on GD18, and fetal and placental tissues were collected. The placental tissue was subsequently homogenized for RNA extraction and cDNA synthesis. Primers for nutrient transporters will be purchased from BioRad. Relative mRNA levels of each nutrient transporter will be quantified using reverse transcriptase quantitative PCR (RT-qPCR).

Results

This analysis will initially focus on glucose and folate transporter expression. Glucose and folate are essential for fetal growth and development, and the placenta expresses various transporters to facilitate their uptake from the maternal blood to meet fetal demand. Decreased expression of glucose and folate transporters has been implicated in FGR. Therefore, the results of this study will compare the expression of various glucose and folate transporters between exposure groups to determine if VPA alters their expression.

Conclusion

Understanding the mechanisms underlying VPA induced fetal and placental toxicity is critical so safer treatment options may be developed, and VPA-treated mothers can achieve healthy, successful pregnancies.

P1.84

Placental Barrier Transport: Identification of Maternal and Infant Determinants of BCRP and MDR1 Transporter Levels

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Objectives

The 'placenta barrier' is comprised of membrane transporters including BCRP and MDR1 that actively efflux chemicals from the syncytiotrophoblast back to the maternal circulation. This function allows BCRP and MDR1 to limit the accumulation of drugs and toxicants and prevents transplacental transfer of substrates. In this study, we evaluated relationships between maternal (gestational weight gain, early pregnancy body mass index, smoking, parity, pregnancy complications, age) and infant factors (birth weight, race, transporter genetics) and placental levels of BCRP and MDR1 proteins in a healthy U.S. cohort.

Methods

Term placentas were collected from healthy participants in the Understanding Pregnancy Signals and Infant Development Study (UPSIDE) birth cohort (Rochester, NY, n=237). BCRP and MDR1 proteins were quantified in frozen villous placenta tissues using quantitative targeted absolute proteomic mass spectrometry. We examined determinants of placental protein concentrations through bivariate analyses and multivariable linear regression models.

Results

Levels of BCRP and MDR1 proteins in term placentas were moderately correlated ($r=0.58$, $p<0.001$). In bivariate analyses, BCRP levels were associated with gestational weight gain, infant race, parity, and the rs2231142 genetic variant. In mutually adjusted models, only parity ($\beta=0.07$; 95%CI:0.02,0.11) and the rs2231142 AC/AA genotype ($\beta=-0.09$, 95%CI:-0.14,-0.04) showed notable associations with BCRP levels. By comparison, in bivariate analyses, MDR1 levels were associated with parity and more weakly, with birthweight, race, smoking, and the rs1045642 and rs2032582 gene variants. In mutually adjusted models, parity ($\beta=0.11$; 95%CI:0.05,0.17), smoking ($\beta=-0.11$; 95%CI:-0.21,-0.01), the rs1046642 TT genotype ($\beta=-0.14$, 95%CI:-0.24,-0.03), and the rs2032582 TT genotype ($\beta=0.10$; 95%CI:-0.02,0.21) were associated with MDR1 levels.

Conclusion

Identifying maternal and infant factors that contribute to the regulation of BCRP and MDR1 levels in healthy, term placentas is the first step in dissecting the impact of disease and environment on the

integrity of the placental barrier. Supported by R01ES029275, R01HD083369, UG3OD023349, UH3OD023349, P30ES005022, UL1TR003017, and UC2HD113039.

P1.85

Reconstruction of 3D human placental barrier in a microfluidic chip

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Objectives

When taking a medicine during pregnancy, the mother-to-fetus transfer of the drugs across the placental barrier, which is formed by the syncytiotrophoblasts, needs to be taken into consideration. It is thus essential to establish the prediction system of the placental barrier permeability for assessing the risk of the fetal drug exposure. Although the rodents are mostly used to evaluate the *in vivo* drug permeability, the problem of the structural and functional differences in the placental barrier between the rodents and human has not been overcome. The purpose of this study is to reconstruct the 3D human placental barrier in a microfluidic device.

Methods

Human trophoblast stem cells were mixed with thrombin and fibrinogen and introduced into a microfluidic device. The trophoblast stem cells were differentiated into the syncytiotrophoblasts in the device. The syncytiotrophoblast marker protein expression was evaluated by immunohistochemistry. To assess the transport activity, fluorescent-labeled extracellular vesicles, human immunoglobulin G, and dextran was introduced into the device and the fluorescent images were taken using a confocal laser scanning microscope.

Results

The continuous culture of trophoblast stem cells in the device led to the 3D structure formation. The fluorescence signals of human chorionic gonadotropin, a marker of syncytiotrophoblasts, were detected the outer layer of the 3D structure, indicating the formation of the syncytiotrophoblast layer. When the fluorescent-labeled extracellular vesicles or immunoglobulin G were introduced into the device, the fluorescence signals were detected in the outer layer of the 3D structure. In contrast, there was no specific fluorescent signal of dextran, a membrane impermeable marker, in the structure. These results suggested the reconstruction of human 3D placental barrier in a microfluidic device, which would be useful for evaluating the placental barrier transport.

Conclusion

We have established the 3D human placental barrier model in a microfluidic chip.

P1.86

Placental extracellular vesicles: their unique characteristics of the blood-brain barrier transport

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Objectives

The placenta actively secrets the extracellular vesicles into the maternal circulation. These placental extracellular vesicles (pEVs) encapsulate the microRNAs, which can modulate the function of pregnant women's body. It is known that pregnancy renders substantial changes in brain gene expression and function. We hypothesized that pEVs mediate the placenta-to-brain transduction of microRNAs across the blood-brain barrier (BBB), thereby modulating the pregnant women's brain function. The purpose of this study was to characterize the pEVs transport system at the BBB as a key mechanism of the placenta-to-brain transduction of microRNAs.

Methods

The pEVs were collected from the culture medium of human placental trophoblast cells (BeWo cells) and rat placental tissue fragments, and analyzed by Zetasizer Nano. The expression levels of microRNAs in the brain and the circulating blood of the pregnant and non-pregnant female mice were determined by quantitative PCR. The BBB transport of PKH67-labeled pEVs was evaluated at 37^{o}C by three-dimensional (3D) human brain microvasculature model called the human BBB on-a-chip, the rat BBB spheroid model, and the human brain microvascular endothelial cells (hCMEC/D3 cells).

Results

The placenta-associated microRNA was detected both in the pregnant mice brain and the blood-circulating vesicles. When the pEVs were perfused in the human BBB on-a-chip, the fluorescent signals were localized in the endothelial cells as well as in the brain parenchymal cells. The pEVs obtained from rat placental tissue fragments were taken up by the endothelial cell layer of the BBB spheroid model. Super-resolution microscopy showed that the pEVs obtained from BeWo cells were localized in the cytoplasm of hCMEC/D3 cells. The uptake was significantly decreased in the virus receptor-knockout hCMEC/D3 cells. These results suggest that the BBB possesses the transport system of pEVs, which involves the virus receptor.

Conclusion

The pEVs undergoes the BBB transport mediated presumably by our newly identified receptor.

P1.87

Dissecting the microenvironment of gestational trophoblastic disease through deconvolution of bulk RNA-seq data

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Objectives

Gestational trophoblastic disease (GTD) describes a group of pre-malignant and cancerous lesions that arise from the trophoblast cells of the placenta. The drivers of GTD remain elusive. In its inherent state, the trophoblast exhibits certain similarities to cancer, such as angiogenesis and invasion of the surrounding tissues. To unveil the mechanisms underlying the transition from healthy placenta to malignant phenotype, this study utilises deconvolution of transcriptomic data to estimate cellular composition of GTD lesions.

Methods

BayesPrism was used to deconvolute transcriptome data from 4 pre-malignant complete hydatidiform moles (CHMs), 2 choriocarcinomas (CCs), 4 placental site trophoblast tumours (PSTTs), and 4 epithelioid trophoblast tumours (ETTs), using single-cell RNA-seq data from cells of the maternal-foetal interface as a reference. BayesPrism was also used to estimate sample-specific gene expression in extravillous trophoblast (EVT), a suspected cancer-initiating cell type. This data was then evaluated for differential gene expression and gene set enrichment analysis.

Results

Epithelial glandular cell content increased with mortality rate, notably higher in PSTT and ETT versus control ($p = 0.038$ and $p = 0.002$, respectively). Immune cell content also rose with disease severity. Unlike healthy and molar samples, cancer subgroups exhibited innate lymphoid cells (ILCs), key in maternal-foetal immune tolerance and trophoblast invasion.

PTEN was significantly upregulated in molar EVT, and its role in hypoxic response in the placenta is further confirmed by the enrichment of the hallmark pathway in CHM. Upregulation of ASPSCR1 and SLC2A1 and downregulation of the oxidative phosphorylation pathway in CHM and PSTT suggest a glycolytic shift. Dysregulated expression of DNA methyltransferases across all disease groups supports the previously suggested role of epigenetics in GTD pathogenesis.

Conclusion

This study is the first to consider both immune and non-immune microenvironment of GTD lesions. These findings can direct clinicians towards identifying novel diagnostic targets for developing personalised therapies against gestational neoplasms.

P1.88

Placental histopathology for early identification of infants at high-risk of neurodevelopmental delays

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Objectives

Preterm birth (PTB) is a major global health concern linked to higher incidence of neonatal mortality and morbidity, with significant implications especially for neurodevelopmental outcomes. Early identification of infants which would require follow-up remains challenging. Our goal was to investigate the association between placental histomorphology, inflammatory markers, and neurodevelopment in late preterm infants.

Methods

We followed a cohort of 141 late preterm infants (i.e. 29-36 weeks' gestation). Maternal demographic and obstetric data were collected. Placenta and fetal membrane were evaluated by histopathology (hematoxylin & eosin – HE staining) and immunohistochemical analysis for immune cells (CD45, CD68 and CD163). Neurodevelopment was assessed up to 24 months of corrected age.

Results

There were 60 infants who had abnormal neurodevelopment at 24 months of age, and their mother had elevated pre-pregnancy BMI, and higher incidence of gestational hypertension/diabetes vs those with normal neurodevelopmental assessment. Placental analysis revealed no correlation between developmental delays and general histopathological assessment. However, increased inflammatory lesions (i.e. deciduitis/villitis) were observed in the placenta of infants with neurodevelopmental delays. Furthermore, increased numbers of CD45+ cells were observed in the placenta of infants with language and cognitive delays ($p<0.05$). The same was observed in fetal membrane with increased CD68+ macrophages in the decidua of infants with an overall abnormal neurodevelopmental score (Bayley scale), especially in relation to language and motor delays ($p<0.01$).

Conclusion

Our study highlights the potential to use the placenta as a tool for identifying preterm infants who may need in-depth clinical monitoring. Further investigation is needed to increase predictive accuracy beyond classical histopathology evaluation.

P1.89

Timing of distal villous fetal vascular malperfusion in the placenta: The clinical significance and placental features

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Objectives

Fetal vascular malperfusion (FVM) can be remote, established, or recent; global or segmental; low grade or high grade. Distal villous FVM is more specific for abnormal clinical and placental phenotypes than large vessel FVM. CD34 immunostaining is diagnostic for the recent FVM. This retrospective analysis compares the value of large vessel (global, partial) and distal villous (complete, segmental) FVM, remote/established, recent and on-going.

Methods

24 independent abnormal clinical and 46 placental phenotypes were retrospectively statistically analyzed among 1002 consecutive cases mostly with congenital anomalies, in which CD34 immunostaining was performed: Group A: 398 cases without distal FVM and none or up to 2 large vessels FVM lesions. Group B: 221 cases with distal villous FVM without clustered endothelial fragmentation by CD34 immunostaining, Group C: 145 cases with clustered endothelial fragmentation by CD34 immunostaining but no clustered sclerotic or mineralized distal villi, Group D: 163 cases with coexistence distal villous lesions of Group B and Group C, and Group E: 75 cases with 3 or 4 lesions of large vessel FVM, but no distal villous FVM lesions.

Results

Established and/or remote FVM had similar clinical/placental associations as recent FVM, but on-going FVM was most commonly high grade and associated with preterm pregnancies, stillbirth, and fetal growth restriction. Large vessel FVM (3 or 4 lesions) occurred in advanced third trimester pregnancies with fetal congenital anomalies, villitis of unknown etiology, and intervillous thrombi but no direct association with abnormal maternal or fetal condition.

Conclusion

In this population of placentas, FVM was the most common pattern of placental injury. Proximal FVM was more common than distal FVM, suggesting the sequence of occurrence and the likely umbilical cord compression etiology. CD34 immunostaining doubles the sensitivity of placental examination, and frequently upgrades the FVM, therefore, it is an important adjunct to placental histology.

P1.90

First trimester combined preeclampsia screening and immune profiling reveal novel specific risk groups for hypertensive pregnancy disorders

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Objectives

First trimester preeclampsia screening by the Fetal Medicine Foundation (FMF) risk assessment calculator is useful for clinical risk stratification and prevention. The resulting risk groups are, however, diverse, and only a subgroup develops disease. Preeclampsia development has a strong immune involvement identifiable by serum cytokine profiling. This study aimed to characterize immunological changes associated with high risk for preeclampsia in the first trimester and identify potential disease-related subgroups.

Methods

The Screentox study performed FMF screening (v2.8) of pregnant women at gestational week 11-14 in 2010-2012. Serum samples were assayed for 27 cytokines by the Bio-Plex Pro Human Cytokine 27-plex Assay and analysed with hierarchical clustering and linear models.

Results

FMF risk assessment of first-trimester pregnancies (n=576) defined 11% (n=65) as high-risk (risk-score >1/100), of which 15% developed preeclampsia and 15% gestational hypertension (GH), compared to 3% and 2% in the low-risk (risk-score <1/100) pregnancies, respectively.

Cytokine profiling revealed three distinct immune groups among the high-risk pregnancies: Low (n=10), Moderate (n=43), and High (n=12). The Low group had lower BMI and serum lipids, and higher PAPP-A, than the other high-risk pregnancies, and resulted in 50% preeclampsia and no GH. In contrast, the High group gave no preeclampsia, but instead 33% developed GH. The largest group, Moderate, resulted in lower disease rate (12% preeclampsia, 14% GH) involving both disorders.

The high-risk subgroups were comparable in terms of s-PlGF levels, uterine pulsatility index or blood pressure. Overall, stronger immune activation was associated with increasing uterine PI and decreasing s-PlGF.

Conclusion

The FMF risk assessment calculator including placenta-specific biomarkers is a useful tool for prediction and prevention of preterm preeclampsia and also seems to predict hypertensive pregnancy complications in general. Cytokine profiling revealed clusters with specific disease outcomes among high-risk pregnancies and may prove a useful supplement to the FMF risk assessment.

P1.91

Cystatin 6 (CST6) and Legumain in preeclampsia

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Objectives

In the search for preeclampsia biomarkers, our *in silico* analysis identified Cystatin 6 (CST6), a cysteine protease inhibitor, as located on the placental surface where it might be released from into maternal circulation. CST6 has a high affinity for the cysteine protease legumain. We aimed to characterise CST6 and legumain in preeclampsia.

Methods

CST6 and legumain mRNA was assessed in placentas from preterm preeclampsia (<34 weeks' gestation) or gestation matched controls. Plasma CST6 and legumain was measured at 36 weeks preceding term preeclampsia diagnosis. Human (cyto)trophoblast stem cells (hTSCs) were differentiated into syncytiotrophoblast or extravillous trophoblast (EVT) or exposed to hypoxia or inflammatory cytokines and *CST6* and *legumain* assessed. Syncytialised hTSCs were treated with recombinant CST6 and sFlt-1 and legumain measured. Finally, *CST6* and *legumain* were examined following TNFα induced endothelial dysfunction and the effect of recombinant CST6 on tube formation determined.

Results

CST6 mRNA was significantly increased and *legumain* decreased in placentas from patients with preterm preeclampsia (n=78) relative to gestation matched controls (n=30, $p<0.0001$, $p<0.05$ respectively). Circulating CST6 significantly increased ($p=0.0084$) in women (36 weeks' gestation) preceding diagnosis of term preeclampsia while legumain levels decreased ($p=0.041$, n=23 vs n=181 controls).

CST6 and *legumain* significantly increased with syncytiotrophoblast ($p=0.0066$ and $p=0.0010$ respectively) and EVT differentiation ($p=0.0618$ and $p=0.0016$ respectively).

CST6 was significantly increased in syncytiotrophoblast exposed to hypoxia (1% O_2 vs 8% O_2) (p=0.0079) but unchanged with TNFα or IL-6 treatment. No change in *legumain* was observed. Treating syncytiotrophoblast with rCST6 did not alter sFlt-1 or legumain secretion.

In HUVECs, TNFα induced *ICAM-1*, *VCAM-1,* and *ET-1,* and significantly reduced *CST6* (p=0.0036) but did not alter *legumain*. rCST6 has no effect on sFlt-1 or legumain secretion from HUVECs and did not alter tube formation.

Conclusion

We provide the first data characterising CST6 and legumain in preeclampsia, demonstrating opposing expression in placenta and plasma. CST6 and legumain may have potential as biomarkers for preeclampsia.

P1.92

IDENTIFYING PLACENTAL MARKERS OF SCHIZOPHRENIA IN GESTATIONAL THC-EXPOSED OFFSPRING

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Objectives

Placental complications leading to fetal growth restriction (FGR) have been linked to differential placental gene expression associated with increased risk of schizophrenia. Our pilot data has demonstrated that edible THC exposure in pregnancy results in FGR and schizophrenic-like behaviour (*e.g.,* decreased pre-pulse inhibition) in male and female rat offspring. However, it remains elusive if prenatal THC exposure induces this schizophrenic signature of placental gene expression. Therefore, our objective was to determine if these established predictive markers of schizophrenia are altered after gestational exposure to edible THC in pregnant rats.

Methods

Pregnant rat dams received edible THC (5 mg/kg)/Nutella or Vehicle/Nutella daily from gestational day (GD)7 to GD19. Maternal measures (*i.e*., maternal food intake, weight gain) were assessed. On GD19 rat dams were sacrificed and placenta tissue was collected from both sexes. Placental RNA was isolated and prepared for qPCR analysis of 30 gene targets identified to be dysregulated in human placentae associated with an increased risk of schizophrenia (Ursini, 2021).

Results

Male and female THC-exposed offspring exhibited significantly lower birth weights and fetal-to-placenta weight ratios with no difference in maternal measures or litter size between groups. Of the top 30% human differentially expressed schizophrenic placental makers, the steady-state mRNA levels of *Rsp10* and *Furin* (involved in protein regulation) and *Rccd1* (involved in chromatin reorganization) were upregulated in both male and female THC-exposed rat placenta at GD19. Furthermore, we found placental *Iqgap1, Eif5, and Vps33b* mRNA levels were significantly increased exclusively in female THC-exposed offspring.

Conclusion

We conclude exposure to edible THC in rat dams led to FGR and altered placental gene expression similar to what has been observed in human placentae linked with heightened schizophrenia risk. Further studies are warranted to examine ontogeny of these targets in the postnatal THC-exposed brain. Supported by the Canadian Institutes for Health Research.

P1.93

Elevated high-sensitive C-reactive Protein in maternal blood: clue to suspect neutrophil migration in extra-placental membranes, but not umbilical cord and chorionic plate

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Objectives

There is a paucity of data about whether maternal high-sensitive c-reactive protein(hs-CRP) progressively increases according to the progression(neutrophil-migration) of acute histologic chorioamnionitis(acute-HCA) in each placental compartment(i.e., extra-placental membranes[EPM], umbilical-cord[UC] and chorionic-plate[CP]) among pregnant women at risk for early spontaneous preterm-birth(PTB). The objective of the current study is to examine this-issue.

Methods

Study-population included 121singleton-PTB(<34weeks) due to either preterm-labor(PTL) or preterm-PROM with both placental-pathology and maternal hs-CRP results within 48 hours before delivery. We examined maternal hs-CRP according to the progression(neutrophil-migration) of acute-HCA in EPM(i.e., group-0, inflammation-free EPM; group-1, inflammation restricted to decidua; group-2, inflammation restricted to the membranous-trophoblast of chorion and the decidua; group-3, inflammation in the connective-tissue of chorion but not amnion; group-4, amnionitis), UC (i.e., group-0, inflammation-free UC; group-1, umbilical-phlebitis only; group-2, Involvement of at least one umbilical-artery[UA] and either the other UA or umbilical-vein[UV] without extension into Wharton's jelly[WJ]; group-3, the extension of inflammation into WJ), and CP(i.e., group-0, inflammation-free CP; group-1, inflammation restricted to subchorionic fibrin[SCF]; group-2, inflammation in the connective-tissue of CP without chorionic-vasculitis; group-3, chorionic-vasculitis).

Results

Maternal hs-CRP significantly and progressively increased with the progression of acute-HCA in EPM(Spearman's rank-correlation test, $\gamma = 0.298$, $p = 0.0009$; Krusal-Wallis test, $p = 0.006$)(Fig.1). However, UC and CP did not show this-trend observed in EPM (Fig.2 and Fig.3).

Fig. 1

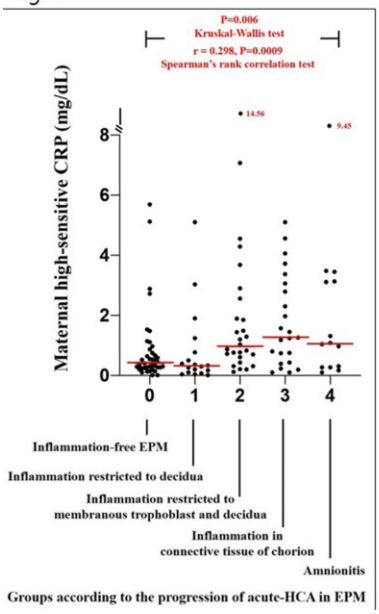


Fig. 2

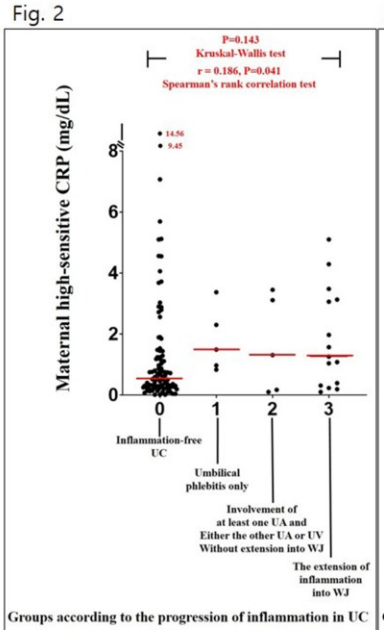
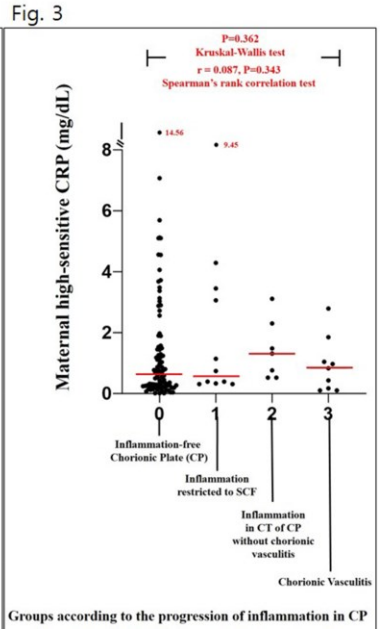


Fig. 3



Conclusion

Maternal hs-CRP significantly and progressively increased according to the progression(neutrophil-migration) of acute-HCA in EPM, but UC and CP. The performance of maternal hs-CRP reflecting neutrophil-migration in EPM should be clearly evaluated in a prospective cohort of patients with either threatened PTL or preterm-PROM. Acute-inflammation in UC and CP indicating fetal inflammatory-response does not seem to be related to maternal hs-CRP.

P1.94

Placental findings from neonates with and without congenital cytomegalovirus and associations with maternal HIV serostatus

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Objectives

Congenital cytomegalovirus (cCMV) affects 1% of children globally, is more common in regions with high HIV prevalence, and may act synergistically with HIV to trigger placental inflammation. However, data on placental effects of CMV are lacking from sub-Saharan Africa. We aimed to determine the prevalence of cCMV in a birth cohort and associations with placental findings.

Methods

We performed CMV PCR on duplicate oral swabs collected <48h after birth from neonates enrolled in a prospective cohort of women with singleton pregnancies in Mbarara, Uganda. We collected placentas for gross and histologic H&E evaluation and characterized findings using Amsterdam consensus criteria. We compared placental findings by neonatal CMV PCR result and maternal HIV serostatus.

Results

Of 440 participants, 231 (52%) were women with HIV (WHIV) taking antiretroviral therapy, and 209 (48%) were HIV-seronegative. A similar proportion of neonates with cCMV were born to WHIV and HIV-seronegative women (10/231 [4.3%] vs 10/209 [4.8%], respectively). Placental disk thickness was lower for CMV- than CMV+ neonates (1.7 vs 1.9 centimeters, *P*=0.06) but mean trimmed placenta weight was similar (455 vs 451 grams, *P*=0.87). Chronic villitis was present in 34/420 (8%) placentas from CMV- and 0/20 (0%) CMV+ neonates (*P*=0.39); and chronic chorioamnionitis in 8/420 (2%) CMV- vs 2/20 (10%) CMV+ (*P*=0.07). Acute chorioamnionitis was present in 155/420 (37%) placentas from CMV- vs 4/20 (20%) CMV+ neonates (*P*=0.16). Fetal vascular malperfusion was seen in 44/420 (10%) placentas from CMV- vs 1/20 (5%) from CMV+ neonates (*P*=0.71); and maternal vascular malperfusion in 131/420 (31%) CMV- vs 3/20 (16%) CMV+ (*P*=0.14). No classical CMV inclusions were seen.

Conclusion

cCMV was common, occurring in 4% of neonates. Placental findings were similar by cCMV status, though chronic chorioamnionitis was more common in the cCMV+ group. Future studies should examine the interactive effects of HIV and CMV on chronic placental inflammation.

P1.95

Comparison of placenta morphology by HIV status and HIV antiretroviral class.

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Objectives

Over 1 million women with HIV become pregnant each year. Most receive antiretroviral therapy (ART). Pregnant women living with HIV (WLWH) on ART are more likely to experience adverse birth outcomes including small for gestational age births. Mechanisms underlying these are not fully understood. The placenta forms the maternal-fetal interface and regulates metabolic processes necessary for fetal growth. Placenta morphology may be an indicator for *in-utero* adaptations to meet fetal needs. We examined placenta morphology by HIV status and ART class based on initial ART in pregnancy – protease inhibitor (PI), non-nucleoside reverse transcriptase inhibitor (NNRTI), or integrase inhibitor (INSTI).

Methods

Term placentas from 125 women (19 HIV-negative, 31 WLWH/PI-ART, 37 WLWH/NNRTI-ART, 38 WLWH/INSTI-ART) participating in the HIV and Zika in Infants and Pregnancy (HIV-ZIP) study were included. Morphometric parameters were computed from placenta digital images. Associations between placenta parameters, HIV/ART status, and birth outcomes were examined using linear regression or Pearson correlation.

Results

Placenta weight and area were lower in WLWH than in HIV-negative women. Compared to HIV-negative women, placenta weight and area were lower in WLWH exposed to PI-ART and NNRTI-ART, but not INSTI-ART. Marginal umbilical cord insertion was more common in WLWH vs. HIV-negative (16.5% vs. 5.3%). ART exposure from conception vs. later in pregnancy was associated with a 4.5-fold (95%CI 1.2–16.5) increase in risk of marginal cord insertion. We observed positive correlations between placenta weight and birth weight (r=0.51–0.78) and head circumference (r=0.42–0.61) z-scores across all groups. Placenta weight was positively correlated with length z-score in the PI-ART (r=0.56) and INSTI-ART (r=0.42) groups. Placenta area was positively correlated with birth weight z-score (r=0.60) and head circumference (r=0.45) in the NNRTI-ART group.

Conclusion

HIV infection was associated with lower placenta weight and area. Among WLWH, those receiving INSTIs had the most favourable placental metrics.

P1.96

Assessing the placental inflammatory profile and association with time between rupture of membranes and birth

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Objectives

Preterm birth (PTB) or birth before 37 weeks of gestation, is the leading cause of perinatal morbidity and mortality, effecting approximately 10% of pregnancies in the United States. PTB can cause lifelong health consequences in surviving newborns, such as neurodevelopmental disorders. The cause of PTB is mostly unknown but is often attributed to the preterm premature rupture of membrane (pPROM). pPROM is frequently observed in cases of spontaneous (laboring, sPTB) and non-spontaneous (non-laboring, nsPTB) preterm birth. While the presence of proinflammatory mediators in the placenta have been associated to PTB, the inflammatory mediator profile in cases of pPROM has not been fully elucidated. Therefore, we assessed the staining intensity and localization of proinflammatory cytokines in the placenta in cases of pPROM compared to term births.

Methods

Immunohistochemistry (IHC) was performed on formalin fixed paraffin embedded (FFPE) placental tissue, looking at previously defined proinflammatory cytokines known to be key drivers of PTB in preclinical models, TNF-α, IL-1β, and IL-6. Semi-quantitative grading of the staining was done by a blinded observer.

Results

Our results showed that staining intensity for TNF-α increases with longer time between ROM and delivery in sPTB, but not in nsPTB. However, this was not observed for IL-1β or IL-6, where there was no association between ROM time and staining intensity in either sPTB or nsPTB. The staining intensity was prominent for IL-1β as compared to TNF-α. Staining for all 3 cytokines was observed especially in the trophoblast layer of the placenta. Within the fetal membrane, there was no association between ROM time and TNF-α, IL-1β or IL-6.

Conclusion

Future directions involve the association of these proinflammatory cytokines to neonatal neurodevelopmental outcomes. We will also use CyTOF to further elucidate the involvement of different cell types in the production of these cytokines in pPROM. Understanding the inflammatory profile associated with pPROM will guide future therapies to mitigate severe inflammation and, subsequently, neonatal development.

P1.97

Hofbauer cells: drivers of the inflammatory profile at the placenta

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Objectives

Placental inflammation, prevalent in preterm birth (PTB), is the main cause of infant mortality and morbidity, and there are currently no available therapies targeting placenta inflammation. Thus, there is great need to understand the cell-specific mechanism of prenatal inflammation at the placenta for development of targeted therapeutics.

Methods

Paraffin-embedded placenta samples from 228 patients (classified: spontaneous PTB [sPTB], non-spontaneous PTB [nsPTB], spontaneous term [sTerm], and non-spontaneous term [sTerm]) were obtained for analysis of placental macrophages, Hofbauer cells (HBCs), by immunohistochemistry (IHC) and imaging mass cytometry (IMC). Fresh biopsies of placenta from uncomplicated term pregnancy and cell suspensions of cytotrophoblasts (CTBs) and HBCs were exposed to inflammatory inducers with production/secretion of inflammatory cytokines measured by ELISA and flow cytometry.

Results

There were significantly elevated number of CD68+ CD163+ HBCs in placentas from PTB, regardless of spontaneous vs non-spontaneous labor classification, vs. Term. IMC analysis found 22 distinct cell populations including HBCs as the only immune cell detected within placenta villi, with some T cells and monocytes in the maternal space only and a higher cell diversity in PTB. In explant models, exposure to classical bacterial or viral PAMPs significantly increased the secretion/production of pro-inflammatory cytokines (IL-1β, IL-6, MCP-1, TNFα). Cell suspensions experiments revealed that HBCs had significantly more IL-6 production compared to CTBs at basal levels. In LPS-stimulated conditions, IL-6 production was significantly increased in HBCs only, compared to the lack of significant upregulation in CTBs.

Conclusion

PTB groups had increased number of macrophages compared to term groups regardless of spontaneous vs non-spontaneous, suggesting that labor itself does not drive the placenta immune profile. Spatial analysis in placenta confirmed HBCs as the only immune cell. Placental analysis by explants and cell suspensions further confirmed HBCs as the driver of the observed inflammatory profile. Future work will investigate interactions between HBCs and trophoblasts.

P1.98

Exploring the Interplay Between the Endocannabinoid and Immune Systems in the Placenta Using Ex Vivo and In Vitro Models

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Objectives

This study investigates the role of the endocannabinoid system (ECS) in placental inflammatory responses. We aim to enhance our understanding of the ECS's interaction with the inflammatory environment in the placenta by analyzing ECS gene/protein expression across various placental models. We further plan to assess the effects of endo- and exogenous cannabinoids on placental inflammatory cytokine release.

Methods

Using qRT-PCR, ddPCR, immunofluorescence, and western blot, we conducted a comprehensive analysis of the ECS in various placental models, including explants from the human term placenta, homogenates from the first and term trimesters, isolated primary trophoblasts, and placental cell lines (BeWo, HTR-8/SVneo, and ACH-3P). Post characterization, placental explants were exposed to treatment with endocannabinoids, specifically AEA or 2-AG, at concentrations of 0.1, 1, 10, and 20 µM for 48 hours. Following this treatment, inflammation was induced using lipopolysaccharide (LPS), and the effects of 2-AG and AEA on the expression and release of pro-inflammatory cytokines were assessed through both qRT-PCR and ELISA.

Results

We observed varied ECS gene and protein expression across different placental models, uncovering notable distinctions. Therefore, an appropriate in vitro model to study placental ECS must be carefully selected. In placental explants, endocannabinoids showed a modest reduction in TNF-α and IL-1β secretion; however, they did not significantly impact gene expression.

Conclusion

Our findings suggest that ECS may modulate placental inflammatory responses through its endocannabinoids 2-AG and AEA. Interestingly, endocannabinoids were observed to slightly reduce specific pro-inflammatory cytokines in placental explants. These findings enhance our understanding of ECS in the placenta and its role in inflammation. Further research using appropriate experimental models is crucial for developing new therapies for managing maternal inflammation and associated complications such as preterm birth. In addition, the effect of prenatal exposure to exocannabinoids on placental immune response needs to be clarified. Funded by GAUK 170-050/235011 and GACR 23-07094S.

P1.99

Mpox virus (MPXV) vertical transmission and fetal demise in a pregnant rhesus macaque model

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Objectives

Adverse pregnancy outcomes are associated with clade I mpox virus (MPXV) infection. However, there is no information on the potential for vertical transmission and fetal harm with clade IIb MPXV, which has recently been circulating in the Western Hemisphere.

Methods

We established a rhesus macaque model of vertical MPXV transmission with early gestation inoculation. Three pregnant rhesus macaques were inoculated by intradermal injection of 1.5 x 10^5 PFU of clade IIb MPXV near gestational day (GD) 30 and animals were monitored for viremia, and maternal and fetal well-being. Animals were euthanized to collect fetal and maternal-fetal interface tissues, and were evaluated for viral loads, infectious viral titers, and histopathology.

Results

MPXV DNA was detected in maternal blood by 5 days post-inoculation (dpi) and skin lesions were apparent by 7 dpi. No fetal heartbeat was detected at 14 dpi and 25 dpi for two dams respectively; the third dam developed significant vaginal bleeding at 5 dpi, deemed an impending miscarriage, and was euthanized. Viral DNA was detected in placental and fetal tissue of both spontaneous fetal demise cases. All fetal tissues from the spontaneous demise cases had high viral DNA burden (10^6-10^9 copies/mg tissue). Some maternal tissues from all the dams had detectable viral DNA at much lower levels than fetal tissues. Infectious MPXV was present in skin lesion swabs from all dams. MPXV was localized to placental villi by ISH and IHC and co-localized with some mesenchymal and Hofbauer cells by immunofluorescence microscopy.

Conclusion

Clade IIb MPXV infection in a pregnant rhesus macaque model during pregnancy results in vertical transmission to the fetus and adverse pregnancy outcomes, similar to clade I MPXV infection in humans. Further studies are needed to determine whether antiviral therapy with tecovirimat will prevent vertical transmission and improve pregnancy outcomes.

P1.100

Evaluation of the NLRP3 inflammasome pathway in the placenta; expression and function across gestational stages and placental models

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Objectives

Inflammasomes are multiprotein complexes associated with pathogen clearance and sterile inflammation. Recent work has shown that the human placenta expresses high levels of NLRP3-inflammasome-associated molecules and secretes large amounts of proinflammatory cytokines IL-1β and IL-18. Moreover, emerging evidence points to the involvement of the NLRP3 inflammasome in pregnancy complications. Nevertheless, there is limited understanding regarding the characteristics of NLRP3 within the placenta. Therefore, this study aims to fill this gap by providing a comprehensive understanding of NLRP3 inflammasome expression and function throughout different stages of physiological pregnancy and in cases of preterm birth. Moreover, we performed NLRP3 characterization in various placental in vitro models.

Methods

The human placenta cohort comprised samples from healthy first-trimester and term pregnancies and preterm deliveries. In vitro models included primary trophoblast cells isolated from human term placenta as well as cell lines BeWo, HTR-8/SVneo, and ACH-3P. Cellular expression of proinflammatory markers and cytokine release were evaluated using qPCR and ELISA, respectively. Additionally, protein expression of the NLRP3 inflammasome components was examined using Western Blot analysis.

Results

We found that the activity of the NLRP3 inflammasome varied throughout pregnancy. Heatmap analysis showed differences in gene expression levels across different stages of pregnancy, with a tendency for increased activity in the early stages. Moreover, we observed substantial transcriptional profile variations within the NLRP3 inflammasome pathway across different placental cell models.

Conclusion

The study demonstrates that the NLRP3 inflammasome exhibits dynamic expression patterns throughout pregnancy, with heightened activity, particularly evident in early gestational stages. In addition, the study highlights significant variations in the NLRP3 inflammasome pathway across different placental cell models, emphasizing the complexity of its regulation. Further research is necessary to elucidate the role of NLRP3 inflammasome within the placenta.

The study was supported by the Czech Health Research Council (Grant number: NU22J-01-00066).

P1.101

Cannabidiol modulates the inflammatory response in the human placenta

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Objectives

Inflammation plays a key role in embryo implantation and parturition, and therefore, the balance between pro-inflammatory and anti-inflammatory responses is vital for healthy pregnancy. Despite the rising use of cannabidiol (CBD) among pregnant women as an anti-inflammatory or anti-nausea medicine, its impact on pregnancy-related inflammation is not well understood. Therefore, in this study, we investigated the effects of CBD on inflammation-related molecules in placental explants and primary human trophoblast cells under inflammatory conditions.

Methods

To investigate CBD's effects on placental anti-inflammatory response, human placenta explants and primary trophoblast cells were exposed to a range of CBD concentrations (from 0.1 to 20 μg/ml) for 24 or 48 hours. Then, LPS was added for 4 hours to trigger an acute inflammatory response. qPCR, western blotting, and ELISA were used to assess changes in gene/protein expression and release of cytokines (IL1-β, IL-6, IL-18, TNFα, NLRP3, Caspase-1).

Results

CBD effectively down-regulated LPS-induced inflammation in both placental explants and primary trophoblast cells. In a dose-dependent manner, CBD reduced gene/protein expression of pro-inflammatory cytokines and mediators and their release from the cells/tissue, suggesting its considerable immunomodulatory capabilities in the placenta.

Conclusion

Taken together, we demonstrate that CBD can modulate the placental pro-inflammatory immune response by preventing the appropriate reaction to an inflammatory challenge induced by LPS in human placental explants. Our findings suggest CBD can interfere with the immune response and disrupt the placental reaction to an inflammatory challenge. These results highlight the need for further investigation to fully understand the impact of prenatal exocannabinoids on pregnancy outcomes.

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P1.102

Effects of Maternal Immune Activation on Tryptophan Metabolism in Rat Placenta and Decidua

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Objectives

In approximately 20% of pregnancies, intrauterine inflammation occurs, with evidence from epidemiological data and animal studies indicating a clear link between maternal inflammation during pregnancy and increased risks of neurodevelopmental and psychiatric disorders in the offspring. Despite robust epidemiological support for the association between maternal inflammation and fetal neurodevelopmental disorders, the underlying mechanisms are unclear. Tryptophan (TRP) metabolism has emerged as a potential mechanism, owing to the neuroactive and immunomodulatory properties of TRP metabolites. Our study aims to evaluate the impact of maternal inflammation on placental and decidual TRP metabolism and the inflammatory-mediated release of kynurenine metabolites and its potential effects on fetal development and programming.

Methods

Pregnant Wistar rats were subjected to inflammation by intraperitoneal injections of Poly (I:C) (5 mg/kg and 15 mg/kg) or LPS (1 mg/Kg) administered on gestational days 17 and 18. Following the second injection, pregnant rats were anesthetized at 4, 24, and 48 hours. Samples of maternal blood, amniotic fluid, placenta, and decidua were collected for analysis. Inflammation marker levels were measured in maternal plasma and amniotic fluid, while gene and protein expressions of key enzymes involved in TRP metabolism were examined in placenta and decidua samples.

Results

Our results show that the inflammatory markers increased in plasma and amniotic fluid. Furthermore, several enzymes in TRP metabolism are affected by inflammation. The KYN pathway was more strongly affected under inflamed conditions: treatment with LPS and/or Poly I:C significantly increased the relative expression of *KMO, KYNU*, *HAAO*, and *QPRT*. Consequently, inflammatory conditions alter the levels of TRP metabolites in the placenta.

Conclusion

Our findings indicate that systemic maternal inflammation disrupts TRP balance in the placenta and decidua, potentially leading to altered release of TRP metabolites into fetal circulation.

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P1.103

Effect of short-chain fatty acids on mouse abortion rate and immune cells in the placental decidua

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Objectives

A decrease in regulatory T (Treg) cells and an increase in Th17 cells in the placental decidua has been reported to be associated with spontaneous abortion. Short-chain fatty acids (SCFAs) are known to have the ability to induce Treg cells. The purpose of this study is to examine whether SCFAs ameliorate spontaneous abortion by modulating decidual T cells using a mouse model of CBA/J×DBA/2.

Methods

Abortion model group (CBA/J x DBA/2), control group (CBA/J x BALB/c), and abortion model group treated with SCFAs (67.5 mM acetate, 25.9 mM propionate, 40 mM butyrate) in the drinking water from day E0.5 to E14.5 of pregnancy were prepared. The percentage of abortion was calculated as the ratio of resorption sites to total implantation sites, and the percentage of decidua T cells was analyzed by flow cytometry.

Results

The abortion rates in the control and abortion model groups were 11% and 32%, respectively, and fetal resorption was observed in 60% and 100% of mothers. The abortion rate in the SCFAs-treated abortion model group was 20%, and the number of mothers presenting with fetal resorption decreased to 64%. The change in the percentage of decidual Treg cells was not significant: 7.8%, 7.4%, and 8.4% in the control, abortion model, and SCFAs-treated abortion model, respectively. The insignificant change was similar for decidual Th17 cells: 1.7%, 2.3%, and 1.8% in the control, abortion model, and SCFAs-treated abortion model, respectively.

Conclusion

It is possible that SCFAs contribute to improved fetal resorption in a mouse abortion model of CBA/J×DBA/2. However, an observed change in decidual T cells was negligible in this abortion model.

P1.104

Regulatory role of Leukemia Inhibitory Factor (LIF) and Oncostatin M (OSM) in the maintenance of cell populations involved in intrauterine inflammation at the fetal-maternal interface

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Objectives

Our study proposes a novel approach using IL-6 superfamily members: Leukemia Inhibitory Factor (LIF) and Oncostatin M (OSM), to treat endometritis and support the immune environment of the endometrium and immune cell activity.

Methods

We investigate the impacts of LIF and OSM pretreatment in two experimental models: in vivo mouse models and ex vivo human placental macrophages. Pretreatment with LIF or OSM followed by LPS-induced endometritis in mice model, was assessed. In placental macrophages, LPS treatment induces M1 macrophage activity, also we assessed LIF and OSM regulatory role in LPS-activated macrophages. Using tissue analysis by histology or immunohistochemistry, protein detection by western blot, immunofluorescence and ELIS assay, and DNA detection by PCR we assessed inflammatory activity and factors in our different experimental models.

Results

From the mice experimental model, in the liver and uterine tissue of pretreated mice groups, we observed a reduction in the LPS-inflammatory response and enhanced tissue repair. In uterine tissue, pretreated groups showed increased Arg-1 expression and decreased iNOS levels, indicative of a regulatory immune response in the uterine environment. Additionally, pretreatment with LIF or OSM led to changes in immune cells such as macrophages through signaling pathways regulation. LIF and/or OSM triggered the expression of regulatory inflammatory signaling pathways and immunometabolism factors in macrophages such as iNOS, Arg-1, CD163, p-STAT3, p-SMAD2, COX-2 and TLR-4.

Conclusion

This integrated approach demonstrates the key regulatory roles of OSM and LIF in pregnancy-related processes, immunity, and inflammation, offering potential therapeutic avenues for endometritis and infertility issues.

P1.105

Prenatal Exposure to Cadmium alters Placental Morphology, Vasculature Development, and Macrophage Subpopulations in Mice

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Objectives

The environmental metal cadmium (Cd) accumulates in the placenta during pregnancy and is associated with fetal growth restriction and preterm birth in rodents and humans. In the placenta, Cd generates oxidative stress which may be important in disrupting placenta development and macrophage responses. We sought to determine whether prenatal exposure of mice to $CdCl_2$ alters placenta development and enrichment of macrophage populations.

Methods

Pregnant C57BL/6Crl mice (n=9-10/group) received distilled water with $CdCl_2$ (0, 5, or 50 ppm) *ad libitum* from gestational day (GD) 7-17. Tissues were collected on GD17 for ICP/MS quantification of Cd concentrations, histomorphologic assessment, and immunohistochemistry.

Results

Exposure to $CdCl_2$ did not affect fetal or placental weight or size, or number of resorptions. Histological analysis of the placentas demonstrated a significant decrease in junctional zone area, with no change in labyrinth zone size, in placentas of dams treated with 50 ppm $CdCl_2$. Compared to vehicle-treated mice, the area of maternal and fetal blood vessels was decreased in placentas exposed to 5 and 50 ppm $CdCl_2$. Immunohistochemical staining for the fetal endothelial surface marker CD34 was significantly increased in the placentas of fetuses exposed to 50 ppm $CdCl_2$. Further, the number of macrophages that stained positive for the marker F4/80 was increased in both the labyrinth and junctional zones of placentas exposed to 50 ppm $CdCl_2$. Conversely, the number of macrophages that stained positive for Iba1, a protein involved in phagocytosis, was decreased in the labyrinth zones of placentas exposed to 50 ppm $CdCl_2$.

Conclusion

In the absence of overt fetoplacental toxicity, exposure to $CdCl_2$ during gestation altered development of placental zones and vasculature in mice as well as shifted the abundance of macrophage subpopulations. This research is supported by R01ES029275, T32ES007148, F31ES032319, P30ES005022, UC2HD113039, and Grover Fellowship.

P1.106

Immunotolerance at the maternal-fetal interface during placental development is regulated by uterine Nodal expression

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Objectives

The maintenance of an immunotolerant state at the maternal-fetal interface is necessary to promote placental and fetal development and is highly regulated by leukocytes within the decidua. The dysregulation of immune populations and their secreted factors has been implicated in many pathological pregnancy complications. Recently, our lab has identified a role for the secreted morphogen Nodal during pregnancy as its conditional uterine deletion in mice resulted in significant subfertility. This was shown as severe implantation failure, fetal loss, preeclampsia-like phenotypes, fetal growth restriction and a greater susceptibility for LPS-induced preterm labour. It was hypothesized that these reproductive complications were immune mediated, and that uterine Nodal expression was necessary for maintaining immunotolerance. Our objective is to characterize the immune landscape of Nodal-deficient females on d10.5 of pregnancy, which coincides with the time of placentation and fetal loss/growth restriction phenotypes.

Methods

The level of expression of 84 inflammatory genes in Nodal knockout mice will be assessed by RT-qPCR, and flow cytometry will quantify the abundance of decidual leukocytes. Single-cell RNA sequencing of CD45+ cells will provide extensive data on leukocyte expression profiles.

Results

On d10.5, Nodal knockout females had increased expression of 25 cytokines, TLR4 pathway components and macrophage-associated factors. Further characterization of leukocyte populations revealed a shift towards the pro-inflammatory “M1” macrophage state in Nodal-deficient mice. Current work is now focused on proposing a mechanism of immunomodulation by Nodal signaling.

Conclusion

The balance between macrophage polarization is critical for the success of artery remodeling and trophoblast invasion, and a dysregulated response has been associated with several pathological complications of the placenta. The sustained, pro-inflammatory state during the expected time of anti-inflammatory tolerance in the Nodal-deficient model demonstrates that Nodal is an important immunomodulator throughout pregnancy. Here, we provide new insights into the mechanisms of placentation, with the overall goal of improving pregnancy outcomes.

P1.107

Differentiation responses to matched stress stimulus exposures show that the differentiation to 1st lineage of TSC is vastly greater than that of ESC.

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Objectives

Embryo loss post-fertilization isn't well-understood. In mouse, trophoblast stem cells differentiate to giant cells (TSC-to-TGC) to produce placental lactogen (PL)1 (human analogue=hCG). PL1/hCG are essential placental hormones for acquiring nutrition. Embryonic stem cells differentiate to extra-embryonic endoderm (ESC-to-XEN). Strong embryotoxic stresses, hyperosmotic sorbitol, and retinoic acid force TSC-to-TGC more than ESC-to-XEN, but other strong-, weak-, and non-embryotoxic stresses need to be evaluated to quantitate the size and breadth of the greater stress response of TSC.

Methods

MuTSC were cultured for 3 days in MEF-conditioned media in Cytation 5 live imager, then Hoechst nuclear-stained and micrographed. Nuclear number, size and ploidy were quantitated using Biotek Gen 5, Cellpose artificial intelligence (AI), and Image J/FIJI. A Cellpose+Cellprofiler pipeline to batch analyze images is in development. MuESC were cultured for 3 days in LIF+serum in the same live imager and analyzed with the same software. In some experiments NOAEL, and IC75 adverse effects doses were used to assay ESC by bulk RNAse. Retinoic acid (RA) and hyperosmotic control stimuli validated TSC-to-TGC and ESC-to-XEN induction.

Results

RA, hyperosmotic sorbitol, and BaP caused differentiation from TSC-to-TGC at much greater population sizes than they caused ESC-to-XEN. TSC-to-TGC had the same categorization as previous ESC tests for non- (penicillin G), weak (salicylic acid/aspirin, Li/Cl) and strong (Methyl Mercury chloride, hydroxyurea), but ESC-to-XEN induction by BaP was nil, as RA and hyperosmotic sorbitol induced less ESC-to-XEN.

Conclusion

For previously categorized strong-, weak-, and non-embryotoxic levels of adverse effects TSC-to-TGC is like previous ESC cytotoxicity tests, but in current outcomes for differentiated subpopulation size, TSC-to-TGC are vastly larger than ESC-to-XEN and should predict miscarriage better.

P1.108

Intelligent single-atom nanozymes for effective and safe therapy of inflammatory diseases in pregnancy

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Objectives

A healthy pregnancy is dependent on a tightly orchestrated inflammatory environment. However, persistent and unregulated Inflammation can be the root of various pregnancy complications; mostly associated with placenta dysfunction, threatening the maternal and fetal well-being. Therapeutic options to treat gestational inflammation are limited to non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics, which suffer from controversial inefficacy and safety data. Hence, we aim to develop a novel therapy by fabricating safe enzyme-mimicking nanoparticles (nanozymes) with anti-inflammatory and antimicrobial activity to treat gestational inflammatory diseases.

Methods

The nanozyme structure, composed of a metal-organic-framework (MOF), will serve as host-scaffold for the adsorption of active elements (e.g. Mn), forming single-atom nanozymes (SAzymes) that can mimic the active center of natural antioxidant enzymes. An infection-responsive SAzyme surface will be engineered with a coating that switches from stealth to bactericidal mode in the presence of pathogens. We will conduct a multi-endpoint toxicological assessment and simultaneously confirm the anti-inflammatory and antimicrobial efficacy using advanced placenta models. Further, we investigate potential biotransformation of SAzymes (TEM, EDX, ICP-OES) and its impact on enzymatic activity.

Results

Our first SAzyme candidate, Mn@PCN224 with Superoxide Dismutase-like (SOD) activity, is used as a pilot nanozyme to perform an initial toxicological, efficacy and stability screening. Mn@PCN224 has so far shown high biocompatibility, without compromising the placenta barrier (NaF) and functionality (hCG) or triggering an oxidative stress response. We observed a cell medium-dependent enzymatic activity decrease, which was correlated to the Mn leakage from PCN224, however, SOD levels remained satisfactory.

Conclusion

The initial Mn@PCN224 screening displayed encouraging biocompatibility findings. The observed biotransformation and reduced enzymatic activity will be counteracted by the planned infection-responsive coating that will restrict the Mn leakage. Further studies are ongoing to understand maternal-fetal particle translocation, potential sub-lethal effects on placental function, early embryotoxicity as well as anti-inflammatory and antibacterial efficacy in placental inflammation/infection models.

P1.109

Effect of Plant-Derived Extracellular Vesicles on Gut-Placental Communication and Function.

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Objectives

FGR is a common pregnancy complication with short- and long-term offspring health consequences. Currently, management options are limited, necessitating development of safe therapeutic interventions. Our group has isolated extracellular vesicles (EVs) from watermelon as a potential plant-derived therapeutic and shown that these EVs beneficially influence aspects of placental cell behaviour *in vitro*. Here we investigate their impact on pregnancy outcomes *in vivo*.

Methods

Pregnant C57BL/6J mice were treated with PBS (control) or a low (5×10^{10} particles/mL) or high dose (5×10^{11} particles/mL) of watermelon EVs (WMEVs) by oral gavage from GD7.5 – GD13.5. Fetal and placental weights were recorded on GD17.5 and maternal fecal samples, intestines and placentas were harvested for 'omic and gene/morphological expression analyses.

Results

Whilst there was no significant impact of WMEVs on GD17.5 fetal weight, placental weight was significantly increased in a dose-dependent manner ($P<0.01$). Expression of key placental nutrient transporter genes - *Slc7a8* (LAT2; amino acid transporter – $P<0.05$) and *Slc6a6* (TAUT; taurine transporter – $P=0.08$) - were also increased in a dose-dependent manner, specifically in placentas from male fetuses. WMEVs also affected the maternal intestinal ecosystem, indicated by changes to the relative abundance of microbial populations including *Bifidobacteriaceae*, and their metabolites (e.g., short-chain fatty acids), and pathway analysis of transcriptomic and proteomic outputs highlighted EV effects on pathways related to immune processes. Further analysis of maternal small intestines showed decreased *Tnf* expression ($P<0.05$) and a dose-dependent reduction in the number of CD45 ($P<0.01$) and CD64 ($P<0.05$) – positive immune cells.

Conclusion

Our data suggest that WMEVs alter gut-placenta communication, likely through several mechanisms including the alteration of the maternal gut microbiome and immune pathways, to beneficially affect placental weight, structure and potentially function. Further work will determine whether EV supplementation in rodent models of pregnancy complications, including HFD-induced FGR, will have a positive impact on fetal weight.

P1.111

Platelet-derived factors modify gene expression profile of human trophoblasts

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Objectives

During human placentation extravillous trophoblasts migrate through maternal endometrium and form trophoblast plugs within the lumen of uterine spiral arteries to prevent maternal blood cells from entering the intervillous space. However, by the middle of the first trimester cells within these trophoblast plugs become loosely cohesive forming capillary-sized channels. So far, we and others have found platelets within these channels, due to their very small diameter. Here, we investigate if activation of maternal platelets has an impact on the differentiation and behaviour of extravillous trophoblasts.

Methods

The human trophoblast cell line ACH-3P and primary trophoblasts isolated from human first trimester placental tissue were used to establish 3D cell culture models, which were either co-incubated with isolated platelets or platelet-derived factors from pregnant women. Changes on the molecular level were measured using standard RNA sequencing (RNA-seq) analysis, qPCR, ELISA and *in situ* padlock probe approach. Several techniques to study trophoblast invasion properties were applied. Platelet activation was assessed via impedance aggregometry measurements.

Results

RNA-seq data revealed that platelet-derived factors cause significant changes in the transcription profile of ACH-3P spheroids. Out of the significantly deregulated genes, we identified those related to embryonic development for further investigation, in particular Leukocyte Associated Immunoglobulin Like Receptor 2 (LAIR2). LAIR2 was significantly upregulated on the RNA and the protein level in the presence of platelet-derived factors. Recombinant human LAIR2 enhanced invasion properties of ACH-3P cells and furthermore inhibited collagen type 1-mediated platelet activation.

Conclusion

Our recent findings suggest that platelet-derived factors significantly change the transcription profile of invading trophoblasts. Among the deregulated genes, LAIR2 seems to promote trophoblast invasion on the one hand, but also to prevent excessive platelet activation on the other. These results tempt us to speculate about a fine-tuned regulatory feedback mechanism between invading trophoblasts and maternal platelets to enable appropriate establishment of utero-placental blood perfusion.

P1.112

Cytochrome c oxidase IV isoform 1 (COX4-1) regulates the proliferation, migration and invasion of trophoblast cells via modulation of mitochondrial function

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Objectives

Spontaneous miscarriage is a common complication of early pregnancy. Previous studies have shown that mitochondrial function plays an important role in establishment of a successful pregnancy. Cytochrome c oxidase subunit 4 isoform 1 (COX4I1), a component of electron transport chain complex Ⅳ, is required for coupling the rate of ATP production to energetic requirements. However, there is very limited research on its role in trophoblast biology and how its dysfunction may contribute to spontaneous miscarriage.

Methods

Placental villi (7-10 weeks gestational age) collected from either induced termination of pregnancy or after spontaneous miscarriage were examined for expression of COX4I1. COX4I1 was knocked down by siRNA transfection of primary isolates of EVT cells. Real-time cell analysis (RTCA) and 5-Ethynyl-2'-deoxyuridine (EdU) were used to detect changes in proliferation ability after COX4I1 knockdown of EVT cells. Migration and invasion indices were determined by RTCA. Mitochondrial morphology was observed via MitoTracker staining. Oxidative phosphorylation, ATP production, and glycolysis in COX4I1-deficient cells and controls were assessed by a cellular energy metabolism analyzer (Seahorse).

Results

In placental villous tissue, COX4I1 expression was significantly decreased in the spontaneous miscarriage group. Knockdown of COX4I1 inhibited EVT cell proliferation, increased the migration and invasion ability and mitochondrial fusion of EVT cells. Mitochondrial respiration and glycolysis were impaired in COX4I1-deficient EVT cells. Knockdown of MMP1 could rescue the increased migration and invasion induced by COX4I1 silencing.

Conclusion

Low expression of COX4I1 leads to mitochondrial dysfunction in EVT, resulting in altered trophoblast function, and ultimately to pregnancy loss.

P1.113

High-throughput arrayed imaging-based CRISPR screen reveals novel regulators of cell-cell fusion and hormone secretion

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Objectives

Cell-cell fusion is a unique differentiation pathway required for cellular function of several human cell types including the proper formation of the multi-nucleated syncytial layer formed by syncytiotrophoblasts during placental development. Defects in syncytiotrophoblast differentiation have been described in pregnancy diseases such as preeclampsia where preeclamptic placentas show impaired cell-cell fusion, decreased expression of endogenous retroviral fusogens such as syncytin-2, and low hormonal hCG production early in pregnancy that correlate with disease severity. Here we aim to build a screening platform capable of interrogating the underlying genetic causes of these phenotypic changes using CRISPR-Cas9-mediated genetic perturbation in an in vitro model of STB differentiation (BeWo cells) coupled with hormone secretion and imaging-based cellular phenotyping.

Methods

The biochemical and morphological phenotypes associated with trophoblast development can be recapitulated using the Forskolin-induced BeWo cell model which exhibits cell-cell fusion, secretion of hCG, and increased expression of fusogens during differentiation. We optimized CRISPR-Cas9 editing in BeWo cells and combined a sensitive imaging-based two-color fusion assay with hCG ELISA readouts to characterize differentiation upon genetic knockout of candidate genes.

Results

We describe the technical optimization to perform arrayed CRISPR screening in a fusing cell type for the first time including optimized gene knockouts in BeWo cells and cell fusion assays at scale. Using high-throughput automation, we measured cell fusion and hCG secretion levels upon knockout of 412 candidate human genes including transcription factors, membrane proteins, and highly expressed placental genes.

Conclusion

Imaging-based screening is a new way to identify genetic regulators of human placental cell fusion in high-throughput and can directly inform on the genotype-to-phenotype relationship of cellular morphologies accompanying differentiation including nuclear enlargement, nuclear clustering and hCG secretion pathways.

P1.114

Characterization and in vitro modeling of trophoblast and villous core networks of human first trimester placental cells

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Objectives

Well-orchestrated interactions between trophoblasts (TB) and villous core cells (VCC) are vital to dynamic morphological and functional adaptations of the early developing human placenta. However, little is known about their characteristics and networks at gestational stages before and after the establishment of maternal blood flow into the intervillous space. Moreover, appropriate in vitro co-cultures modeling TB and VCC interactions have not been established yet. Therefore, we herein created structural as well as cellular and molecular landscapes of 6/7th and 10/11th week placentae and designed 3D in vitro models to investigate signaling pathways governing VCC cell fates in early placentation.

Methods

We employed improved cell isolation methods for harvesting the full repertoire of placental cells from 6/7th and 10/11th week tissues (n=9). Tissues and single cells were used for single-cell RNA sequencing (scRNA-seq), flow cytometry (FC), whole tissue immunofluorescence (IF), and the establishment of 3D co-culture models.

Results

scRNA-seq analysis revealed that clusters of villous cytotrophoblasts (vCTBs), proliferative vCTBs, common fibroblasts, perivascular fibroblasts (FIB2), and endothelial cells (EC) differed in their gene expression profiles between 6/7th (early) and 10/11th (late) week tissue. Investigating VCCs by FC and IF, we also observed shifts in cell numbers and distribution between early and late week placentae. While we noticed appropriate vessel structures formed by EC and FIB2 throughout the entire placentae at 10/11th weeks, FIB2 and EC were more randomly dispersed within the villous core of early weeks. Accordingly, we established 3D co-culture models of FIB2 and EC under well-defined conditions to investigate early placental vasculogenesis.

Conclusion

In summary, we detected significant differences of TB and VCC between 6/7th and 10/11th week placentae. Moreover, we developed 3D in-vitro models of VCC to investigate processes involved in VCC cell fates and vasculogenesis in early placentation.

This study is supported by the Austrian Science Funds (P34588-B, P36159-B).

P1.115

Trophoblast-specific overexpression of Adiponectin Receptor 2 inhibits placental mitochondrial respiration in mice

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Objectives

Maternal circulating levels of adiponectin are negatively associated with birth weight and animal experiments have demonstrated that maternal adiponectin restricts fetal growth by inhibiting placental insulin and mTOR signaling. The cellular effects of adiponectin on trophoblast function are predominantly mediated by adiponectin receptor 2 (AdipoR2) expressed in the maternal facing plasma membrane of the trophoblast. We have recently shown that trophoblast-specific *AdipoR2* overexpression (OX) in mice inhibited placental mTORC1 signaling, reduced placental amino acid transport and caused fetal growth restriction. Previously we have reported that mTORC1 is a positive regulator of trophoblast mitochondrial respiration. The effect of trophoblast-specific *AdipoR2*-OX on placental mitochondrial respiration remains unknown. We hypothesized placental-specific *AdipoR2*-OX in mice decreases placental mitochondrial respiration.

Methods

At Embryonic day (E) 3.5, blastocysts from super-ovulated, time-mated B6D2F1 females were collected and transduced with lentivirus with constructs for *AdipoR2*-OX or SCR sequences. Blastocysts were surgically transferred to pseudo-pregnant CD-1 recipient dams. Following euthanasia at E18.5, we separated the labyrinth and the junctional zone and analyzed them separately. High-resolution respirometry was used to determine the mitochondrial respiration (n=4-6/group). Western blotting was used to measure the protein expression of components of the electron transport chain and of the TOM (translocase of the outer membrane) complex, which translocates proteins produced from nuclear DNA through the mitochondrial membrane for use in oxidative phosphorylation. Statistical significances of differences were determined by an unpaired t-test. P values < 0.05 were considered significant.

Results

In the labyrinth, *AdipoR2* overexpression significantly inhibited placental mitochondrial complex I respiration (P=0.019) and complex IV respiration (P=0.036). Moreover, placental *AdiopoR2*-OX decreased TOM22 protein expression (P=0.035). In the junctional zone, neither mitochondrial oxidative phosphorylation nor protein expression of mitochondrial complexes were significantly different between control (SCR) and *AdipoR2*-OX groups.

Conclusion

Placental *AdipoR2*-OX inhibits mitochondrial respiration in the labyrinth zone, which may contribute to fetal growth restriction.

P1.116

WWTR1 Controls Human Extravillous Trophoblast Development by Governing Mitochondrial Function

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Objectives

During human placentation, the development of invasive extravillous trophoblast cells (EVT) from cytotrophoblast (CTB) progenitors is associated with systemic increase in oxygen exposure. The remodeling of spiral arteries by a subset of EVTs known as endovascular EVTs ensures the optimum amount of blood and oxygen reaching the growing fetus which is necessary for a successful and healthy pregnancy. However, molecular mechanisms that ensure adaptation of developing EVTs with the altered oxidative stress is poorly understood. Earlier, we reported that the WW domain containing transcription regulator 1 (WWTR1), a transcription co-factor and a Hippo signaling pathway component, plays a role in promoting self-renewal in CTBs and is required for their differentiation into EVTs. Here, we investigated whether WWTR1-mediated regulation of mitochondrial energetics underlie EVT development.

Methods

We used human trophoblast stem cells (hTSCs) as our experimental model system. Electron microscopic studies were implemented to understand alteration in the mitochondrial ultrastructure associated with EVT development. Oxygen consumption rates for EVT cells were determined by mitochondrial stress test. Oligomycin, an inhibitor of ATP synthase complex, was used to abrogate the mitochondrial electron transport chain (ETC) function during EVT development. Unbiased single-cell RNA-sequencing analysis was done to investigate the WWTR1-gene expression dynamics that control mitochondrial integrity during EVT maturation.

Results

Oligomycin disrupted EVT differentiation signifying the requirement of proper ETC function in this process. We show that mitochondrial oxidative phosphorylation is essential for EVT maturation and WWTR1 regulates this process by orchestrating gene expression program in developing EVTs. Our single cell RNA sequencing revealed a WWTR1-IFI27 regulatory axis that controls mitochondrial energetics ensuring EVT maturation.

Conclusion

We identified a novel regulatory axis where WWTR1-mediated regulation of mitochondrial function ensures EVT development.

P1.117

PATTERNS OF VEGF/KDR AND AQP3 EXPRESSION IN THE MATERNO-FETAL INTERFACE AND VASCULARIZATION OF MOUSE PLACENTA AFTER PERIGESTATIONAL ALCOHOL CONSUMPTION UP TO EARLY ORGANOGENESIS

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Objectives

VEGF/KDR and aquaporin-3 (AQP3) are involved in the maternal-fetal interface development and placental vascularization, through roles in trophoblast migration and differentiation, labyrinthine and maternal vascular remodeling, syncytial formation and apoptosis regulation. We aimed to analyze the expression pattern of VEGF/KDR and AQP3 in the junctional zone (JZ) and labyrinth (Lab) of mouse placenta after perigestational alcohol consumption (PAC) until early organogenesis, and the potential links with probable trophoblastic fetal-face anomalies of the placenta.

Methods

Ethanol 10%/water was administered to female mice for 15 days before and up to day 10 of gestation (D10), and gestation continued without ethanol exposure until D13 (TF). Control females (CF) were administered with ethanol-free drinking water.

Results

In TF, the Fetal/Placental weight index and the % of abnormal placentas with normal or abnormal fetuses increased ($p<0.05$), with an increased JZ area and reduced labyrinthine one ($p<0.05$) (PAS, *ImageJ*) vs CF. The 87-100% of JZ and lab of TF-placentas had disorganized labyrinth, low lab-vascularization, abnormal trophoblast giant cells (TGCs), interdigitated JZ into lab, and low count of PAS+ cells in JZ. Caspase-3 expression (immunohistochemistry) was increased ($p<0.05$) at the JZ-decidual interface of TF vs CF. In the CF and TF, VEGF, KDR, and AQP3 were immunolocalized mainly in the syncytial trophoblast of the labyrinth and the TGC and glycogen cells of JZ. VEGF immunoexpression (OD, *ImageJ*) increased in the labyrinth and JZ of TF-placentas vs CF ($p<0.05$), but KDR was reduced in the TF-lab vs CF. AQP3 immunoexpression was higher in labyrinthine syncytiotrophoblasts, JZ-TGCs, and glycogen cells from normal and abnormal TF-placentas than in CF ones ($p<0.05$).

Conclusion

The altered D13-placental growth, JZ-dedifferentiation, and abnormal Lab-vascularization after PAC may be due to trophoblastic VEGF/KDR and AQP3 deregulation, suggesting that these mechanisms may induce defective fetal growth and morphogenesis at term.

P1.118

Mitochondrial pyruvate flux regulates trophoblast differentiation

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Objectives

Trophoblast differentiation into syncytiotrophoblasts is associated with marked metabolic, epigenetic, and transcriptional remodeling. Mitochondria are thought to play critical roles in these processes. Here, we test the hypothesis that the mitochondrial pyruvate flux mediated by the mitochondrial pyruvate carrier (MPC) regulates multiple domains of trophoblast differentiation.

Methods

Using trophoblast stem cells (Okae; CT-29 and CT-30) in the self-renewing or syncytiotrophoblast state, we chemically disrupt the mitochondrial pyruvate carrier and assess the impact on multiple domains of differentiation. Specifically, we use untargeted static mass spectrometry, qPCR, ELISA, western blotting, and confocal imaging to assess the impact on multiple domains of trophoblast differentiation in 2D culture and 3D organoids.

Results

MPC expression decreases with differentiation, and we find that disruption of MPC enhances gene expression of several markers of syncytiotrophoblast differentiation regulating HCG (CGA) and cellular fusion (ERVW-1 and ERV-FRD1) and decreases expression of cytotrophoblast marker, TEAD4. Together, this suggests that the loss of MPC expression promotes differentiation. Cytotrophoblast differentiation into syncytiotrophoblasts is associated with decreased histone acetylation. We find that MPC inhibition further attenuates differentiation-dependent decreases in histone acetylation at lysine 9 and 27 in both the cytotrophoblast and syncytiotrophoblast states. This decrease in histone acetylation is associated with altered cellular metabolism, including decreased relative abundance of TCA cycle intermediates and epigenetic precursors, including citrate and acetyl-Co-A, most notably in the syncytiotrophoblast state. MPC inhibition results in smaller organoids with a disordered expression of cytotrophoblast and syncytiotrophoblast markers, suggestive of dysregulated trophoblast differentiation.

Conclusion

Mitochondrial nutrient flux regulates multiple domains of trophoblast differentiation with disruption of MPC enhancing expression of genes involved in trophoblast differentiation, impairing global histone acetylation, and decreasing key TCA intermediates. Further, we find that MPC inhibition dysregulates organoid formation. This work highlights a critical link between mitochondrial nutrient metabolism, epigenetic modifications, and trophoblast differentiation.

P1.119

Cannabis cigarette smoke vs Δ9-THC: Differential impacts on syncytiotrophoblast stress and mitochondrial function

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Objectives

The bioactive components of cannabis, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), impact the formation of the materno-fetal interface by disrupting the differentiation of cytotrophoblasts (CT) into syncytiotrophoblasts (ST). Mitochondria are crucial in facilitating this differentiation process and are impaired by cannabinoids. However, less is known about the effects of THC, delivered in cigarette smoke, on trophoblast and mitochondrial function. We hypothesized that cannabis cigarette smoke conditioned media will have differential effects on trophoblast cell function compared to THC alone.

Methods

BeWo b30 cells were exposed to smoke conditioned media (SCM) from cannabis cigarettes (14%THC:1% CBD) or 10µM THC for 48hrs during the process of fusion, which was initiated by the addition of forskolin. Following 48 hrs, cells were subjected to gene and protein expression analysis. We investigated pathways for responsible for cellular differentiation, cellular stress, mitochondrial function, and endocannabinoid system (ECS) signaling.

Results

The expression of trophoblast fusion (*ERVW1* and *CGβ)* were attenuated by both treatments. Interestingly, we observed a decrease in the expression of stress markers (*Hsp60* and *SOD1)* in THC treated cells but a significant increase in SCM treated cells. Similarly, *TFAM*, a marker of mitochondrial stress was upregulated by 3.9-fold in SCM, but not affected by THC treatment. The expression of the ECS receptor *TRPV1* was upregulated by 3.3-fold in cannabis smoke exposed cells compared to THC treatment. We expect increases in cellular ROS and decreased in mitochondrial ETC function in cannabis smoke exposed cells compared to delta-9-tetrahydrocannabinol, which will highlight differences in oxidative stress responses.

Conclusion

We demonstrate differential impacts on syncytiotrophoblast function following SCM and 10 µM THC treatment. These findings highlight the need to evaluate whole cannabis smoke on placental function and point to potential mechanistic differences for whole cannabis smoke vs single component treatment.

P1.120

Regulatory action of progesterone receptor membrane component 1 (PGRMC1) in shear stress-induced microvilli formation and trophoblast differentiation

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Objectives

Villous trophoblasts floating in the intervillous space of the placenta are continuously exposed to fluid shear stress by maternal blood flow throughout pregnancy. Recent studies have shown that the shear stress promotes the differentiation of trophoblasts to the syncytiotrophoblasts and the formation of microvilli which have glucose transporter on the apical surface of the syncytiotrophoblasts. We have previously reported that the downregulation of progesterone receptor membrane component 1 (PGRMC1), originally identified as a progesterone binding protein, stimulates syncytiotrophoblast differentiation, including hCG production and cell fusion. Using a microfluidic device, we explored the roles of PGRMC1 in the fluid shear stress-induced trophoblast differentiation and microvilli formation.

Methods

A microfluidic device equipped with a vitrified collagen membrane was engineered to replicate placental villi. BeWo cells transfected with control or PGRMC1 siRNA were seeded on the membrane inside the device and cultured for 1 hour or 3 days under non-flowing static and fluid shear stress (1.1 μL/min) conditions. The formation of microvilli was evaluated by fluorescence immunostaining using an anti-Ezrin antibody. Furthermore, we investigated the impact of reducing PGRMC1 expression on the phosphorylation of AKT, especially in the microvilli formation by shear stress, along with the expression of hCG and syncytialization-associated gene ERVFRD-1 (syncytin-2).

Results

The fluid shear stress increased the number of microvilli on the apical surface of BeWo cells, which was accompanied by the phosphorylation of AKT. Knocking down PGRMC1 expression inhibited the shear stress-induced microvilli formation and AKT phosphorylation. In addition, shear stress enhanced the expression of hCG and ERVFRD-1, and PGRMC1 knockdown further promoted their expression.

Conclusion

These results suggest that the downregulation of PGRMC1 expression facilitates trophoblast differentiation for the development and maintenance of placental villi, while PGRMC1 plays a role in shear stress-induced microvilli formation to nourish the fetus.

We have identified specific genes in each pathology, providing evidence that these pathologies are not only distinct in their clinical manifestations but also in their placental profiles, which could be leveraged to understand where their underlying mechanisms diverge and could offer therapeutic targets.

LB.1

Patterns of trophoblast invasion in deep uterine arteries in accreta placentation

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During normal placentation, extravillous trophoblast (EVT) invades and, in synergy with maternal leukocytes, transforms the walls of uterine spiral arteries in the decidua and inner myometrium, a process that is largely complete by 20 weeks. The resulting expanded channels allow blood to enter the intervillous space at low pressure, thus avoiding disruption of the villous tree. Previous caesarean section imposes a risk of placenta accreta syndrome (PAS) in subsequent pregnancies. Here, EVT invasion extends from scar sites into deeper myometrium, where larger arteries are encountered. This is associated with the development of intraplacental lacunae on ultrasound, thought to arise when jets of blood enter the intervillous space from large feeder arteries. It is therefore of interest to examine the susceptibility of deeper arteries to colonisation by EVT and maternal inflammatory cells. Placental bed and scar biopsies obtained at or near term (11 patients, 79 biopsies) were processed for histopathology and stained for EVT or inflammatory cells. Arterial profiles positioned within 5mm of villous tissue at scar sites were included in the analysis (54 lumina).

EVT colonisation of scar tissue was much more extensive than in adjacent myometrium ($p<0.001$). The scar-myometrial interface was ragged, with arterial segments sometimes incorporated in scar fields. Large arteries were classified as remodelled (20.3%), partially remodelled (22.2%) or unremodelled (29.6%). Unremodelled arteries were present in 10/11 patients while 6/11 had one or more fully remodelled vessels. CD45 cells were frequently associated with arteries undergoing remodelling.

These results suggest that the deeper arteries are less susceptible to remodelling than spiral arterial segments downstream. Caution is, however, required in interpretation as EVT are fed into the placental bed from anchoring villi, thus the extent of EVT invasion is likely to vary depending on the stage of early pregnancy at which anchoring sites form within the adjacent scar.

LB.2

Adenoviral delivery of stabilized HIF-2α mutant in primary cytotrophoblasts: a novel in vitro model for syncytiotrophoblast stress and preeclampsia research

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Preeclampsia, a condition arising from placental dysfunction, affects approximately 10% of pregnancies and significantly increases morbidity and mortality rates for both mothers and infants. Despite its profound societal and economic burdens, current therapeutic interventions for placental dysfunction are lacking. The prevailing hypothesis suggests that impaired invasion and incomplete uterine artery remodeling during early gestation compromise placental perfusion, leading to abnormal blood flow, persistent hypoxia, and trophoblastic stress. Understanding this stress is critical for developing novel and targeted therapies. Current *in vitro* models of placental dysfunction typically involve culturing cells under low-oxygen tensions, which likely induce multiple unknown cellular adaptations. Our research has highlighted the crucial role of hypoxia-inducible factor (HIF)-2α, a transcription factor stabilized under low-oxygen tension. To investigate its specific involvement in impairing trophoblast differentiation and inducing angiogenic imbalance, we stabilized HIF-2α by mutating the prolyl residues responsible for its degradation under normal oxygen tension. Using an adenovirus-based delivery system, we overexpressed stabilized HIF-2α in primary trophoblasts isolated from term human placentas. The mutant HIF-2α protein was stabilized under normoxia and successfully transactivated its response element in a luciferase reporter assay. Under 21% O_2, overexpression of HIF-2α impaired cytotrophoblast differentiation into syncytiotrophoblasts, evidenced by decreased *CGB* and *GCM1* expression compared to GFP-transduced primary trophoblasts. Additionally, this overexpression induced an angiogenic imbalance, with increased *sFLT1* and decreased *PGF* expression, mirroring the effects observed in cytotrophoblasts cultured under 2.5% O_2. These findings confirm that HIF-2α alone is sufficient to cause trophoblastic stress and underscore the relevance of our adenoviral system for studying the pathophysiology of placental dysfunction and testing novel targeted therapies for preeclampsia.

LB.3

Studies of the Endocrine Disrupting Effects of Novel and Emerging Pesticides Using a Physiologically Relevant Cell-Based Feto-placental Model of Human Steroidogenesis

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Introduction: With the increasing global population, demand for food is rising, resulting in increased pesticide use. This exposes humans to varying pesticide doses through occupation, diet, or household applications. For many pesticides introduced in the last 5 to 10 years, little is known about their potential endocrine toxicities, particularly in pregnant persons who require precisely regulated steroidogenesis. **Objectives:** To evaluate the effects three classes of pesticides: the neonicotinoid insecticides flupyradifurone and sulfoxaflor, the herbicides clopyralid, picloram, and triclopyr, and the fungicides azoxystrobin and pyraclostrobin in a human feto-placental co-culture system. This co-culture reflects the steroidogenic cooperation between the fetal adrenal zone and placental trophoblasts, essential for a healthy pregnancy. The focus is on the regulation of the human cytochrome P450 19 enzyme (CYP19, aromatase), responsible for converting androgens into estrogens. **Methods and results:** H295R and BeWo cells were placed co-culture and exposed to pesticides at concentrations ranging from environmentally relevant doses to toxicologically significant levels (0.1, 0.3, 1, 3, 10, or 30 µM for 24-h). The cytotoxicity of these concentrations was assessed using a WST-1 assay. No significant toxicity was observed. The steroid hormones (progesterone, DHEA, androstenedione, estradiol, estriol, estrone, and β-HCG) in the feto-placental co-culture system were quantified by ELISA. The effect of the pesticides on steroidogenic enzyme gene expression was assessed by RT-qPCR. Findings indicate a discernible alteration in steroid hormone levels with the selected pesticides, particularly at certain concentrations, suggesting potential steroidogenic disruption effects. **Conclusion:** Our results highlight the potential endocrine disruptive effects of selected pesticides which may pose a risk during pregnancy.

LB.4

Automatic inflammatory lesions detection in placenta whole-slide images using self-supervised learning

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Context: Placental lesions can explain adverse outcomes in the neonates and can predict recurrent complications in subsequent pregnancies. However, placenta whole-slide image (WSI) analysis is not performed systematically due to the time and specialized skills required. Our goal is to propose an automated tool to assist pathologists in the detection of these lesions.

Method and results: Because precise and reliable annotations are very limited, we use contrastive self-supervised learning to obtain a representation of placenta WSI patches. This representation space is used to compare unseen WSIs with a lesion-specific prototype vector defined using a small set of annotated WSIs. A semantic similarity map is computed at the slide level comparing all the patches to the prototype in the representation space. The similarity map is then refined using a simple propagation method to take into account spatial patch proximity. This framework is evaluated on a dataset of 167 WSIs (108 and 59 from healthy and pathological placentas respectively) annotated by three senior pathologists. We demonstrate that using only one labeled pathological placenta as a support set for the prototype definition, we can achieve AUROCs of more than 0.96 at the patch level and 0.93 at the slide level. Moreover, we show that the learned representation offers interesting inter-lesion generalization capabilities.

Conclusion: The proposed method is efficient, automatic, fast (< 45 seconds per slide) and easily explainable: we find relevant regions of interest highlighting placental inflammation by searching for regions similar to examples provided by pathologists. In practice, this assistance tool could save pathologists considerable amounts of time and eliminate inter-operator variability. This would enable a more systematic examination of placental tissue at the end of pregnancy.

LB.5

Melatonin inhibits nutrient transfer in a placenta cell model of ischemia

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Introduction: Melatonin is known for its roles in the regulation of the circadian cycle and its powerful antioxidant properties. Human trophoblast cells are able to synthesize *de novo* melatonin and express its receptors (MT1 and MT2). Melatonin supplementation during pregnancy exerts antioxidant properties, improves vascularization, and reduces inflammation in the placenta. *In vivo* results suggest that melatonin supplementation increase feto-maternal glucose and amino acid transfer.

Objective: The aim is to study the effect of melatonin treatments on BeWo cells under normoxia or hypoxia-reoxygenation. Because melatonin affects BeWo cells inversely to primary cells, we hypothesize that it will inhibit nutrient transfer in this model.

Methods: BeWo cells were cultured for 24h in normoxia (8% O_2). Melatonin was then added at different concentrations (0, 10pM, 1nM, 100nM, 10µM and 1mM) with 20µM of forskolin every 24h for 48h. For the last 24h of culture, cells stayed under normoxia or were subjected to hypoxia (0,5% O_2) for 4h and then reoxygenated (normoxia) for 20h. Cells were snap-frozen, mRNA was extracted and retrotranscripted using commercial kits. Taqman low-density arrays (qPCR) were used to quantify the expression of 71 genes involved in glucose, amino acids, lipids, vitamins, and minerals transport. Results were analyzed using nonparametric ANOVA followed by fdr correction (n = 4 per group).

Results: No differences in gene expression were observed between groups for cells cultured under normoxia. For cells exposed to hypoxia-reoxygenation, the group exposed to the highest concentrations of melatonin showed a decreased expression of 12 (10µM) and 30 genes (1mM). Under-expressed genes were involved in glucose, amino acids (especially tryptophan), long-chain fatty acids as well as zinc transport.

Conclusion: This study suggests that melatonin treatment decreased the expression of genes involved in nutrient transport in BeWo cells only after an episode of stress.

LB.6

P-glycoprotein Transporter Expression on Placental Cell- and Size-Specific Extracellular Vesicles Measured by Nanoscale Multiplex High-Resolution Flow Cytometry

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Objectives: Circulating extracellular vesicles (EVs) have surface markers that represent placental cell-specific phenotypes. In turn, placental EVs may provide blood-based biomarkers that reflect placental health and relative risk for later pregnancy complications. Since P-glycoprotein (P-gp) plays an important role in maternal-fetal transport, we hypothesized that variance in placental cell-specific EV expression of P-gp in maternal blood may be a potential biomarker for integrity of the placental barrier.

Methods: As a proof-of-principle study, we tested culture media from BeWo cells and P-gp expressing HEK cells collected after 72 hours as positive controls for P-gp positive EVs. We employed a highly sensitive and specific nanoscale flow cytometry approach to multiplex antibodies on EVs in control media, stained PBS negative controls, and first trimester plasma samples (11 weeks) for PLAP, HLA-C, P-gp, and EV tetraspanins. Sample volume was standardized with 200nm bead PBS buffer to dilute test samples with results in triplicate reported as nanocounts/1000 beads.

Results: Media from BeWo cells showed abundant PLAP+/HLA-C- (syncytiotrophoblast, STB) EVs with fewer numbers of HLA-C+ (extravillous trophoblast, EVT) EVs. First trimester plasma samples were similarly enriched with predominantly STB EVs, but also contained 10-fold greater concentrations of EVT EVs compared with BeWo cells. Co-expression of P-gp on these EVs was more abundant in BeWo than HEK cells. Moreover, P-gp was detected on EVs from both EVTs and STBs in first trimester plasma. Potentially significant was the observation that placental STB and EVT EVs from a woman who later developed severe preeclampsia did not express P-gp.

Conclusion: This pilot study demonstrates the ability to image and quantitate the transport protein P-gp on placental cell-specific EVs. Early data suggest there may be variance related to early onset preeclampsia, which now warrants further study.

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LB.7

Reproducibility of first trimester placental volume assessment with fully automated placental segmentation using the OxNNet toolkit

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Background

First trimester placental volume (PV) is proposed as a predictor of fetal growth restriction and pre-eclampsia, but clinical utility and large-scale research are hindered by the time-consuming process of placental segmentation. OxNNet — a fully convolutional neural network — performs rapid and fully automated placental segmentation, and two ongoing large-scale cohort studies will explore the potential of PV as a population-level screening test (FirstPLUS and OxPLUS). Variation in image quality could influence the reliability of measurements, and it is critical that measurements are reproducible to be considered for population-level screening. Here we aim to assess both the intra-operator and inter-operator reproducibility of OxNNet derived PV.

Methods

An opportunistically selected subgroup of participants in the OxPLUS study underwent 3D placental ultrasound between 11+2 and 14+1 weeks' gestation. Assessments were either performed twice by Operator 1, or once each by Operator 1 and Operator 2. The ultrasound machine was reset between assessments and assessors were blinded to each other's performance. Data were transformed as appropriate for the calculation of intraclass correlation coefficients (ICC), and Bland-Altman plots constructed with accompanying descriptive statistics using mean difference ± 1.96 standard deviations (SD) as the limit of agreement.

Results

The subgroups included 64 and 56 cases for intra-operator and inter-operator assessment respectively. The intra-operator ICC was 0.90 (95% CI 0.84-0.94) and the mean difference was 1110 mm^3. 92.2% (n=59) of cases were within agreement limits of -17670 mm^3 to 19890 mm^3, with 4.7% (n=3) above and 3.1% (n=2) below. The inter-operator ICC was 0.79 (95% CI 0.66-0.87) and the mean difference was 1750 mm^3. 96.4% (n=54) of cases were within agreement limits of -26100 mm^3 to 29559 mm^3, with 3.6% (n=2) above and none below.

Conclusion: The findings suggest OxNNet-derived PV has excellent intra-operator and good inter-operator reproducibility, supporting its potential use in population-level screening.

LB.8

Functional modelling of early syncytiotrophoblast development highlights the impact of gene flow from Neanderthals.

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Early placental cells are susceptible to evolutionary modification[1]. Gene flow from *Homo neanderthalensis* affects development[2] and may impact the placenta. Placental cell differentiation can be modelled using hypergraphs of the relationships between the expression of many genes to measure the dynamic and functional organisation of the transcriptome[3].

A data set of human preimplantation single cell RNAseq data (n=2280 cells, oocyte to day 14) was assembled by meta-analysis. From this we generated a trajectory from trophectoderm (days 5-7) to early syncytiotrophoblast (STB), cytotrophoblast (CTB) and extravillous trophoblast (EVT). Hypergraph models of transcriptomic function were produced, and the top 150 most connected hypervariable genes (n=8000) were used as a mechanistic framework. The impact of recent hominid evolution was assessed using Loss-of-Function Observed/Expected Upper-bound Fraction (LOEUF) constraint as a measure of evolutionary pressure and gene flow from *H. neanderthalensis* by the presence of introgressed genes.

In the CTB/EVT there was a reduced number of constrained genes (LOEUF < 0.2) and in the STB cluster no change compared to controls (CTB/EVT: 3.3% vs. 9.5%, p= 0.016) indicating low recent evolutionary impact on the CTB/EVT. Gene flow from *H. neanderthalensis* was also unchanged in CTB/EVT. However, greater gene flow in STB compared to controls (STB: 16.0% vs 8.3%, p= 0.008) demonstrated significant impact on the early development of STB. Introgressed genes were associated with placental development (adj.P=2.3×10^{-3}) and included *EGFR* and *TFEB* which are known to impact STB differentiation.

We demonstrate that in the earliest stages of placental development there is limited impact of recent hominid evolution on CTB/EVT, but we show evidence for gene flow between *H. neanderthalensis* and *H. sapiens* that impacts the STB lineage. These findings relate recent evolutionary pressure to function in early placental development.

[1]Wildman etal. *PNAS* 103:3203-8; [2]Wei etal. *eLife* 12:e80757; [3]Battiston etal. *Nature Physics* 17:1093-98

LB.9

Texture Analysis of Ultrasound Images for the Detection of Fetal Growth Restriction

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Introduction: Fetal Growth Restriction (FGR) affects an estimated 5-10% of all pregnancies worldwide. FGR can be attributed to impaired placental function, leading to an inadequate supply of nutrients and oxygen to the fetus. However, traditional ultrasound analyses to detect FGR have focused on fetal rather than placental health. The objective of this project is to develop a computational method for placental texture analysis in prenatal ultrasound imaging, offering an approach to detecting changes in placenta structure.

Methods: Ultrasound imaging was performed on patients with (N = 11) and without (N = 11) FGR. Placental images were manually segmented from the ultrasound scans using ITK-Snap, and rectangular regions of interest (ROIs) were saved for analysis. A Python program was used for the analysis, which extracted normalized pixel intensities and each ROI to analyze the distribution of intensities. Additionally, the Gray-Level Co-occurrence Matrix (GLCM) method was employed to assess the textural characteristics of the placenta, including contrast, homogeneity, dissimilarity, correlation, and energy quantification.

Results: A sensitivity study of the ROI area found similar pixel intensity distributions between the FGR and control groups in a defined area of 6,950 pixels. However, when the area increased to 30,000 pixels, distinctions emerged, with FGR images showing smaller normalized pixel intensities compared to controls ($p=0.0189$). The Gray Level Co-occurrence Matrix (GLCM) analysis further differentiated the groups, with FGR placentas exhibiting lower dissimilarity ($p < 0.001$) but higher homogeneity ($p < 0.001$) and energy ($p < 0.001$), suggesting a more uniform texture compared to controls.

Conclusion: This study demonstrates the potential of image processing techniques in prenatal diagnostics by focusing on US imaging of placental health. Future work integrating such computational tools into routine prenatal screening could improve the assessment and management of placenta-related complications.

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LB.10

Exogenous Fibrin Induces Pathology and Diminished Placental Perfusion in the Rhesus Macaque

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Pathologists have long associated gross and histologic lesions with placental malperfusion. Increased fibrin deposition, inflammation, and calcification have historically been linked to altered maternal blood perfusion and adverse pregnancy outcomes (APOs) - though the location, quantity and combination of lesions that result in pathological malperfusion to the fetus remains unknown. MCP-1 is a potent chemokine that attracts a host of immune cells. Tisseel is a biological glue used in surgery applications, in which fibrinogen and thrombin combine to form fibrin. The study aim was to model placental malperfusion by inducing inflammation (MCP-1) or fibrin (Tisseel) at the placenta. On gestational day (GD) ~100 we injected MCP-1(0.5 mL, n=4), Tisseel (1.5 mL, n=2), or saline (0.5 mL, n=4) into the placental villi of macaques. MRIs were taken after injection (GD ~115) and before tissue collection (GD ~145) to determine changes in placental perfusion after treatment. At GD ~155 (full term = 165) fetal and placental tissues were collected for biometric and histological analysis. Whole slide imaging was used to quantify fibrin deposition, inflammatory injury, and calcification, with respect to location (chorionic plate, placental villi, trophoblastic shell, or decidua) in each cotyledon. MCP-1 had no effect on fetal biometrics, placental pathology, or placental blood flow compared to controls. Tisseel cotyledons showed increased fibrin deposition ($p=0.039$), chorionic inflammation ($p=0.001$), and calcification at both the chorionic plate ($p=0.002$) and placental villi ($p=0.025$). Additionally, placental blood flow decreased following Tisseel treatment, compared to uniform increases in saline controls. MCP-1 treatment alone was insufficient to induce placental pathologies and altered perfusion observed in APOs, while Tisseel led to increased placental pathologies and decreased placental blood flow. Together, these data illustrate that increased placental fibrin can induce additional placental pathologies commonly observed in APOs, and these pathologies directly coincide with diminished maternal blood flow in pregnancy.

LB.11

Chromosome 19 microRNA cluster expression in macaque primary and trophoblast stem cell-differentiated trophoblasts

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Introduction: The placenta-specific chromosome 19 miRNA cluster (C19MC) is unique to primates and plays a vital role in early embryo and placental development. The C19MC is expressed in macaque placenta, however, the cell-type specific expression of these miRNAs is undefined. To determine the expression of C19MC miRNAs in different types of trophoblasts, we evaluated miRNA expression in primary trophoblasts and in trophoblast stem cells (TSC) cultured in vitro.

Methods: Primary cytotrophoblasts (pCTB) were dispersed from macaque placentas (n=10) collected on gestation days 39-75. A subset of pCTBs was differentiated to primary syncytiotrophoblasts (pST) and cultured to derive TSC. TSC were differentiated into extravillous trophoblasts (EVT) and syncytiotrophoblasts, grown in 2D (ST2D) and 3D (ST3D) culture. The miRNA expression profiles of pCTB, pST, TSC, and TSC-differentiated ST2D, ST3D, and EVT were evaluated by miRNA-sequencing.

Results: Principal component analysis of C19MC expression segregated primary and TSC-derived trophoblasts, although C19MC miRNAs were expressed in all cell types. C19MC expression was higher in pST than pCTB, with significantly increased expression of 17 of 38 detected mature C19MC miRNAs in pST. When comparing primary and cultured trophoblasts, we found that 19 C19MC miRNAs were upregulated in TSC versus pCTB. A total of 12 and 10 C19MC miRNAs had significantly higher expression in ST2D and ST3D compared to pST, respectively. Few C19MC miRNAs were differentially expressed between TSC and their subsequently differentiated ST and EVT, however, miR-516a-5p and miR-520a-5p were more highly expressed in differentiated trophoblasts compared to TSC.

Discussion: The characterization of miRNA expression within *in vivo* and *in vitro* trophoblasts may provide valuable insights into their role in trophoblast differentiation and function. Additionally, comparing placental miRNA expression in macaque versus human EVTs may help us understand key species differences and provide crucial information for improving the modeling of human pregnancy pathologies in macaques.