Nursing in the periconceptional period alters placental gene expression and subsequent foal growth

Emilie Derisoud, Orianne Valais, Luc Jouneau, Alexia Durand, Michèle Dahirel, Audrey Prézelin, Yan Jaszczyszyn, Laurence Wimel, Pascale Chavatte-Palmer

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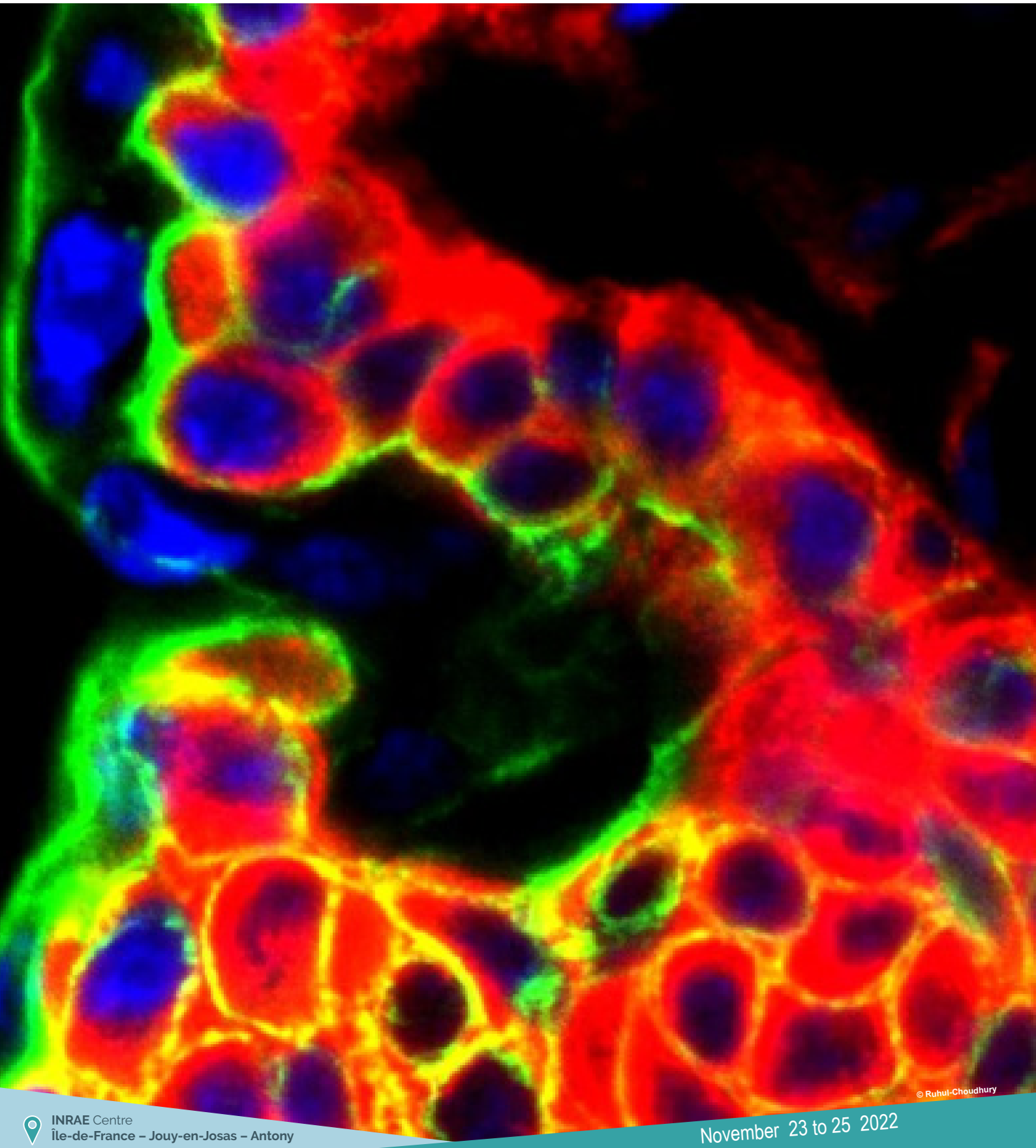

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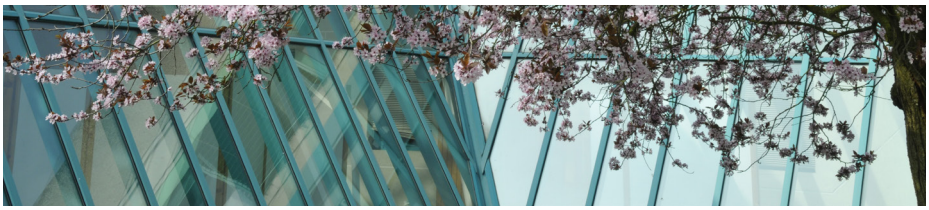

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CHARACTERISATION OF PROTEIN O-GLCNACYLATION REVEALS NOVEL REGULATION OF SECRETORY PATHWAY AND DNA DAMAGE PROTEINS DURING SYNCYTIOTROPHOBLAST DIFFERENTIATION

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Abstract

*Early stage investigator (up to three years post-PhD) - Yes

Background: Placental dysfunction underlies many obstetric diseases and is associated with impaired trophoblast dynamics, including differentiation of cytotrophoblast to syncytiotrophoblast (STB), and activated stress response pathways. Post-translational modification of nucleocytoplasmic proteins with O-linked acetyl-glucosamine (O-GlcNAcylation) occurs dynamically, and in response to stress. O-GlcNAcylation is mediated by a pair of enzymes: O-GlcNAc transferase (OGT) and removal enzyme O-GlcNAcase (OGA). Despite confirmed O-GlcNAcylation of thousands of proteins, the effects on trophoblast biology are largely unknown.

Aim: To identify dynamically O-GlcNAcylated proteins during STB differentiation and investigate how O-GlcNAcylation regulates protein activity during this process.

Methods: O-GlcNAcylated proteins were enriched by succinylated wheat germ agglutinin (sWGA)-binding from human trophoblast stem cell (TSC) lysates at stages of induced differentiation to STB (TSC, early STB differentiation [day 2], and mature STB [day 8]). Enriched and total proteins were identified by mass spectrometry. OGT inhibitor OSMI was used to verify O-GlcNAcylation-dependent enrichment. Candidate O-GlcNAcylated proteins were investigated by immunofluorescence microscopy in cultures treated with OSMI and the OGA inhibitor TMG.

Results: Total protein expression changes over TSC-STB differentiation reflect protein folding and metabolic pathways previously associated with STB function. 403 proteins were differentially enriched by sWGA-binding between TSC, early STB differentiation and mature STB ($p<0.05$). OSMI diminished enrichment in 49/403 proteins, and of these 29 have mapped O-GlcNAc sites. Mature STB was associated with reduced O-GlcNAcylation of 37/49 of these proteins, while early differentiation exhibited an intermediate state. A cluster of O-GlcNAcylated proteins in early differentiation included DNA damage-responsive kinase PRKDC (9 GlcNAc sites) and secretory COP1 complex delta subunit ARCN1 (5 GlcNAc sites). Immunofluorescence microscopy revealed OSMI- and TMG-responsive changes in Golgi association (ARCN1, $p<0.05$) and protein phosphorylation (PRKDC, $p<0.05$) during STB differentiation, suggesting that O-GlcNAcylation regulates the activity of these proteins.

Conclusion: Differentiation of TSC to STB reveals distinct patterns of O-GlcNAcylation. 49 differentially O-GlcNAcylated candidates were identified, with ARCN1 and PRKDC validated as differentially localised and phosphorylated respectively during STB differentiation in an O-GlcNAcylation-dependent manner. This is the first study to characterise O-GlcNAcylation during trophoblast differentiation, with ARCN1 and PRKDC identified as novel regulators in trophoblast dynamics. Understanding how stress-induced O-GlcNAcylation regulates STB differentiation and other trophoblast functions will provide mechanistic understanding to advance prevention and treatment options for placental dysfunction.

PUTATIVE PPARγ ISOFORMS IN PLACENTA: A SEQUENCE ANALYSIS AND RNA DETECTION IN BeWo CELLS AND PLACENTAL VILLI

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Abstract

*Early stage investigator - No

Context:

Peroxisome proliferator-activated receptor γ (PPARγ) encoded by the PPARG gene on chromosome 3p25.2 in humans, is a ligand-depend transcription factor belonging to the nuclear receptor family and contains a DNA (DBD) and a ligand binding domain (LBD). In various tissues, PPARγ controls cell differentiation and proliferation and its dysregulation can conduct to tumor development. Its implication in placenta function is today recognized in controlling trophoblast invasion and fusion. Actually, four RNA isoforms encoded by nine exons are described. Their specific roles and implication in placenta functions and cytotrophoblast cells, like fusion and invasion, are not clear. Moreover, the international data bases (NCBI and Ensembl) show a lot of different PPARG transcripts. The function of each transcript is not known yet. A sequence analysis is a prerequisite to highlight their roles in cytotrophoblast functions and in PPARγ activity regulation.

Methods: The coding sequence (CDS) and the protein sequence of PPARG transcripts described in NCBI and Ensembl data bases were extracted, analyzed and compared to PPARγ2 CDS and protein sequences. Nine primers which can target eight PPARG mRNA isoforms present in the NCBI Data base, were designed and validated by sequencing. PCR analysis was performed on BeWo cells, placenta villi and HUVEC cells. BeWo choriocarcinoma cells is a model for syncytium formation in placenta. Placental villi were isolated from elective caesarean section of term placenta.

Results: NCBI data base describes 16 PPARG transcripts for 8 RNA isoforms and 32 RNA transcripts coding for 26 putative proteins. The comparison of the CDS isoforms obtained from Ensembl and NCBI data bases identified 20 different CDS. The smallest is 123 bp long for 41 aa and the longest is PPARγ2 with 1518 bp for 505 aa. The identification of the DBD and LBD regions exhibits three categories of protein structure. Only three isoforms have the DNA binding domain (DBD) and the Ligand binding domain (LBD) sequence in full lengh. Nine of them contain the complete DBD sequence and five the LBD sequence. Six sequences have neither DBD nor LBD.Over the eight CDS isoforms described in NCBI data base, seven mRNA isoforms were detected in BeWo cells and four in placental villi.

Conclusions:

Twenty PPARγ isoform candidates are present in the two data bases NCBI and Ensembl. Identification of the DBD and LBD regions indicate a large diversity. This variety of possible PPARγ isoforms suggest different functions or roles in the regulation of PPARγ activity.

NOTCH3 IS A KEY REGULATOR OF HUMAN TROPHOBLASTSELF-RENEWAL AND DIFFERENTIATION

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Abstract

*Early stage investigator - Yes

Objectives:
Correct development of the human placenta and differentiation into its various trophoblast subtypes is inevitable for a successful pregnancy. On the one side, villous cytotrophoblasts (vCTBs) differentiate into the extravillous trophoblast (EVT) lineage invading the maternal uterus. In this regard, individual Notch receptors have been discovered that control development or migration of EVTs. On the other side, the role of NOTCH signaling during fusion of vCTBs into hormone-secretingsyncytiotrophoblasts(STBs),representingtheinterfacebetweenthematernalandfetalcirculation,hasbeenpoorlyelucidated. In the present study we provide evidence that NOTCH3 signaling is a key regulatory pathway controlling trophoblast expansion and cell fusion of purified placental vCTBs and human trophoblast stem cells (TSCs).

Methods:
Immunofluorescence, qPCR and Western blotting were performed to unravel the expression of individual Notch receptors, membrane-anchored ligands and mastermind-like (MAML) transcriptional co-activators in CTBs and STBs of first trimester placental tissue, primary cells, TSCs and TSC-derived organoids. In addition, TSC clones expressing Dox-inducible dominant-negative (DN)-MAML1, an inhibitor of canonical Notch signalling, or constitutively active NOTCH3 intracellular domain NOTCH3-ICD, the transcriptionally active form of the receptor, were created. DN-MAML1 clones were subjected to RNA-Seq and downstream targets were confirmed by qPCR and Western blotting.

Results:
The data suggest that NOTCH3/NOTCH3-ICD is the predominant receptor in first trimester placental tissues, vCTBs, organoids and TSCs, co-expressing JAG1, DLL1, MAML1 and MAML3. In contrast, NOTCH proteins are absent from STBs. Inhibition of the NOTCH3-dependent pathway using DN-MAML1 overexpression revealed upregulation of STB-specific genes, while factors associated with proliferation and stemness were downregulated. Moreover, constitutive active NOTCH3-ICD signaling showed the vice versa effect, maintenance of the vCTB or TSC phenotype and downregulation of STB formation.

Conclusion:
NOTCH3-ICD orchestrates a transcriptional program controlling maintenance and expansion of vCTBs and TSCs, while fusion into STBs is inhibited.

The present study is supported by the Austrian Science Fund (grant P-31470-B30)

TEMPORAL AND SPATIAL MAPPING OF VILLOUS TROPHOBLASTS AND STROMAL CELLS DURING HUMAN FIRST TRIMESTER PLACENTA DEVELOPMENT

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[2]Medical University of Vienna, Department of Obstetrics and Fetal-Maternal Medicine, Maternal-Fetal Immunology Group, A-1090 Vienna, Austria

Abstract

*presenting author, early stage investigator - Yes

Objectives: During pregnancy, the placenta constantly adapts to the requirements of the developing fetus through well-coordinated growth and communication between the trophoblast epithelium and the underlying villous core. Nonetheless, very little is known about trophoblast-stroma interactions that support early human placentation. Moreover, the spatial and temporal dynamics of villous core cell compositions remains still largely unknown. Hence, we aim to identify different stromal cell types as well as their locations along first trimester placental development as a prerequisite for studying specific interactions in 3D trophoblast/stromal cell co-culture models.

Methods: To address these tasks we have developed an enhanced tissue processing protocol. Precisely, we performed four subsequent digestion steps with various enzymes to isolate the full spectrum of trophoblast and stromal cells from 6/7th (early) and 10/11th (late) week placental tissues (time points before and after oxygen switch in the intervillous space). In order to construct a detailed cellular map of villous cell populations we conducted flow cytometry, immunofluorescence analyses, qPCR, and western blot analyses as well as single cell RNA-sequencing (scRNA-seq) of five samples from each early and late week placentae.

Results: Preliminary analyses of scRNA-seq data sets revealed an overall 10-fold increase of the stromal cell compartment between early and late placentae with a simultaneous decrease in the trophoblast population. In order to unravel specific cellular locations, we prepared tissue specimens allowing us to define two principle areas within early and late placental tissue. The area close to the chorionic plate predominantly contains large stroma cell-rich villi, while the basal plate area facing the maternal decidua is characterized by small-sized villi occasionally containing trophoblast cell columns. Using immunofluorescence analyses, we were able to detect differences in the localization of CD14^{pos} (Hofbauer cells), CD31^{pos} (endothelial cells), and aSMA^{pos} cells depending on the spatial localization within early and late gestational placentae. Hofbauer cells, for example, were largely absent in early placentae and, if any, only detectable in large-sized villi close to the chorionic plate.

Conclusion: Taken together, we observed a dynamic change in stromal cell compositions and locations during first trimester placental development indicating an adaptation of placental morphology and function supporting fetal growth and wellbeing.

This study is supported by the Austrian Science Fund (grant P-34588).

COULD A PRECONCEPTION/PREGNANCY COMBINED MYO-INOSITOL, PROBIOTIC AND MICRONUTRIENT SUPPLEMENT ALTER PLACENTAL POLY-UNSATURATED FATTY ACID (PUFA) METABOLISM WHICH CAN INFLUENCE PARTURITION?

Victoria K B Cracknell-Hazra, Oliver C. Watkins, Mohammad Omedul Islam, Preben Selvam, Reshma Appukattan Pillai, Hannah EJ Yong, Anne K Bendt, Amaury Cazenave-Gassiot, Keith M Godfrey, Markus R Wenk, Wayne Cutfield, Rohan M Lewis, Shiao-Yng Chan

Abstract

Objectives | The poly-unsaturated fatty acids (PUFA), docosahexaenoic acid (DHA) and arachidonic acid (AA), are vital for fetal-placental development, labour, and long-term offspring health. While PUFA metabolism and maternal metabolic health have been implicated in the pathogenesis of preterm birth the role of nutrition remains uncertain. The NiPPeR RCT investigating a combined nutritional supplement taken pre-conception and throughout pregnancy reported reduced incidence of preterm birth. We hypothesised that preconception maternal metabolic health influences placental PUFA metabolism and that the NiPPeR intervention moderates this association.

Methods | Term placentae were collected from singleton pregnancies in the NiPPeR trial in Singapore (11 control, 12 intervention). Placental explants were incubated with ^{13}C-labeled-AA and ^{13}C-labeled-DHA for 48 hours. Newly synthesized ^{13}C-labeled-lipids were quantified by liquid-chromatography-mass-spectrometry (LCMS). Enrichment of labelled lipids (i.e., the turnover of existing lipids to newly synthesised ^{13}C-lipid) was calculated. Preconception HOMA2-IR (insulin resistance, a marker of metabolic health) was calculated. Data was analysed by linear regression with Benjamini-Hochberg correction for multiple testing. Statistical significance was set at $P<0.05$.

Results | 19 newly synthesised DHA-containing lipids and 23 newly synthesised AA-containing lipids were quantifiable. In the control group (taking a standard supplement) increasing pre-conception HOMA2-IR positively associated with 9 AA lipids including diacylglycerol 38:4 (P=0.002), phosphatidylcholine 40:8 (P=0.004) and phosphatidylcholine 36:4 (P=0.004), and negatively associated with triacylglycerol 52:5 (P=0.018). In the intervention group, alterations in all lipids were moderated towards a physiological mean; only two remained positively associated but less strongly [phosphatidylinositol 40:4 and 38:4]. No significant changes were seen in DHA lipid enrichment with increasing preconception HOMA2-IR in either the control or intervention groups.

Conclusions | The impact of preconception maternal insulin resistance on placental lipid metabolism is fatty acid specific, specifically affecting AA metabolism. This association appears to be moderated by the nutritional intervention. Since AA phospholipids are precursors of prostaglandins and eicosanoids critically involved in parturition, alterations in placental AA metabolism could be a mechanism for the reduction in preterm births observed with the intervention. This highlights the potential for a nutritional intervention to reduce complications in pregnancy through influencing placental lipid metabolism.

15-HYDROXYPROSTAGLANDIN DEHYDROGENASE (HPGD) IN HUMAN EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION

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Abstract

*Presenting author; early stage investigator - No

Objectives: Correct extravillous trophoblast (EVT) formation and function is a crucial prerequisite for uncomplicated pregnancies ensuring maternal and fetal wellbeing. Recently, we identified transforming growth factor-β (TGF-β) signaling as a key mechanism for EVT maturation, establishing secretory features of interstitial EVTs (iEVTs). RNA sequencing data of in situ EVT populations, placental EVTs (pEVTs) and iEVTs, and *in vitro*-derived trophoblast organoid EVTs (ORG-EVTs) revealed that TGF-β-inhibited EVTs failed to express various iEVT-specific genes, including HPGD. HPGD is a key enzyme for catabolizing the prostaglandins PGE2 and PGF2α and was reported to be expressed at the maternal-fetal interface. Interestingly, HPGD knockout studies in mice provoked early pregnancy loss at day 8.5 indicating a crucial role of this enzyme during pregnancy. However, little is known about localization and regulatory mechanisms of HPGD during human placental development.

Methods: To verify HPGD expression, we conducted immunofluorescence, Western blotting and qPCR analyses of first and third trimester placental and decidual tissue as well as third trimester fetal membranes. Furthermore, we inhibited or activated TGF-β signaling in isolated first trimester EVTs for 72 hours or in ORG-EVTs for 10 days, and subsequently performed qPCR and Western blot analyses using cellular protein extracts and cell culture supernatants.

Results: Immunofluorescence of placental and decidual tissue, derived from 6/7th and 10/11th week, revealed that HPGD was exclusively expressed in placental EVTs (pEVTs) of the distal cell column as well as in the majority of iEVTs. In third trimester tissue, we detected HPGD in iEVTs as well as in chorion trophoblasts of the fetal membranes. Furthermore, qPCR analyses of isolated trophoblast subpopulations revealed that the enzyme was present in EVTs but absent from syncytiotrophoblasts, and villous cytotrophoblasts. We additionally stained for proteins that we recently identified as TGF-β-induced iEVT-specific secreted proteins including diamine oxidase (DAO) and pappalysin-A2 (PAPPA2) and determined that DAO- and PAPPA2-positive cells co-expressed HPGD. *In-vitro* experiments of isolated EVTs and ORG-EVTs further confirmed that TGF-β activity is required for HPGD expression and subsequent secretion.

Conclusions: In summary, we revealed a TGF-β-dependent expression of HPGD in pEVTs coinciding with the localization in the majority of iEVTs throughout gestation. The data suggest a crucial role of this enzyme in EVT-controlled prostaglandin homeostasis within the maternal decidua.

The present study is supported by the Austrian Science Fund (grant P-34588).

VIEW INSIDE A CURRENT STUDY ON SENESCENCE OF ENDOMETRIUM IN RIF PATIENTS

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Abstract

Uterine Natural Killer (uNK) cells in the endometrium are described to be required for successful implantation and placentation. In contrast to cytotoxic peripheral blood NK cells endometrial uNKs are immunoregulatory. [1] Nevertheless, patients with fertility problems show lower or higher uNK cell concentration compared to a mid range in fertile women. [2] The aim of this study is to determine, whether indications of senescence can be found in the endometrium of patients with Recurrent Implantation Failure (RIF) and if so whether the expression of this senescence markers is associated with the age of the patient and/or the uNK cell concentration. The number of CD56+ uNK cells, CD138+ plasma cells and CD16+ cells as well as the expression of the senescence markers p16 and DIO2 were assessed by immunohistochemistry in endometrial biopsies from 63 RIF patients and 10 healthy controls (day 19-22). Pathologic and CD138 positive patients were excluded.

An exemplary proteome analysis was performed using mass spectroscopy by using the eFASP method. Multiplex immunofluorescence staining was performed to visualise the spacial relationships between the markers p16, DIO2, CD56, CD31 and cytokeratin. Initial analyses show increased expression of senescence markers in RIF patients compared to healthy controls. In the groups with high uNK cell counts (>300 cells/mm2), a higher senescence marker expression was found than in the group with low concentrations (<100 cells/mm2).

This findings will be validated by further data analysis of this study.

Keywords: endometrium; senescence; recurrent implantation failure (RIF); natural killer cells; CD56; CD138; CD16; p16; DIO2; CD31.

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MODULATING RESISTANCE TO FETOPLACENTAL BLOOD FLOW ALTERS MATERNOFETAL OXYGEN TRANSFER

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Abstract

*Early stage investigator - No

Introduction: Qualities of fetoplacental blood flow include net blood flow rate as well as villous distribution linked to blood flow matching between the two circulations. Ex vivo dual perfusion of the human placenta enables an assessment of the relationship between resistance to fetoplacental blood flow and oxygen (O_2) transfer across the placental barrier. O_2 transfer is largely dependent on flow rates through the placenta. We hypothesise that compounds that modulate vascular tone, such as antihypertensives, alter O_2 transfer across the placental barrier and dysregulate maternofetal blood flow matching, which may affect fetal growth.

Methods: Placental lobules from normal human pregnancies were perfused ex vivo, first with Earle's Bicarbonate Buffer (EBB) alone (open-circuit, 60 mins), followed by gravitational flow with 20% fetal blood haematocrit/80% EBB (control phase; closed-circuit, 15 mins) in the maternal circulation at 14mL/min. A single cannula in the intervillous space (IVS) with an acentric lobule position was used, leaving the distal side of the lobule with reduced IVS perfusion. An optode monitored O_2 in this area. The fetal circulation was perfused with a peristaltic pump (open-circuit) at 6ml/min with EBB alone throughout (N=3). Following the control phase perfusion, the vasodilatory compounds sodium nitroprusside (SNP), was added to the maternal haematocrit/EBB perfusate at 10-5 and 10-4M. Real-time PO_2 readings were taken in the fetal-circulation afferent and efferent to the placental lobule, and afferent to the IVS. Real-time fetal-side inflow hydrostatic pressure (FIHP) was recorded afferent to the chorionic plate arterial cannula.

Results: Maternal-side haematocrit/EBB perfusion had no effect on FIHP compared to EBB alone (32.0 ± 0.9 vs 33.3 ± 0.7 mmHg, respectively). Addition of SNP 10-5 and 10-4M to the maternal-side haematocrit/EBB perfusate reduced FIHP by a maximum value of 27.2% (23.3 ± 7.2 mmHg; P=0.1000) and 36.6% (20.3 ± 8.1 mmHg; P=0.1000), respectively. The addition of SNP to the IVS increased fetal venous PO_2 by 25.7%, from 21.4 ± 0.7 to a maximum value of 26.9 ± 13.3 mmHg, whilst reducing IVS PO_2 values distal to the maternal cannula by 64.2 % (P=0.1000). Temporally matched mean fetal venous PO_2 values negatively correlated with mean FIHP values (Fig 1).

Conclusions: With 90% certainty, SNP reduced vascular resistance in the fetal-side villous circulation, which led to an increase in O_2 acquisition in the fetoplacental circulation. The reduction in IVS PO_2, measured via the O_2 optode distal to the IVS maternal cannula, suggests a re-distribution of villous blood flow. Whilst SNP appeared to have a negative effect on FIHP, further work will include temporal control experiments in the absence of SNP. This study provides a first insight into how antihypertensives, that may differently impact on the fetoplacental blood flow, as seen in the clinic, might also affect placental O_2 transfer for fetal growth.

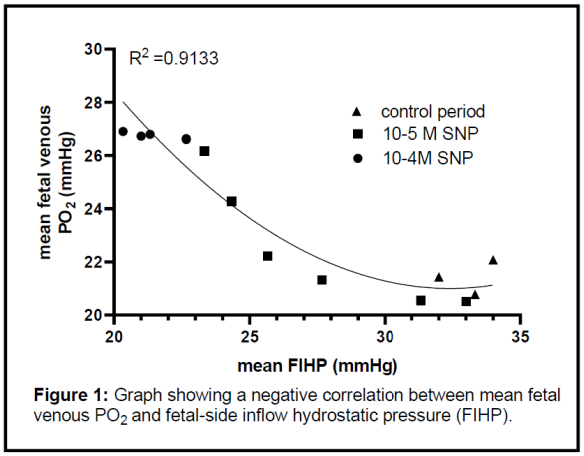

Figure 1: Graph showing a negative correlation between mean fetal venous PO_2 and fetal-side inflow hydrostatic pressure (FIHP).

LIVER X RECEPTOR ACTIVATION ATTENUATES OXYSTEROL INDUCED INFLAMMATORY RESPONSES AND VASCULAR DYSFUNCTION IN PLACENTAL ENDOTHELIAL CELLS

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Abstract

*Early stage investigator

Introduction: Oxysterols are oxygenated derivatives of cholesterol with pro-inflammatory properties. They serve as endogenous ligands for nuclear receptors namely Liver X Receptor (LXR). Elevated levels of oxysterols (eg: 7-keto-cholesterol, (7-ketoC) 7β-hydroxycholesterol, (7β-OHC)) have been observed in pregnancy pathologies like gestational diabetes and pre-eclampsia. These pathologies are often associated with dysfunction of the placental vasculature. LXR activation has been known to inhibit inflammatory pathways. This study investigates the role of oxysterols on alterations of the placental vasculature by looking at inflammatory responses of primary fetoplacental endothelial cells (fPEC). The ligand-specific activation of LXR and its regulation of downstream genes linked to inflammation and endothelial functions are particularly investigated in fPEC.

Methods: Primary fPEC were isolated from term placentas and treated with oxysterols or synthetic LXR agonists TO901317 (TO) for 24h. Intracellular cytokine profile, pro-inflammatory protein levels, and p65-NFκB nuclear translocation and cell adhesion molecule expression were analyzed by qPCR, immunoblotting, immunofluorescence and FACS respectively. Cellular barrier functions and membrane stiffness were investigated using electric cell substrate impedance sensing and Atomic Force Microscopy respectively.

Results: 7-ketoC and 7β-OHC activated TLR-4 inflammatory signaling cascade by significant enhancement of p38, ERK, JNK, p65-NFkB phosphorylation, cytokine transcription (IL-6, IL-8) and ICAM-1 expression in fPEC (**p<0.01). Moreover, oxysterols induced calcium flux, depolarization of mitochondria, plasma membrane stiffness and loss of endothelial intercellular junction integrity (**p<0.01). Addition of LXR agonist TO significantly inhibited the TLR-4activation and downstream phosphorylation events and inflammatory gene transcription. Additionally, LXR activation rescued and maintained the monolayer barrier integrity of fPEC (*p<0.05). TLR-4 inhibitor (Tak-242) diminished the oxysterol induced inflammatory responses (**p<0.01). Inhibition of LXR activation by antagonists prevented the protective effects of TO confirming the essential role of LXR in regulating vascular function and inflammation.

Conclusion: Our findings suggest that 7-ketoC and 7β-OHC induce inflammation and vascular dysfunction in fPEC, although oxysterols serve as LXR agonists. In contrast, LXR synthetic agonist TO exerted protective effects against oxysterols by inhibiting TLR-4 signaling cascade thereby preventing fPECs to switch to a pro-inflammatory phenotype in the presence of oxysterols. We speculate that, LXR activation by oxysterols could be a result of a negative feedback mechanism which could subside the chronic inflammation occur otherwise. TO activate LXR target genes as ABC-transporters and thereby affecting cellular cholesterol homeostasis. In turn this imbalance may inhibit oxysterol induced TLR-4 activation in fPEC.

THE PLACENTA - A NEW SOURCE OF BILE ACIDS DURING PREGNANCY

Edgar Ontsouka[1,*], Mariana Schroeder[1,2], Linda Ok[3], Cathy Vaillancourt[3], Deborah Stroka[4], Christiane Albrecht[1,2]

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Abstract

*Early stage investigator

Bile acids (BA), e.g. cholic acid in humans or muricholic in mice, are endogenous ligands specifically interacting with cell-surface receptors that mediate, among others, cellular proliferation, smooth muscle contraction and vascular development. BA are synthesized from cholesterol mainly in the liver via the classic (or neutral) and alternative (or acidic) pathways. The classic pathway is initiated by the microsomal cytochrome P450 (CYP) 7A1 and converts cholesterol to 7a-hydroxycholesterol, while the alternative pathway starts with hydroxylation of the side chain of cholesterol producing an oxysterol. Beside originating from the liver, BAs are reported to be synthesized in the brain.

We aimed at determining the potential role of the placenta as an extrahepatic source of BA. To this end, we screened the expression profile of selected enzymes and transporters, constituents of the hepatic BA-synthesis machinery, in human term (mean gestational age: 39.4 weeks) and CD1 mouse (late gestational age) placentas. The placental mRNA profiles of these targets were compared to identify potential species-specific differences. Additionally, mouse placenta and mouse brain data were compared to determine whether alternative BA synthesis is analogous to extra-hepatic tissues.

Our results showed that the mRNA coding *CYP7A1*, *CYP27A1*, *CYP46A1* and *BAAT* were lacking in human placentas, while corresponding homologs were detected in the murine placentas. Conversely, mRNA coding *Cyp8b1* and *Hsd17b1* were undetected in murine placentas, while its homologs were expressed in the human placenta. *CYP39A1/Cyp39a1* and cholesterol 25-hydroxylase (*CH25H/Ch25h*) mRNA were detected in both species. When comparing extra-hepatic tissues, the presence of *Cyp8b1* and *Hsd17b1* mRNA was only detected in the brain.

Our data demonstrate that the placenta is an extra-hepatic source of BA, and that BA-synthesis-related genes are placentally expressed in a species-specific manner. Moreover, *CYP8B1/Cyp8b1*, *HSD17B1/Hsd17b1*, and *BAAT/Baat* are determinants discriminating between BA synthesis in human and murine placentas. These new findings imply that placentally synthesized BA could serve as endocrine and autocrine stimuli, which may play a role in fetoplacental growth and adaptation, thereby impacting pregnancy outcome.

EFFECT OF CHOLESTEROL ON AMPK PROTEIN EXPRESSION AND ACTIVATION IN HUMAN MSPH PLACENTAS, BEWO, AND PHT CELLS

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Abstract

During pregnancy, maternal physiological hypercholesterolemia (MPH) occurs as an adaptive response to maintaining fetal lipids demands. However, in some cases, an increase in cholesterol plasma concentration exceeds 280 mg/dL, leading to maternal supraphysiological hypercholesterolemia (MSPH). MSPH has been connected to placental vascular dysfunction, and atherosclerosis development in fetal arteries, linked with an increased risk of cardiovascular diseases and hypercholesterolemia during the whole offspring's life. AMP-activated protein kinase (AMPK) is a crucial sensor of cellular energy, stress, and metabolism, involved in many physiological pathways. Moreover, it has been reported to attenuate atherosclerosis development. AMPK placental dysregulation has been related to other metabolic pregnancy complications, such as diabetes mellitus. However, the role of placental AMPK under MSPH conditions has never been described. Therefore, we investigated changes in AMPK protein expression and phosphorylation (active form, pAMPK) between MPH and MSPH human placentas and after low-density lipoprotein (LDL), high-density lipoprotein (HDL), oxidized low-density lipoprotein (ox-LDL), and metformin (MET) treatment in BeWo and PHT cell cultures. Our results revealed significant upregulation of AMPK and pAMPK protein expression in MSPH placentas, which correlated with total cholesterol and LDL maternal plasma concentration. In BeWo cells all studied lipoproteins induced pAMPK protein expression, but not AMPK protein expression. Contrary, in PHT cells, LDL, ox-LDL and MET induced expression of both proteins. In summary, we demonstrate cholesterol-induced AMPK activation and upregulation as an adaptive mechanism, equilibrating altered metabolic conditions in the fetoplacental unit during MSPH.

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ANALYSIS OF TRAFFIC-RELATED PARTICULATE MATTER IN PERFUSATES OF *EX VIVO* PERFUSE HUMAN PLACENTAL LOBULES

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Abstract

* presenting author; early stage investigator - Yes

Introduction: Traffic-related air pollution (TRAP) nanoparticles levels associate with childhood cancer probability. *Ex vivo* dual perfusion of the human placenta permits the investigation of transplacental transfer of nanoparticles from the maternal to the fetal circulation. Firstly, reliable method development is needed to quantify and characterise TRAP nanoparticles within perfusates sampled from the maternal and fetal circulations. We tested the hypothesis that a nanoparticle filter-capture method, followed by scanning electron microscopy with energy dispersive X-ray analysis, provides a reliable means to quantify TRAP nanoparticles in perfusates prior to future transfer studies.

Methods: A placental cotyledon from normal pregnancy was collected following informed written consent (ethics: LREC 15/NW/0829), and dually perfused *ex vivo* with supplemented Earle's bicarbonate buffer, including 3.5% (w/v) dextran (70K) in closed-circuit (following a 1-hour open-circuit washout period). The maternal reservoir contained - TRAP particles liberated by sonication from a PM2.5 pre-filter from a NO2 air quality monitoring station. T= 0 hour maternal and fetal reservoir perfusates were sampled. 1 mL of each was forced through polycarbonate filters (0.1µm pores), using a syringe driver at 0.2 mL/min. The filters stubs were coated with a 5nm layer of carbon to eliminate charge interference noise, and 9 inter-mesh micrographs were systematically taken (800x magnification) followed by particulate analysis per field of view (FOV). Particle chemical identities were determined, and analysis of variance of particle numbers with a running mean of FOVs determined accuracy of representation of the filter population.

Results: Nine FOVs at 800x magnification yielded a reliable estimate of the filter population of nanoparticles (>0.1 µm) within 5% variance of the running mean. Particulate counts per FOV were 27±5.3 and 6.7±3.3, for the maternal and fetal reservoirs, respectively (mean±SE) at zero hours. Corresponding chemical characterisation revealed the elements: O, Al, Si, Ca, Fe, Au in the maternal perfusate and O, Al, Si, Ca, Cr, Fe in the fetal perfusate found in >10% of particles. It was not possible to gauge particulate carbon levels, due to the need carbon-coat the filters. Na, a positive control for perfusate, could not be detected, suggesting an adequate filter washing.

Conclusions: This method development has enabled quantification and elemental composition of TRAP nanoparticles >0.1µm within perfusates used in *ex vivo* dual perfusion of the human placenta, with a background noise of 25%. Analysis of perfusates from further experimental time points, with additional ultra-fine particle analysis, will provide an understanding of whether TRAP particles traverse the placenta barrier. This will enable a better understanding of how environmental pollution affects fetal and neonatal health in later life, including genomic effects from particle adhered carcinogens.

HYDROXYCHLOROQINE EFFECT ON CYTOKINE INDUCED ACTIVATION OF FETO-PLACENTAL ENDOTHELIAL CELLS

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Abstract

*Early stage investigator - Yes

Hydroxychloroquine (HCQ) is a well-known drug with different anti-inflammatory and immune-regulatory effects. It is widely used for different autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome. HCQ has been associated with a lower risk of preeclampsia and higher gestational age at time of delivery in pregnant lupus patients who continued taking HCQ treatment during pregnancy. Although, HCQ crosses the placenta, its safety during pregnancy has been confirmed. Therefore, HCQ seems to be a promising candidate for treatment of pregnancy related disorders connected to cytokine activation of endothelial cells. We aim to investigate the effect of HCQ in model of multi-cytokines induced endothelial dysfunction of the placenta.

Primary feto-placental arterial endothelial cells (fpECAs) were isolated from the chorionic plate of six healthy term placentas. For analysis, cells were incubated in the presence or absence of 1 µg/mL HCQ and/or cytokine mix (MIX) (interleukin (IL)-6 10 ng/mL; TNF-α 10 ng/mL; IL-1β 1 ng/mL). Because TNFα, IL-6 and IL-1β were used as challenge, gene and protein expression of IL-8 and leukocyte adhesion molecules (ICAM-1, VCAM-1 and selectin E (SELE)) were measured with RT-qPCR as well as FACS and/or western blot, as a readout for endothelial activation. To test the HCQ effect on the endothelial functionality of fpECAs, a wound-healing assay was used, while xCelligence real-time cell analysis was performed to test the effect on endothelial barrier integrity. Statistical analyses of data are done in GraphPad Prism version 9.4. by use of paired repeated measurements one-way ANOVA with Sidak post-hoc or Friedman test with Dunn's post hoc.

Cytokine mix led to a rise in mRNA and protein levels of IL-8, ICAM-1, VCAM-1, while increasing effect on SELE was noticeable only on mRNA level. In case of cells treated only with HCQ alone, there was a significant downregulation of VCAM-1 and SELE on mRNA level. Interestingly, reduction of IL-8 by HCQ was significant on both, mRNA and secretory level. Treatment of HCQ together with cytokines reduced total protein levels of VCAM-1, thus it did not affect IL-8, ICAM-1 and SELE. The wound closure ability of fpEPCAs challenged with cytokine mix was significantly reduced, while the combined treatment with HCQ resulted in a moderate but not significant improvement compared to control. Moreover, real-time impedance analysis showed that addition of cytokine mix with and without HCQ led to a loss of endothelial barrier integrity after five hours. HCQ alone did not affect neither wound healing ability nor endothelial barrier integrity.

Although HCQ did not prevent activation of endothelial cell, with exception of VCAM-1, it did not impair it either. This additionally confirms the safety of this drug. Our study also suggests that the effect of HCQ on basal levels of IL-8, VCAM-1 and SELE is independent of cytokine cascade.

PLACENTAL EXTRACELLULAR VESICLES COULD MODIFY HEMATOPOIESIS DURING PREGNANCY

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Abstract

*Early stage investigator

Introduction: During pregnancy, major adaptations of the maternal immune system take place in order to successfully combat pathogens but tolerate the semi-allogenic fetus. Pregnancy is also marked by an increase in the erythrocyte count. While numerous studies are focusing on the end-point immune cell phenotypes in the peripheral blood and the uterine environment, little is known about the effects of pregnancy on the hematopoiesis, the process of differentiation of hematopoietic stem cells (HSCs) that reside in the bone marrow (BM) into different blood cell lineages. Extracellular vesicles (EVs) secreted from the placenta are important messengers for fetal-maternal communication and can alter the function of recipient cells.
In this pilot study we aim to investigate the uptake of placental EVs by HSCs and their effects on HSC differentiation into immune cell types and erythrocytes.

Methods: One-sided human *ex-vivo* term placenta perfusion was performed as a source of placental EVs. Different populations of EVs, namely pooled EVs, small EVs (sEVs) and large EVs (lEVs) were enriched by ultracentrifugation. EV samples were characterized in regard to protein content (BCA assay), marker expression (Western blot) and particle size (nanotracking analysis, cryo-TEM). CD34+ HSCs were obtained from peripheral blood of healthy female donors. Uptake of EVs by HSCs was analyzed by flow cytometry (FC), confocal microscopy and detection of placenta-specific miRNA by qPCR. HSCs were treated with different concentrations of EVs and allowed to differentiate for 14 days. FC analysis (CD34, CD3, CD14, CD19, CD56, CD235a, DAPI) and colony forming unit (CFU) assay was performed on day 0, 7 and 14 of culture to assess the differentiation stage of the EV-treated cells.

Results: Placenta perfusions were successfully performed and different populations of EVs enriched. Placental origin of the EVs was confirmed by PLAP expression. sEVs show high expression of CD63, Alix and TSG100, whilst lEVs are enriched for CD47. Both populations are taken up by the HSCs. There is a time-dependent decrease of CD34+ HSCs concomitant with an increase of more mature cell types within the 14 days of the study. Preliminary results showed that compared to the untreated control, placental EV-treated HSCs resulted in a higher percentage of CD235+ erythrocytes and CD14+ monocytes while there was a decrease in the percentage of CD3+ T-cells after 7 days.

Conclusion: Placenta perfusion is a valuable source of placental EVs. CD34+ HSCs are able to internalize placental EVs. Our results are consistent with the reports in the literature showing that pregnancy immune phenotype is characterized by an increase in erythrocytes and monocytes and a decrease in T-cells. These *in vitro* findings may indicate that placental EVs may target the BM and shape the hematopoiesis therein.

PLACENTAL UPTAKE OF VITAMIN D; A POTENTIAL MEDIATOR OF FETAL GROWTH?

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Abstract

*Early stage investigator - Yes

Suboptimal maternal 25-hydroxyvitamin D (25(OH)D) levels during pregnancy associate with reduced fetal growth and increased risk of poor postnatal health. We previously show that within the human placenta, 25(OH)D is metabolised into its more active form, 1,25-dihydroxyvitamin D [1,25(OH)2D] and that both can be taken up into the placenta potentially via endocytosis, however the exact uptake mechanisms are unclear.

We investigated whether 25(OH)D and 1,25(OH)2D are taken up by human placenta or cell lines via endocytosis.

Term human placental villous fragments (n=5 per treatment) were cultured for 8 h with 20 μM 25(OH)D + vitamin D binding protein plus the endocytic inhibitors 5 mM amiloride and 10 μM cytochalasin D, which block pinocytosis and clathrin-dependent endocytosis respectively. The vitamin D-mediated up-regulation of *CYP24A1* mRNA expression in placental fragments, as measured by qPCR, was significantly reduced by amiloride and cytochalasin D ($p<0.05$), suggesting that human placenta takes up 25(OH)D via endocytosis.

Further experiments in HEK293 cells used siRNA to knockdown the expression of genes involved in endocytosis. Cells were cultured with 1 μM 1,25(OH)2D for 24 h and uptake was measured with a luciferase reporter assay using vitamin D response elements from the human *CYP24A1* promoter. Clathrin knockdown increased the vitamin D luciferase reporter assay response ($p<0.05$, n=5).

Our data indicate that vitamin D is transported into the placenta by a selective and controlled active mechanism not just via diffusion, however these observations indicate that cellular uptake and signalling of vitamin D is complex. Insufficient placental transport of vitamin D may decrease placental gene expression and limit the fetal supply, affecting fetal development.

PLACENTAL UPTAKE OF GLUTAMINE AND GLUTAMATE IS REDUCED FOLLOWING EXPOSURE TO TERT-BUTYL HYDROPEROXIDE (TBOOH), AN INDUCER OF OXIDATIVE STRESS

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Abstract

*Early stage investigator - Yes

Background: Fetal growth restriction (FGR) describes a fetus that fails to achieve its genetic growth potential and is a major risk factor for stillbirth. We have demonstrated that placental uptake of glutamine and glutamate, key amino acids for fetal growth and placental metabolism, is reduced in FGR although the mechanisms responsible are unknown. FGR is associated with elevated placental oxidative stress (OS) and an agent that induces OS, tert-Butyl hydroperoxide (tBOOH), inhibits amino acid uptake into BeWo cells. However, the possibility that OS underlies the reduced placental uptake of glutamine and glutamate in FGR has not been investigated. Here we test the hypothesis that OS reduces glutamine and glutamate uptake into placental tissue from normal pregnancy.

Methods: In this study, an *in vitro* model that mimics placental OS seen in FGR was used. Placental explants from normal pregnancy (N=6) were cultured for 7 days and exposed to 1mM tBOOH or 1mM hydrogen peroxide (H_2O_2) on days 5 and 6. Ouabain-sensitive (transporter-mediated) uptake of ^{14}C glutamine and ^{14}C glutamate was measured for 24h between days 6-7 (shown previously to represent steady-state intracellular accumulation). Culture medium was collected daily to measure indicators of endocrine function (hCG) and cellular stress (IL6 secretion and lactate dehydrogenase (LDH) release). Immuno-histochemistry to assess syncytiotrophoblast integrity (Cytokeratin 7 (CK7)), apoptosis (M30) and lipid peroxidation (4HNE) was assessed in explants at day 7. Data are expressed as % of control and analysed by Wilcoxon signed rank test.

Results: Both tBOOH and H_2O_2 significantly increased lipid peroxidation, consistent with elevated OS (16x and 6x respectively; $p<0.05$). tBOOH reduced the uptake of glutamine and glutamate by 90(66-100)% and 95(40-99)% of control (median and range: $p<0.05$), inhibited hCG secretion by 75(5-81)%, andincreased IL6 secretion to 5774 (1420-16365)% of control ($p<0.05$). tBOOH increased apoptosis to 275 (100-433)% of control ($p<0.05$) but did not alter CK7 staining or LDH release. Despite elevating OS, H_2O_2 did not affect glutamine and glutamate uptake or any of the other variables measured.

Conclusion: 1mM tBOOH induced OS and markedly inhibited glutamine and glutamate uptake. In view of the lack of effect of H_2O_2 on amino acid uptake, the reduction in uptake following tBOOH exposure might not be directly attributed to oxidative stress. Furthermore, 1mM tBOOH also had detrimental effects on other variables that can influence nutrient transport. Future work will investigate the cellular mechanisms through which tBOOH alters placental function, and the concentration dependency, to aid our understanding of whether oxidative stress has direct effects on glutamine and glutamate uptake.

DNA METHYLTRANSFERASE 3B IS ESSENTIAL FOR PLACENTAL DEVELOPMENT

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Abstract

DNA methylation is a repressive epigenetic modification that is essential for development, exemplified by the embryonic and perinatal lethality observed in mice lacking de *novo* DNA methyltransferases (DNMTs). Here we characterise the role for DNMT3A, 3B and 3L in gene regulation and development of the mouse placenta. We find that each of the DNMTs establishes unique aspects of the placental methylome through targeting to specific genomic regions based on underlying chromatin features. We demonstrate that loss of *Dnmt3b* results in de-repression of germline genes in trophoblast lineages and impaired development of the placental maternal-foetal interface. Using single nuclei RNA sequencing, histological analysis and immunofluorescence, we find that loss of *Dnmt3b* does not lead to abnormalities in lineage specification *per se*, but rather to defective formation and vascularisation of the placental labyrinth. Using *Sox2*-Cre to delete *Dnmt3b* in the embryo, leaving expression intact in placental trophoblast cells, we were able to rescue the placental phenotype and, consequently, the embryonic lethality, as *Dnmt3b* null embryos could now survive to birth. We conclude that de *novo* DNA methylation by DNMT3B during embryogenesis is principally required to regulate placental development and function, which in turn is critical for embryo survival.

SEX-DIFFERENTIAL EFFECT OF MATERNAL OBESITY ON PLACENTAL NUTRIENT SENSING PATHWAYS

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Abstract

*Early stage investigator

Introduction: The global obesity epidemic does not spare women of childbearing age, predisposing their offspring via fetal programming to a wide range of metabolic diseases and perpetuating a vicious cycle of obesity. Maternal obesity up-regulates the mechanistic target of rapamycin (mTOR) signaling pathway in placenta, a key regulator of nutrient transfer to the fetus. This placental dysfunction is often related to fetal overgrowth. Additionally, AMP activated protein kinase (AMPK), another nutrient sensor, is downregulated in placenta of obese mothers with infants born large for gestational age. Yet, with the exception of extreme birthweights, little is known about placental adaptations to maternal obesity. Moreover, literature evidences suggests that the role of fetal sex, particularly in placental nutrient sensing pathways, should be seriously considered. We hypothesized that placental mTOR and AMPK pathways are differentially regulated by fetal sex in response to maternal obesity.

Method: We enrolled pregnant women in a prospective placenta bio-collection cohort. Term placental tissue was collected from women with pre-gravid obesity (OB, BMI=33.6±4.1 kg/m2, 12 female/15 male infants) and normal BMI (N, BMI=21.5±2.1 kg/m2, 13 female/19 male infants). They gave birth to babies whose birth weight (BW) was considered appropriate for gestational age and not different between the two groups of mothers (OB, BW=3473±430g, N, BW=3344±355g). Expression of proteins involved in the mTOR and AMPK pathways were determined in the placenta by Western Blot and analyzed by unpaired multiple t-test. Correlations between protein expression of mTOR and AMPK pathway were studied using the Spearman test.

Results: We found a reduced expression of 4-EBP1, the final protein of the mTORC1 pathway, and a trend towards an increased expression of rictor (mTORC2) in male placentas of obese mothers. Contrary to other tissues, AMPK activation was positively associated with mTORC1 activation in placentas of women carrying a male infant. Phosphorylated-AMPK was decreased in female placentas of obese women, however without any correlation with mTOR, as if it was shunted downstream by other mTORC1 regulators.

Conclusion: This is the first report to show a differential regulation in placental nutrient sensing pathways depending on fetal sex in response to maternal obesity without fetal overgrowth. In male placentas, the trend towards the increased expression of mTORC2 while mTORC1 is under-expressed suggests reduced protein synthesis, and a predominant influence of insulin pathway. The female placentas show higher sensitivity to the nutritional intake of obese mothers *via* AMPK. The full impact of obesogenic environment and the importance of maternal feeding during pregnancy on these placental nutrient sensing pathways deserve to be investigated in order to adjust guidelines for the personalized nutritional management of such pregnancies for improving long term fetal health.

Key-words: obesity, placental mTOR pathway, sexual dimorphism

SEX-SPECIFIC ADAPTATIONS OF THE HUMAN PLACENTA TO MATERNAL OBESITY

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Abstract

*Early stage investigator

Maternal obesity is characterized by chronic, low-grade inflammation, oxidative stress, and dyslipidemia. This lipotoxic environment have been associated with poor pregnancy outcomes and increased the risk for the offspring to develop metabolic syndrome later in life. At the fetal-maternal interface, the placenta plays an essential role in the exchanges between the mother and the fetus. Different studies have demonstrated that maternal obesity without gestational diabetes (GD) impacts placental structure but also its major functions including endocrine function, nutrient exchanges. However, birthweight is not affected by the maternal obesogenic environment. So, the placenta has the potential to interpret environmental signals, such as excessive nutrient availability, and adapt itself in order to support fetal growth and development. In this context, to better understand molecular mechanisms implicated in this placental adaptation, we compared lipid, inflammatory, and oxidative status of maternal-placental-fetal triad between obese and non-obese women. Firstly, by using a lipidomic approach, we showed i) a higher polyunsaturated fatty acid (PUFA) concentration specifically in maternal plasma and placenta from obese women compared to non-obese group, ii) a lower sphingolipid (ceramide) concentration specifically in maternal plasma from obese women compared to non-obese group, and iii) a sex-specific disruption of some derivate of ceramides (i.e. lactosylceramide, dihydroceramide, and sphingomyelin) in cord plasma and placenta from obese women. Secondly, our preliminary results demonstrated that maternal obesity seemed to increase oxidative stress as suggested by the apparition of a molecular target generated after lipid peroxidation in female placenta specifically. Thirdly, by using a transcriptomic approach, we found a lower expression of enzymes involved in antioxidant defense (as CAT and GPx8) and lipid metabolism (as FADS1/2 and ELOVL1/4) in female placenta from obese women once again.

Finally, we showed that maternal obesity significantly reduced placental inflammatory status (cytokine secretion and signaling pathway) in male placenta from obese women only. In conclusion, our present results clearly showed that maternal obesity without GD is associated with placental lipid metabolism, inflammation, and oxidative stress modifications in a sex-specific manner and highlighted, in part, the molecular mechanisms involved in the placenta's adaptations to a harmful maternal environment.

OBESOGENIC DIET IN PREGNANCY PROVOKES DISTURBANCES IN PLACENTAL IRON HOMEOSTASIS IN ASSOCIATION WITH FETAL GROWTH RESTRICTION

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Abstract

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Metabolic diseases during pregnancy, including obesity and gestational diabetes mellitus (GDM), adversely impact fetal growth and maternal postnatal health. Despite this, the mechanisms underlying poor pregnancy outcomes in women with metabolic diseases are unclear. Recently, we have shown that women with GDM exhibit altered levels of iron homeostasis genes in their placenta that led to reduced iron uptake *in vitro*. To explore mechanisms affecting placental iron homeostasis, we used a well-established mouse model of maternal metabolic disease, in which pregnant dams show excess adiposity, glucose intolerance and insulin resistance. We hypothesised materno-fetal iron handling would be disrupted and relate to stress signalling pathways in the placenta. To test this hypothesis, we analysed iron levels in placenta and maternal liver, and levels of oxidative stress, expression of iron handling genes and activation of signalling pathways involved in cellular stress/ferroptosis in the placenta of mice with maternal metabolic disease.

From day (D)1 of pregnancy, C57Bl/6 female mice were fed a standard chow or a high fat, high sugar diet (HFHS; 3x fat and ~5x sugar content of standard chow). Mice were sacrificed at D16 or D19 for collection of tissues. Tissue iron-levels were determined using a ferrozine-based assay and iron deposition in the placenta using Prussian Blue staining. Lipid peroxidation, protein carbonylation and antioxidant capacity were determined by MDA, carbonylation quantification and GSH activity assay. Protein levels of stress kinases/ferroptosis markers (ERK, JNK and p38MAPK) were analysed by immunoblotting and expression of iron homeostasis genes by RT-qPCR. Data were analysed by Mann-Whitney test, $\alpha=0.05$.

HFHS diet reduced the ratio between placental and maternal liver iron content, and decreased iron deposition in the maternal decidua at D16. There was reduced abundance of ERK2 and activated p38MAPK in the placenta of HFHS mice at D16. At D19, placental iron content and expression of iron transporter genes *Dmt1*, *Zip14*, and *Fpn1* were reduced and activation of p38MAPK was enhanced. There were no differences in maternal iron status, or placental JNK activation, lipid peroxidation, protein carbonylation or antioxidant capacity in HFHS versus control dams at either gestational age. The HFHS diet reduced fetal growth at both time points and reduced placental weight at D19.

Placentas from HFHS mice may protect the fetus from excessive oxidative iron levels, even with relative high iron status of the mother (as represented by the reduced placental-maternal ratio in tissue iron levels). There were fewer iron deposits in the HFHS decidua, compromised p38MAPK activation and disturbed placental iron transfer capacity in late gestation. These results suggest that impaired placental iron transport may contribute to adverse perinatal outcomes in pregnancies complicated by GDM or maternal obesity.

THE IMPACT OF EXTRACELLULAR VESICLES DERIVED FROM WATERMELON ON PLACENTAL FUNCTION AND PREGNANCY OUTCOMES IN AN *IN VIVO* MODEL

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Abstract

*Early stage investigator - Yes

Background: FGR is a common pregnancy complication associated with adverse outcomes for the offspring. The difficulty in identifying the underlying placental developmental/functional anomalies with precision, necessitates treatment of women at risk of developing this complication, yet some of these women will transpire to have normal pregnancies. This means that any potential treatment must have minimal, ideally zero, risk. Identification of treatments that pose a minimal risk to pregnancy is therefore needed. Our group has successfully isolated extracellular vesicles (EVs) from watermelon as a potential plant-derived therapeutic supplement and shown that these EVs beneficially influence key aspects of placental cell behaviour *in vitro*. This study aimed to investigate their impact on pregnancy outcomes *in vivo*.

Methods: Female C57BL/6J mice were time-mated with visualisation of the vaginal plug designated as gestational day (GD) 0.5. Pregnant mice were randomly allocated to receive PBS control (N=7) or watermelon EVs (WMEV; N=8, 5 x 109 particles/mL) by oral gavage once daily from GD7.5 – GD13.5. Maternal bodyweight and food and fluid intake were monitored throughout pregnancy. Dams were sacrificed at GD14.5, or GD17.5. Litter size, number of resorptions, maternal organ weights and fetal and placental weights were recorded. Placentas harvested at GD17.5 were stained with haematoxylin and eosin and total placental area, as well as the areas of the decidua, junctional zone and labyrinth, were quantified using Image J.

Results: Treatment with watermelon EVs had no significant impact on maternal feeding or maternal bodyweight during pregnancy. No effect of treatment was observed with respect to maternal organ weights, litter size, resorption number, sex distribution or fetal weight at either harvest time-point. Whilst placental weight was unaffected at GD14.5, a significant increase in placental weight was observed at GD17.5 in response to watermelon EV treatment (P=0.003). Junctional zone : total placental area was significantly increased in the WMEV group compared to control (P=0.03). The ratios of labyrinth : total area and decidua : total area were not significantly altered by WMEV treatment.

Conclusion: The lack of effect on maternal food and fluid intake, maternal bodyweight and litter size suggests treatment with watermelon EVs does not adversely affect pregnancy progression. Increased placental weights and an increase in the area of the junctional zone in response to watermelon EVs suggests altered placental structure and potentially beneficial effects *in vivo*. It remains to be determined whether EV supplementation in rodent models of pregnancy complications, including FGR, will have a positive impact on fetal weight.

PETN ENHANCES STRESS RESISTANCE IN ENDOTHELIAL AND TROPHOBLAST CELLS

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Abstract

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Objective: Preeclampsia (PE) and fetal growth restriction (FGR) are pregnancy complications characterized by placental and endothelial dysfunction. To date there is no treatment for PE and FGR. Drugs targeting endothelial function have been tested in clinical trials, so far without significant success. In a clinical trial we tested the effect of the NO-donor pentaerythrityltetranitrate (PETN) on the course of pregnancies complicated by placental dysfunction. PETN has vasodilatative and vasoprotective properties and thus qualified to target endothelial and vascular dysfunction in PE and FGR. Although, in our randomized controlled trial PETN did not prove to significantly influence the development of FGR or perinatal death, it significantly attenuated the severe course of pregnancies at risk for placenta-associated pregnancy complications by reducing the rate of pregnancy-induced hypertension and preterm birth by 30 % each. In addition, intake of PETN stabilized the sFlt-1/PlGF ratio, which indicated a pro-angiogenic effect. On a cellular level, PETN is known to induce HO-1 expression. HO-1 is one of the most important guardians against cellular stress. This cellular effect could well explain the effect of PETN in pregnancies with endothelial and trophoblast dysfunction. We aimed to further study the anti-oxidative properties of PETN related to endothelial and trophoblast dysfunction in PE.

Material and Methods: We studied serum from our study participants to measure sFlt-1 and PlGF by routine laboratory procedures. PETN concentration was quantified using LC-MS. In addition, we determined the anti-oxidative capacity of serum with the help of an antioxidant assay kit. Furthermore, we investigated the effect of PETN *in vitro* on cellular stress resistance. For this purpose, we measured ROS production using MitoSOX staining and of sFlt-1 by ELISA in endothelial cells stressed with thrombin and trophoblast cells stressed using H_2O_2 or hypoxia.

Results: Data analysis currently in progress shows a significant advantage for taking PETN in pregnancy among the diseased women in individual clinical parameters. In our cell culture studies, we could show that PETN protected endothelial cells from thrombin-induced ROS production and endothelial dysfunction. Reduced ROS production was also demonstrated in trophoblast cells stressed with H2O2 or hypoxia. PETN strengthened the antioxidant defense mechanisms of endothelial and trophoblast cells with PETN-induced expression of HO-1 thought to be a key regulator of such effects.

Conclusion: The results of these studies suggest that PETN has the potential to enhance stress resistance in trophoblasts and endothelial cells. This may lead to protection of the maternal endothelium from both pregnancy-related dysfunction and long-term damage.

THE FGF/ERF/NCOR1/2 REPRESSIVE AXIS CONTROLS TROPHOBLAST CELL FATE

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Abstract

*Early stage investigator - No

Mouse trophoblast stem cells (TSCs) recapitulate aspects of placental development and provide an excellent *in vitro* model to study molecular mechanisms controlling cell fate decisions. The withdrawal of the self-renewal inputs Fgf/Erk and Nodal/Activin leads to TSCs exiting multipotency and their differentiation into various trophoblast cell types. However, little is known about how attenuation of Fgf signalling drives transcriptional changes that result in TSC differentiation. Here, we demonstrate that upon inhibition of the Fgf/Erk pathway in TSCs the Ets2 repressor factor (Erf) interacts with the Nuclear Receptor Corepressor Complex 1 and 2 (NCoR1/2) and recruits it to key trophoblast genes. Genetic ablation of *Erf* or *Tbl1x* (a component of the NCoR1/2 complex) leads to mis-expression of Erf/NCor1/2 target genes resulting in a TSC differentiation defect. Mechanistically, Erf regulates expression of these genes by recruiting the NCoR1/2 complex to their enhancers and modulation of H3K27ac. Taken together, our findings uncover how the Fgf/Erf/NCoR1/2 repressive axis governs cell fate and placental development, providing a new paradigm for Fgf-mediated transcriptional control.

DISTINCT NEUTROPHIL PHENOTYPES IN FIRST TRIMESTER PREGNANCY

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Abstract

*Early stage investigator - Yes

Objectives: Pregnancy is an immunological challenge for mother and fetus, and the establishment of immune tolerance at the feto-maternal interface is highly regulated and accompanied by leukocyte infiltration. Function of macrophages and NKs has been well described; however other leukocyte populations, including neutrophils, have been identified to play a crucial role in first trimester pregnancy. Neutrophils form an essential part of the innate immune response and have the ability to regulate adaptive immunity. Further, neutrophils have been implicated in the establishment of maternal tolerance through the induction of regulatory T-cells and neutrophil depletion led to abnormal development of the feto-maternal unit in mouse models. Therefore, our study aims to elucidate the phenotypes, functions and interactions of neutrophils migrated into decidua basalis of first trimester pregnancies as well as their impact on development of pregnancy complications in humans.

Methods: First, we evaluated neutrophil infiltration in decidua basalis in comparison to decidua parietalis using immunohistochemistry and flow cytometry. Further, we focused on the evaluation of the complexity and function of neutrophils and other immune cells in the feto-maternal interface (macrophages, NK cells, T-cells,..). We screened for 360 surface markers, comparing matched decidua basalis, decidua parietalis and blood immune cells from first trimester elective abortion samples using flow cytometry. Differentially expressed surface molecules identified in the screen and neutrophil phenotyping markers (e.g. CD11b, CD10,...) were validated.

Results: Preliminary data suggest abundant neutrophil infiltration in decidua basalis, in contrast, low infiltration of neutrophils in decidua parietalis was observed. In a flow-based surface marker screen (360 molecules) I identified unique expression patterns of neutrophils in decidua basalis when compared to matched decidua parietalis and blood. Based on their biological relevance we validated 30 of them in combination with already known neutrophil markers such as CD66b, CD10, CD63, CD62L, CXCR4 and CD11b. We identified four neutrophil subpopulations in decidua basalis and could confirm 14 differentially expressed novel markers expressed by neutrophils infiltrated in decidua basalis. We also observed higher amounts of neutrophil stimulating cytokines in decidua basalis, when compared to decidua parietalis.

Conclusion: In conclusion, we identified four unique neutrophil populations which are present in higher abundance in the invaded feto-maternal interface (decidua basalis). We will further evaluate if these subpopulations represent distinct pro- and anti-inflammatory functions and their role in the establishment of immune tolerance. With this project we aim to deepen our knowledge on neutrophil phenotype and function in first trimester pregnancy and if they contribute to development of pregnancy complications and to miscarriages.

THE PLACENTA HAS AN ACTIVE ROLE IN MAINTAINING FETAL HOMEOSTASIS FOLLOWING MATERNAL IMMUNE ACTIVATION

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Abstract

Activation of the maternal immune system during pregnancy does not only affect maternal health, but essentially also the development of fetus, and the allocation of maternal-fetal resources. The maternal body needs to balance between i) defending itself and the fetus by mounting an immune response, and ii) investing resources into continued growth, metabolism and development to ensure successful pregnancy. The placenta is a key interface for adjusting this balance, as it works both as a barrier and a selective filter, able to regulate nutrient exchange and crucial metabolic and inflammatory communication between maternal and fetal circulation. However, the mechanisms to integrate these different functions and allow for immediate response and adaptation in the case of maternal immune activation remain unclear. Moreover, how placental signalling acutely affects fetal health during maternal inflammation is uncertain. To untangle these mechanisms, we exposed the lung epithelium of late-pregnancy C57BL/6 mice to the endotoxin lipopolysaccharide (LPS) to mimic an acute bacterial infection. Profiling the transcriptome, proteome and lipidome of mother, fetus and placenta across 24 h after exposure, provided a fine-grained picture of the dynamics of this adaptation. Strikingly, the placenta first reacts to systemic inflammation by strengthening its tissue integrity to combat potential infections and simultaneously dampens growth. Afterwards, a return to homeostasis is marked by loosened extra-cellular matrix stiffness, heightened biosynthesis and expression of ER stress genes. This mechanism successfully protects the fetus from inflammation, as we observe no immune response in the fetal liver transcriptome. Instead, we observed fetal liver metabolic alterations to maintain homeostasis, as it increases lipid and amino acid catabolism and inhibits cholesterol metabolism. These metabolic adaptations may occur as a response to i) temporary maternal fasting, caused by LPS-induced fever and lack of nutrient intake, visible as increased lipid oxidation and catabolism across 24h in the maternal liver and ii) placental regulation of fetal metabolism and maternal-fetal resource allocation. The insights from our multi-omics study on the active control of allocated resources by the placenta will help to diagnose problems in placenta homeostasis in the future.

FUNCTIONAL HETEROGENEITY BETWEEN DECIDUA BASALIS AND PARIETALIS MACROPHAGES IS CONTROLLED BY PLACENTATION

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Abstract

*Early stage investigator - Yes

In early pregnancy, so-called extravillous trophoblasts (EVTs) invade and remodel the maternal uterine lining, also referred to as decidua basalis (decB). The non-invaded pregnant uterus is termed decidua parietalis (decP). During placentation, semi-allogenic EVTs interact with maternal immune cells to initiate spiral artery remodeling. However, the close interplay between trophoblasts and maternal immune cells poses the risk of compromising successful pregnancy. To study placentation-dependent immunological adaptions, we established a tissue sampling protocol to collect first-trimester decidual decB and donor-matched decP tissues. Strikingly, we identified an accumulation of tissue-resident macrophages in decB, whose phenotype, transcriptome, and secretome are distinct from macrophages isolated from decP. Decidua basalis-associated macrophages (decBAMs) express high levels of CD11c and CD44, secrete IL-10, CXCL1, CXCL5, and M-CSF, and are efficient inducers of regulatory T cells. In contrast, decidua parietalis-associated macrophages (decPAMs) have high MHC class II molecule levels, show a motile phenotype, enhance phagocytosis, and activate naïve T cells. We further find that conditioned media from EVT (EVT-CM) cultures suppresses this antigen-presenting cell (APC) –like phenotype. Here, EVT-CM significantly reduced the motility, the phagocytic activity of decPAMs, and their potential to activate naïve T cells. In summary, we identified a decB-specific macrophage population, which we termed decBAM, that exerts a pregnancy-tolerant phenotype shaped by placentation.

This study was supported by the Austrian Science Fund (grant P 33485 to J.P.).

ROLE OF THE PROKINETICINS IN PERINATAL INFLAMMATION: CASE OF CHORIOAMNIONITIS

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Abstract

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Chorioamnonitis (CA) is an infection of the utero-placental unit by pathogens such as, group-B Streptococcus (GBS) or Escherichia coli. CA is the main cause of preterm birth (< 37 weeks of gestation (WG)), and is responsible of 70% of perinatal mortality and 50% of neurologic effects[1]. The prokineticins are a family of proteins composed of two members, Prokineticin 1 (PROK1) and Prokineticin 2 (PROK2), also called EG-VEGF and BV8, respectively. PROKs act *via* two GPCR receptors, PROKR1 and PROKR2. The PROK members and their receptors are expressed in numerous tissues, including placenta, and play a key role in placental development during the first trimester of pregnancy and have been reported as potential quiescence factors during the third trimester, before the onset of labor[2]. Using a rat model of CA, our team demonstrated that 1) PROKR1 and PROKR2 expression decreased after injection of GBS; 2) PROK1 expression increased upon GBS injection and decreased latter-on. In addition, preliminary data from our team demonstrated that mice pre-injected with PROK1 and subsequently injected with lipopolysaccharide (LPS) at 10µg/ml, delivered normally, compared to those injected with LPS only who delivered in the frame of 16 hours upon injection. To get more insights into the mechanism by which PROKs may control CA-mediated preterm delivery, we used the rat cell model, RCHO-1, that mimics two key states of the trophoblast, the proliferative and the differentiated state that correspond to the stem cell and trophoblastic giant cell (TGC), respectively. In this paper, we used the differentiated RCHO-1 cells that we first validated as TGC cells (85%), through the assessment of the expression of prolactin gene. The cells were then treated with LPS during 24, 48h and 72h and analyzed for their phenotypes and for the expression of PROK1 and its receptors. We did not observe any adverse effect on the morphology of these cells after stimulation with LPS. Using RT-qPCR and Western Blot analysis, we demonstrated that LPS treatment significantly decreased PROKR1 expression at 48 and 72 h. Using ELISA test we also demonstrated that LPS treatment significantly decreased PROK1 secretion after 24h in the conditioned media of RCHO-1 cells. This decrease was followed by an increase at 48h. These results strongly suggest that placental inflammation directly involves the PROK family members and substantiate the observations and data found in the rat and mouse models of CA. Ongoing experiments are in progress to better characterize this role in an inflammatory context of pregnancy such as CA.

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PLACENTAL NLRP3 INFLAMMASOME SIGNALING IN GESTATIONAL DIABETES AND THE THERAPEUTIC POTENTIAL OF METFORMIN (new project introduction)

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Abstract

*Early stage investigator

Gestational diabetes mellitus (GDM) is an obstetric complication affecting up to 20% of pregnancies, and its prevalence is set to increase due to the global rise in maternal obesity. GDM is characterized by maternal insulin resistance, chronic inflammation, and impaired placental function. Epidemiological studies have established that untreated GDM is associated with adverse perinatal outcomes and long-term sequelae for the offspring, including the development of obesity and type 2 diabetes. Nonetheless, the molecular mechanisms underlying the pathophysiology are poorly understood. NLRP3 inflammasome plays critical role in protective immune functions, however, its excessive activation is associated with chronic inflammation that may result in the development of diabetes (including obesity-induced insulin resistance) or atherosclerosis. Importantly, studies have shown a complex relationship between host lipid metabolism and NLRP3 inflammasome activation. Recently, it has been shown that the placenta expresses high levels of the NLRP3 inflammasome and secretes NLRP3-associated pro-inflammatory cytokines IL-1ß and IL-18. Interestingly, metformin, an alternative first-line agent to insulin in the treatment of GDM, has attracted interest due to its potential to modulate inflammasome activation and affect lipid metabolism. Considering the milieu associated with GDM, our central hypothesis is that GDM promotes a lipotoxic placental environment, leading to hyperactivity of placental NLRP3 inflammasome machinery and contributes to chronic, low-grade inflammation. We speculate that besides anti-hyperglycemic effect, metformin has additional therapeutic actions by inhibiting NLRP3 inflammasome activation.

This project recently received funding by the Czech Health Research Council (grant no. NU22J-01-00066).

THE IMMUNOLOGICAL SIGNATURE OF FETO-PLACENTAL ENDOTHELIAL DERIVED EXOSOMES ON THE FETUS

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Abstract

Introduction: In pregnancy, the mother does not reject the placenta because antigens expressed by the feto-placental unit do not activate the immune system of the mother. The placental tissue releases small vesicles, exosomes, into the maternal circulation carrying a specific cargo of proteins, nucleic acids and lipids. These exosomes support an immune-tolerant environment for the growing fetus by communicating with the maternal immune system. Placental exosomes are also released from the placenta to the fetus. Although the communication between exosomes and the maternal immune response is well studied, the interaction of such vesicles and the fetus has not been studied yet. Therefore, our study aims to investigate the characteristics and role of feto-placental-endothelial derived exosomes (fpExo) in the context of preeclampsia (PE). Offspring from PE pregnancies are at higher risks of thrombocytopenia and neutropenia allows to suppose that the fetal innate immunity is triggered by fpExo already *in utero*.

Methods: fpExo from media supernatants of normal, PE and preterm fpECs were isolated by differential ultracentrifugation (100,000g, 22h, 4°C pellet). All fpExo enriched fractions were characterised by size with nanoparticle tracking analysis and by specific proteins for exosomes namely, Alix, Syntenin, CD9, CD63 and CD81 (Immunoblotting). Small RNA of fpExos was classified and quantified by sequence approach. A customized lectin microarray was used to profile glycosylation pattern of fpExos.

Results: fpECs secrete exosomes with a mean size of 125.3±3.9 nm and express Syntenin, Alix and tetraspanins CD9, CD63 and CD81. Moreover, on protein level we could determine potential placental specific endothelial markers, such as Siglec-6, a leptin and sialic acid binding protein of the immunoglobulin superfamily and the endothelial marker CD31. The glycosylation profiling showed that fpExos from normal and PE fpECs differ significantly in their glycosylation signature and are enriched with sialic acids compared to the fpECs membrane fractions. miRNA-sequencing revealed that fpExos are released with a high content of hsa-miR-21-5p and hsa-miR-126-3p.

Conclusion: These preliminary results suggesting that signature of fpExos highly resembles origin and state of the parent fpECs. Given that, glycans are important for communication within cells; obtained signature on fpExos may indicate an interplay of these vesicles and respective receptors on fetal immune cells and thereby effectively priming fetal innate immune system already *in utero*.

INFLUENCE OF BETA-HYDROXYBUTYRATE ON MOTILITY, CELL METABOLISM AND THE EXPRESSION OF PRO-INFLAMMATORY FACTORS OF BOVINE CARUNCULAR EPITHELIAL CELLS *IN VITRO*

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Abstract

*Early stage investigator - Yes; presenting author

It is a common reception that in high-yielding dairy cows a negative energy balance (NEB) and a decreased reproductive performance co-occur. In calf hepatocytes and endometrial cells (BEND) beta-Hydroxybutyrate (BHBA), as one of the primary characteristics of a NEB, increments the levels of NF-κB regulated cytokines. These include, for instance, tumor necrosis factor-α (TNFα) and Interleukin-6 (IL-6), which are prominent for the inflammatory and immunity response. Moreover, it is described that BHBA increases the oxidant status and leads to higher concentrations of reactive oxygen species in the plasma of dairy cows.

A bovine caruncular epithelial cell line (BCEC) was used for the experiments. It is suitable to explore fetal-maternal communication, infections, and metabolic modulations of pregnancy-associated diseases. The cell line was isolated and established from the placentomes of pregnant cows, exhibits the morphological and functional characteristics of epithelial cells and forms an epithelial barrier.

To investigate the in vitro impact of BHBA during pregnancy, especially in the period of implantation and placentation, the BCEC was stimulated with 0.6mM, 1.2mM, 1.8mM and 2.4mM BHBA for 24h and 36h. For each experimental group, cells incubated in serum-reduced medium served as controls. Examination of cell metabolism, cell motility and the expression of pro-inflammatory factors have been carried out after the BHBA incubation. The cell metabolism was analysed by an MTT-Assay and the motility by a live-cell imaging program. To investigate the mRNA expression of TNFα, IL-6 and NF-κB a quantitative real-time PCR was utilized.

The results indicate a significant negative effect on cell metabolism and the motility at a concentration of 2.4mM BHBA. TNFα shows a significant increase in mRNA expression correlating with higher BHBA concentrations. However, IL-6 and NF-κB were not altered in their mRNA expression compared to the control group.

The decreased BCEC metabolism and motility as well as the increased TNFα mRNA expression could lead to a restricted function of maternal caruncles. This might result in a poor interaction between maternal caruncles and fetal cotyledons which could cause early pregnancy losses.

BOVINE PLACENTAL TROPHOBLAST GIANT CELLS AND HYBRID CELLS IN RELATION TO THE FETOMATERNAL INTERFACE – A MORPHOLOGICAL CHARACTERIZATION BY 3-DIMENSIONAL RECONSTRUCTION

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Abstract

*Early stage investigator

Trophoblast giant cells (TGC) are a particular feature of the bovine placenta. They represent ~15% of cells in trophectoderm and are continuously formed during gestation. TGC are able to migrate towards the maternal epithelium (ME) and fuse with uterine epithelial cells (UTC) to form hybrid cells (HC). The aim of our study was to investigate morphological features of TGC and their connection to the ME in a 3-dimensional research approach.

Placenta samples from 6 cows (gestation days 120, 150, 205, 266, 278 and 284) were fixated in Karnovsky and embedded in Durcupan. Seven stacks of 3000 sections (60 nm thickness) each were created by serial block face scanning electron microscopy. Editing, analyses and segmentation were accomplished using the software Microscopy Image Browser. Visualization was done with the software Imod and volume calculations with the software Amira. The methodology was supported by the recording of high-resolution transmission electronic microscopy images. TGC analysis included following criteria: contact to fetal basement membrane (FBM), membrane protrusions to FBM, contact to ME, formation of pseudopodia in direction to ME, presence of membrane-bound granules, double lamellar bodies, mitochondria, rER and number of cell nuclei. For three stacks (150, 205, 284) the cell volumes (CV) and total nuclear volumes (TNV) were calculated. The CV displayed a wide range between 2048 and 14708 μm^3. TNV varied between 853 and 3395 μm^3. TGC occurred in majority as binuclear cells. Few TGC with one or three nuclei were also observed. Based on morphological criteria, TGC could be distinguished into young, maturing and mature cells. Young TGC still had close large contact with the FBM, whereas in the maturing and mature TGC the contact was only small or no longer present. Maturing and mature TGC with a TNV of approximately 800 μm^3 began to form contacts with ME. Contacts were mostly established by formation of pseudopodia. Some pseudopodia contained a distinct organelle-free area delimited by a membrane-like structure. The contact of TGC with the ME decreased during gestation. A surprisingly large proportion of TGC still had contact to the FBM. This was usually no longer extensive like in the young TGC but in form of membrane protrusions. Contact with FBM initially increased from ~55% in the second half of gestation, to 100% in late pregnancy and decreased again to ~75% at term. Several TGC had contact to the fetal BM and the ME simultaneously. After fusion with one or more UTC, HC were dispersed in the ME, and had two to 17 cell nuclei. HC with two nuclei could be identified as degenerated hybrid cells. The proportion of HC increased towards end of pregnancy.

Therefore, contrary to previous studies, TGC can form contacts with the FBM and the ME simultaneously. Not only young but also immature and mature TGC maintain their contacts to the fetal BM longer than expected. In HC, a distinction could be made between vital and degenerated cells.

mTOR SIGNALLING PATHWAY AS A KEY-MODULATOR IN PLACENTA DEVELOPMENT: CELL FUNCTIONALITY AND GENE EXPRESSION OF TROPHOBLAST ADAPTIVE RESPONSE DURING THE EARLY STAGE OF PREGNANCY IN SHEEP

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Abstract

During the early stage of placentation in sheep (15-23 Days), normal conceptus development is affected by trophoblast cells (oTCs) functionality whose dysregulation leads to early pregnancy loss, characterised by inadequate nourishment and oxygen supply. In this crucial developing period, oTCs metabolism is mostly supported by endometrial histotrophic factors, including FGF2, involved in cell differentiation and function through the modulation of specific cellular mechanisms. Among the others, mTOR is known as cellular "nutrient sensor", but its downstream regulation in oTCs is still poorly understood. Therefore, our hypothesis was that oTCs adapt to overcome poor-growth factors in the uterine environment through mTOR signalling pathway modulation. The main aim was to set up an *in vitro* culture system from early sheep placenta in order to study trophoblast adaptive response in suboptimal environments occurring in impaired pregnancy.

Firstly, primary oTCs from 21-days old sheep placenta collected at the slaughterhouse were characterised by cell morphology, immunofluorescence and PCR to detect specific trophoblast markers. Then, oTCs primary cells and oTr cell line were subjected to different treatment (50 ng/ml FGF2, 100 nM rapamycin – mTOR inhibitor – for 24h) to study their effects on cell functionality, gene and protein expression profile.

oTCs showed mainly mononuclear cells with epithelial cell-like growth and placental morphological properties, such as bi- and multinucleated syncytium-plaques, expressing peculiar trophoblast markers and progesterone release in culture medium. Cell functionality tests relieved similar results in both cell systems. FGF2 promoted cell proliferation and migration in normal culture conditions, whereas mTOR-inhibition induced a significant decrease. Invasion activity wasn't influenced by FGF2 when mTOR signalling pathway was activated. On the contrary, when it was inhibited, cell invasiveness was affected by FGF2. mTOR-inhibition leaded to a significant low-cell motility both in presence and absence of FGF2, but its supplementation seemed to restored oTCs activity even when mTOR was prevented, as confirmed by mTOR phosphorylation even in presence of the inhibitor. Interestingly, mTOR-inhibition influenced endocrine trophoblast marker regulation, indeed oPL expression wasn't affected by FGF2 supplementation as observed in the control, while IFN-τ was drastically reduced.

Present findings support that FGF2 acts to modulate oTCs behaviour by regulating mTOR signalling pathway. The study provides new insight regarding how mTOR-inhibition influences trophoblast migration and invasion activity, essential for conceptus development. Moreover, mTOR involves in the expression of hormonal trophoblast markers, suggesting that it plays a crucial role in the early placenta growth and foetal-maternal cross-talk.

Key words: sheep, early placenta development, trophoblast, mTOR, FGF2.

EFFECT OF MATERNAL EXPOSURE TO A COCKTAIL OF FOOD CONTAMINANTS ON FETO-PLACENTAL DEVELOPMENT AND GROWTH IN A RABBIT MODEL.

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Abstract

*Early stage investigator - Yes

Pregnant women are daily exposed to many food contaminants, including endocrine disruptors. This exposure can have consequences on feto-placental development and growth and on the offspring's health in the long-term. In this context, this study was designed to evaluate the effect of maternal exposure by gavage to a cocktail of eight food contaminants, during the folliculogenesis and/or pre-implantation development (FED and ED groups, respectively) on feto-placental development (via ultrasound monitoring), fetal metabolism (blood biochemistry), placental structure (stereological analysis) and function (transcriptomic analysis by RNAseq) in a rabbit model. This study is part of the ANR FEDEXPO program (https://anr.fr/Project-ANR-17-CE34-0015).

In order to mimic human exposure, the contaminant cocktail was defined from the characterized exposome of HELIX and INMA mother-child cohorts, and included substances from the phthalate family (DEHP), organochlorine pesticides (pp'DDE, β-HCH and HCB), brominated flame retardants (BDE-47), bisphenols, (BPS) and perfluoroalkylated substances (PFOS and PFOA).

Maternal exposure to this cocktail had no significant effect on biometrical parameters evaluating fetal development and growth at 14 and 28 days post conception (dpc), and on placental morphometry at 28 dpc in the FED and ED groups compared to the control group. Only the placental volume increased significantly at 28 dpc for the FED group compared to ED group ($p=0.035$). Fetal blood biochemistry exhibited a decrease of triglycerides ($p=0.035$) and HDL cholesterol ($p=0.028$) levels in FED fetuses compared to control and to ED groups, respectively. However, multifactorial analysis (MFA) on these different datasets (feto-placental biometry, fetal biochemistry and placental structure) indicated that the three groups were well separated. In addition, numerous placental genes were differentially expressed in the FED and ED groups compared to the control group, among which genes involved in lipid metabolism and in PPAR signalling pathways, such as *DGKG*, *PLA2G4E* (underexpressed for ED group compared to control), *APOA2*, *APOA4*, *ELOV6*, *STARD4* (overexpressed for FED compared to control group). Genes were also differentially expressed between the two exposed groups (FED vs ED), among which *APOA1*, *APOA2* (overexpressed in FED) and *NPC1L1* (underexpressed in FED group).

Altogether, these results indicate that maternal exposure to food contaminants during key windows of development altered fetal development and blood biochemistry as shown by well separated ellipses represented the groups. Moreover, this exposure altered placental gene expression with potential repercussions on the offspring's phenotype in the long term, especially on metabolism.

DECIDUALIZATION IN THE DOG: FOCUSING ON MATERNAL-DERIVED DECIDUAL CELLS FOR UNDERSTANDING THE MAINTENANCE AND TERMINATION OF CANINE PREGNANCY.

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Abstract

*Early stage investigator - No

In the domestic dog, the conceptus-maternal interaction results in the formation of the invasive, species-specific endotheliochorial placenta. Despite sharing several similarities with other invasive placentation types, such as decidualization, the canine placenta is characterized by shallow invasion compared with the haemochorial placentation of humans and rodents. This places the canine placenta between noninvasive epitheliochorial placentation and the more invasive hemochorial type, making it an interesting model for investigating morpho-functional aspects of placentation. By focusing on the mechanisms involved in the maintenance of canine pregnancy, our studies have shed new light on the canine placenta as an endocrine organ From a functional perspective, the dog is the only domestic animal species that does not produce placental steroids, although the placenta responds to circulating steroids by expressing progesterone (P4) and estrogen receptors. Of particular interest are the maternal stroma-derived decidual cells, the only cells of the canine placenta expressing the nuclear P4 receptor (PGR). The natural or antigestagen-mediated withdrawal of P4/PGR signaling activates the luteolytic cascade and leads to parturition/abortion, associated with the release of PGF2α from the trophoblast. This indicates a functional interplay between the fetal and maternal compartments during induction of parturition. As a functional consequence (revealed by our transcriptomic analysis), the luteolytic cascade within the placenta is associated with apoptotic signaling, disruption of vascular integrity and increased immune response. Among P4-regulated genes are those involved in the production of prostaglandins (PTGS2), cortisol metabolism (HSDB11B2) and vascular function (ICAM1, TGFβ, VEGFA). Decidualization in the dog is embryo-induced and is associated with mesenchymal-epithelial transition, as revealed by our electron microscopic analyses and by applying an *in vitro* model of canine decidualization with dog uterine stromal (DUS) cells. As a sign of this morpho-functional transition, decidual cells are surrounded by a layer of interstitial matrix containing COL4, while retaining the expression of vimentin. DUS cells, developed in our laboratory to enable in vitro studies on canine decidualization, undergo cAMP-mediated decidualization and show increased levels of decidualization markers, e.g. PTGES, IGF1 and PGR. In a recent transcriptomic and kinomic study, including the antigestagen-mediated effects, we demonstrated that whereas decidualization led to the upregulation of over 1800 differentially expressed genes involved in cellular proliferation and adhesion, mesenchymal-epithelial transition, extracellular matrix organization, and vaso- and immunomodulation, these effects could be mostly reversed by antigestagens. Cumulatively, the most recent findings provide new clues underlying the importance of P4/PGR signaling for canine decidual cell function.

NURSING IN THE PERICONCEPTIONAL PERIOD ALTERS PLACENTAL GENE EXPRESSION AND SUBSEQUENT FOAL GROWTH

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Abstract

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To be profitable, equine breeders aim to produce a foal/mare/year, meaning that mares are bred while still nursing their previous foal. Most studies show that fertility as embryo/fetal mortality is increased in nursing mares. Long-term effects, however, have not been investigated. This project aimed to analyze the effect of nursing on placenta and foal growth. Multiparous Saddlebred mares aged 10-16 years were bred with the semen of one unique stallion on the 2nd heat after foaling (Nursing, N, n=6) or after having been left barren for 1 year (Barren, B, n=14). They were then managed as one herd in pasture and stalled individually in the same barn from around 6th month of gestation until foaling (11 months gestation). Placentas were recovered at foaling, measured and weighed. Stereology was performed using MercartorPro software on HES-stained paraffin embedded samples. Paired-end RNA-sequencing was performed on frozen placenta (Illumina, NextSeq500). Differential expression was analyzed (DESeq2) using a false discovery rate (FDR) <0.05 cutoff. Gene Set Enrichment Analysis was performed using KEGG, GO BP and REACTOME databases. Other data related to foal growth, metabolism and placenta were analyzed using a linear model with permutations using R software. A Frequently Sampled IV Glucose tolerance test was performed in mares at 300d of gestation and in foals at 6, 12 and 18 months of age. Lactation and foal growth were monitored until, respectively, weaning (≈6 months) and 18 months of age. N were more sensitive to insulin at 300 days of gestation (in median, 2.54 for N vs 0.78L/(mUI*min) for B, p<0.05). Gestation length was reduced (-8d) in N vs B. Although no morphological nor structural difference were observed, 38 genes were differentially expressed (23 and 15, respectively, over and under-expressed, FDR<0.05) in N placentas, among which 4 were directly involved in Wnt signaling pathway (WNT7, SFRP1 and LRP6 over-expressed and TRABD2A under-expressed in N placentas). Among the 226 perturbed pathways (183 GO BP, 16 KEGG and 27 REACTOME), only 13 were enriched in N placentas, mainly related with protein synthesis. Gene sets enriched in B placentas were involved in cell division and the regulation of inflammation and innate immunity. Although foal weight was similar at birth, post-natal growth was reduced in N, foals being lighter by 24kg at 18 months of age (p<0.05). No difference in carbohydrate metabolism was observed. Milk production, as analyzed through individual milking, was reduced at the end of the lactation period in N (N: 212.80 ±92.41 vs B: 401.62 ±100.64g, p<0.05) although milk quality was similar.

In conclusion, nursing at the beginning of gestation is associated with reduced gestation length and functional placental adaptation probably compensating for the increased insulin sensitivity in N mares and enabling the birth of a normal weight foal. Reduced lactation potential, however, impaired long-term foal growth.

MULTISCALE CORRELATIVE IMAGING OF HORSE AND ZEBRA PLACENTAL VI

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Abstract

*Early stage investigator

Despite having a single evolutionary origin, the mammalian placenta exhibits wide interspecific morphological and structural variation. Equine (horses and their kin) placentas display branching villi which sit in apposition with maternal tissue and represent the site of fetomaternal nutrient and waste exchange. Three-dimensional imaging techniques have recently identified and quantified novel structures in human placental villi, however similar tools have yet to be broadly applied to other species. Such approaches have the potential to both expand our understanding of comparative placentation and better resolve the structural composition of the studied taxa. Using scanning electron microscopy (SBF-SEM) of horse and zebra placenta, we demonstrated the presence of stromal macrovesicles previously only observed in human placental villi. Here, we also present a workflow for correlative three-dimensional imaging of equine placental villi by combining x-ray microtomography (microCT) and SBF-SEM. This allows calculation of the total surface area of the equine placenta including microvilli. Through this workflow, we quantify the villus structure across multiple orders of magnitude in horse and zebra placentas. These morphometric data, including volume, surface area, and branching angle, help us to better resolve equine placental organization and contribute towards a holistic understanding of equine placental function.

THE GLYCOSYLPHOSPHATIDYLINOSITOL (GPI)-ANCHOR BIOSYNTHESIS PATHWAY IS INVOLVED IN REGULATING MOUSE PLACENTATION

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Abstract

*Early stage investigator - No

Objective:
To characterise the role of the GPI-anchor biosynthesis pathway in the regulation of mouse trophoblast stem cell self-renewal and differentiation.

Methods:
Using CRISPR/Cas9 technology, we deleted two genes, *Pigl* and *Pigf*, encoding fundamental proteins for the correct GPI biosynthesis in mouse trophoblast stem cells and studied their role in the regulation of early mouse placentation.

Results:
Phosphatidylinositol Glycan Anchor Biosynthesis Class L (PIGL) and F (PIGF) proteins are essential components of the glycosylphosphatidylinositol (GPI) biosynthetic pathway that culminates in the generation of the GPI glycolipid. The GPI anchor is then post-translationally transferred to proteins in the endoplasmic reticulum (ER) and relocated as GPI-anchored proteins (GPI-APs) to the cell surface where they play essential roles in cell signaling transduction. Our previous work identified the *Pigl* and *Pigf* genes as essential for placentation, highlighting the fact that GPI-anchor biosynthesis pathway is critical for early placentation. Here, we analyse the functional contribution of PIGL and PIGF to the GPI biosynthesis pathway in regulating mTSC self-renewal and differentiation. We first characterized the dynamics of *Pigl* and *Pigf* expression during trophoblast differentiation by performing a time-course experiment. Whereas *Pigf* was highly expressed in mTSCs under stem cell conditions and was strongly downregulated during trophoblast differentiation, *Pigl* expression did not significantly change across this differentiation time course. The deletion of *Pigl* or *Pigf* did not affect stemness. However, under differentiation conditions, $Pigl^{-/-}$ and $Pigf^{-/-}$ mTSCs failed to upregulate markers of syncytiotrophoblast, whereas differentiation towards trophoblast giant cells was promoted, indicating that GPI biosynthesis pathway proteins may play a role in the lineage determination towards syncytiotrophoblast. These data point to an essential role for GPI biosynthesis pathway proteins in modulating trophoblast differentiation towards syncytiotrophoblast and identify GPI-APs as novel players in regulating early placentation.

TRPV2 IS INVOLVED IN TROPHOBLAST BRANCHING OF THE MOUSE PLACENTA

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Abstract

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INTRODUCTION |

The placenta mediates nutrient supply to sustain fetal growth during pregnancy. A large exchange area is created by extensive branching morphogenesis of the trophoblasts. Pivotal to this labyrinth formation is the differentiation and branching of Syncytiumtrophoblast layer II (SynTII). However, little is known regarding the underlying pathways that underpin placentation. Calcium signals are well suited to translate signals from the environment into a cellular response. Previously, we identified a crucial role of the calcium-permeable channel TRPV2 in placental development as Trpv2-/- mice display intra uterine growth restriction (IUGR) and lethality. Here, we investigated the role of TRPV2 in placental development and trophoblast branching.

RESULTS |

Global genetic ablation of Trpv2 results in IUGR and embryonic lethality at E18.5. Deletion of Trpv2 in the embryo proper or in the junctional zone of the placenta was compatible with normal development, suggesting that labyrinthine Trpv2 is indispensable for normal fetal development. In the labyrinth, Trpv2 is highly expressed in SynTII trophoblast cells. Moreover, the labyrinth morphology of Trpv2-/- placentas was severely disturbed, with regions where that are void of both SynTII cells and fetal vessels. RNAsequencing of wildtype and Trpv2-/- placentas revealed that branching morphogenesis and angiogenesis pathways were affected. Interestingly, Crispr/Cas9-mediated deletion of Trpv2 in Trophoblast Stem Cells resulted in impaired differentiation of the SynTII labyrinth lineage.

CONCLUSION |

Our observations suggest that loss of TRPV2 in mice disrupts the differentiation of SynTII trophoblasts, which likely contributes to defects in labyrinth morphogenesis, nutrient supply, and thus compromised fetal growth.

PHYSIOLOGICALLY-RELEVANT CULTURE MEDIUM INCREASES MITOCHONDRIAL FUNCTION AND PROMOTES PROLIFERATION AND DIFFERENTIATION OF HUMAN TROPHOBLAST STEM CELLS (hTSCs)

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Abstract

*Early stage investigator - Yes

Introduction:
Despite advances in cell culture systems, researchers have continued to use culture media based on historic formulations. For example, Dulbecco's modified Eagle's Minimal Essential Medium (DMEM), the most widely used culture media reported in the literature, is based on a formulation in 1959. Standard culture media such as DMEM contain non-physiological levels of nutrients and lack defined concentrations of many metabolites present *in vivo*. Despite this, nearly all cell culture-based studies on human trophoblasts have been performed using this media. Recently, Tardito *et al.* (Sci Advances 2019) developed a culture medium (Plasmax), with nutrients and metabolites with concentrations comparable to the human *in vivo* environment. To understand the impact of the physiological metabolic environment on hTSC growth, differentiation and metabolism, we compared the effects of culturing cells in a physiologically-relevant medium (Plasmax) versus the standard, widelyused medium (DMEM-F12).

Methods:
hTSCs (n>4) were cultured in DMEM-F12 or Plasmax and maintained in their undifferentiated state or differentiated into syncytiotrophoblast (STBs) or extravillous trophoblasts (EVTs). Cells were counted daily over 4 days to assess their proliferation rate. Differentiation was determined by qPCR analysis of STB and EVT markers at different stages of differentiation. Energy metabolism was examined by LC-MS analysis of glycolytic and tricarboxylic acid (TCA) cycle intermediates and cellular respiration on an extracellular flux analyser.

Results:
Compared to DMEM-F12, hTSCs grown in Plasmax increased their proliferation by 60% ($P=0.003$). STB or EVT differentiation, as determined by expression of STB (ERV-W, CYP19A1; $P<0.01$) and EVT (ERBB2, HLA-G; $P<0.001$) markers, increased in cells cultured with Plasmax. Lastly, hTSCs in Plasmax demonstrated greater energy metabolism as demonstrated by increased intracellular production of glycolytic and TCA cycle intermediates as well as elevated basal respiration and oxidative phosphorylation.

Conclusion:
Physiological medium (Plasmax) improves the metabolic fidelity of hTSCs resulting in increased proliferation and differentiation compared to standard medium (DMEM-F12). We propose that the physiological concentrations as well as the greater complexity of nutrients and metabolites in Plasmax allow for a better *in vitro* model system for trophoblast biology.

TOWARDS A HUMAN-BASED PLACENTA-EMBRYO CHIP FOR DEVELOPMENTAL TOXICITY AS-SESSMENT OF NANOPARTICLES

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Abstract

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Nanoparticles (NPs) are emerging materials enabling a wealth of novel applications, but evidence is increasing that NPs can have adverse effects on pregnancy and health in later life. The mechanisms of developmental toxicity of NPs are poorly understood but likely involve direct toxicity from particles that crossed the placental barrier as well as indirect toxicity from particle accumulation in placental tissue and interference with placental function and signaling. Since the placenta is the most species-specific organ, animal studies may not deliver relevant results for human applications. Consequently, there is a great need for alternative human-based ad-vanced *in vitro* models for developmental toxicity assessment.

We have recently developed a microphysiological model combining a placental barrier model (BeWo tropho-blast cell line) with murine embryoid bodies in a user friendly microfluidic platform1. We currently further im-prove the biological model: 1) for the placenta, we work on establishing co-cultures of primary human cyto-trophoblasts (CTBs) and endothelial cells; 2) as a supportive membrane to cultivate placental cells, we explore highly permeable electrospun membranes with superior permeability to NPs compared to conventional track-etched polymer membranes and 3) we replace murine EBs with human induced pluripotent stem cell (iPSC)-derived EBs.

In static insert cultures, we have successfully established placental trophoblast/endothelial cell co-cultures2 and a highly permeable chitosan/PEO electrospun cell culture support3, which will be transferred to the micro-fluidic chip platform. Primary human CTBs were isolated from human term placenta to form a confluent mon-olayer of syncytialized cells. To verify the predictive value of the model for NP transport studies, in particular the impact of flow conditions and a primary syncytiotrophoblast layer, we will study size-dependent transloca-tion of differently sized polystyrene NP in static versus dynamic conditions as well as in BeWo versus primary CTB monolayers and compare the results to those obtained with a human *ex vivo* placenta perfusion model.

Overall, reproducing the extensive interplay between placenta and embryo in a microphysiological model will allow for a systematic assessment of placental transport as well as direct and indirect embryotoxicity to gain novel mechanistic insights, which are important for designing safe NPs.

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STUDY OF PREECLAMPSIA-DERIVED EVs ON THE BLOOD-BRAIN-BARRIER INTEGRITY AND MICROGLIA ACTIVATION USING STATIC AND MICROFLUIDIC MODELS

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Abstract

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Aim | Extracellular vesicles (EVs) are recognized as important players in materno-fetal communication. EV concentration increases in plasma of normal pregnancy (NP) throughout gestation and is higher in patients with preeclampsia (PE). PE is characterized by hypertension and inflammation being a predisposing factor for cardiovascular conditions in life. The brain is one of the early targets of hypertension-induced damage and this is associated with cerebrovascular and neurological impairment. EVs are associated with neuroinflammation through the activation of microglia and with neurodegenerative conditions. Previously, we have reported that exogenous EVs can be internalized by endothelial cells in the BBB and exert microglia activation. However, whether PE-derived EVs are involved with central nervous system injury is unknown. Here we investigated the effects of PE-derived EVs on the human blood-brain barrier (BBB) and microglia using static and microfluidic models.

Methods | EVs obtained from plasma of NP and PE diagnosed patients by differential ultracentrifugation were characterized by nanotracking analysis (NTA), western blotting and cryo-electron microscopy (cryo-TEM). The uptake of PKH67-labeled EVs by hCMEC/D3 endothelial cells and HMC3 microglia cells, the localization of tight junction proteins, and some microglial activation markers were visualized by confocal microscopy. The effect of PE-derived EVs in the BBB permeability (FITC-Dextran assay) and the transendothelial electrical resistance (TEER) was assessed by culturing hCMEC/D3 cells in a transwell-based static model. The effect of PE-derived EVs in the BBB integrity was evaluated using a microfluidic-supported biochip.

Results | Plasma from PE contained higher sEV and lEV concentrations compared to NP as measured by NTA and visualized by TEM. EVs isolated from maternal plasma contain placenta-specific markers including HLA-G, PLAP, and miR-519d-3p and their levels differ between PE and NP samples. EVs were taken up by endothelial cells and microglia cells. PE-derived EVs decreased TEER and increased FITC-Dextran permeability of the BBB under static conditions. The BBB continuity was impaired and a decreased expression of the BBB-tight junction proteins (ZO-1, **β-catenin**, **claudin-5**, and occludin) was observed after stimulation with PE-derived EVs under flow conditions. EVs crossed the *in vitro* BBB and were uptake by microglia cells increasing the activation markers CD11b and Iba1.

Conclusion | Our results suggest that placental EVs may reach the BBB and be taken up by endothelial cells. PE-EVs increase BBB permeability by decreasing endothelial resistance and tight-junction proteins. Those EVs may cross the BBB and be taken up by microglial cells inducing their activation and release of inflammatory components. EVs could be the link between PE and the cerebrovascular and neurological impairment observed in those patients.

MUTLIPLEXING SENESCENCE MARKERS IN THE PLACENTA: ELUCIDATING PREMATUREAGING IN PREGNANCY PATHOLOGIES BY SPATIAL PHENOTYPING

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Abstract

Background

During the 40 weeks of pregnancy, developing of placenta is associated with aging and an increased expression of senescence associated proteins. Cellular senescence is characterized by a permanent state of cell cycle arrest and shows an upregulation of cyclin-dependent kinase inhibitors like p16 and p21. Cellular senescence can be induced by many stimuli like oxidative stress leading to a higher production of reactive oxygen species (ROS). The ROS production is counteracted by antioxidants like superoxide dismutase (SOD). Although placental aging is a physiological process, premature aging of the placenta can be a pathological state and is a hallmark of several placentaassociated pregnancy complications such as fetal growth restriction (FGR) and preeclampsia (PE).

Aims: The study aims to establish a multiplex immunofluorescence (mIF) protocol applicable on placental samples to simultaneously analyze the spatial expression pattern of senescence markers in different tissue compartments. Investigating different placental diseases, the role of dysregulated aging in placental pathologies will be elucidated and putative targets for diagnosis and therapy will be defined.

Materials and Methods: Formalin-fixed paraffin-embedded placenta samples (total n=30) including healthy term controls (n=5), healthy term controls delivered by caesarean section (n=5), placentas from late onset (n=5) and early onset FGR-pregnancies (n=5), placentas from pregnancies complicated by PE (n=5), and placentas from pregnancies where PE was accompanied by FGR (n=5) are included in the study. Based on Tyramide Signal Amplification, mIF enables the simultaneous detection of multiple proteins of interest in the same tissue section. Using the Opal™ 6-Plex Detection Kit (AKOYA Bioscience), the expression of the senescence markers p16, p21, SOD and IL6 in different placental cell populations is analyzed. The placental cell populations are differentiated by the following phenotype markers: Vimentin (placental stroma), Cytokeratin (trophoblast), β-hCG (syncytiotrophoblast), E-cadherin (cytotrophoblast), CD31 (endothelium). On each slide, one senescence marker is combined with five phenotype markers. Image acquisition is done with the Vectra Polaris™ fluorescence whole slide scanner and inForm® Tissue Analysis Software (both from AKOYA Bioscience) is used for quantification of senescence marker expression as well as the identification of the different cell types involved in premature aging.

Results and discussion: We could successfully establish a 6plex antibody panel for mIF studying placental aging. The quantitative and spatial analysis of six different markers per sample is possible and currently ongoing enabling us to describe different patterns of cells involved in premature placental aging especially in FGR and PE. In PE, the outer layer of the placenta, the syncytiotrophoblast, might be particularly involved, where premature senescence leads to shedding of activating material into the vascular system of the mother, while in FGR fetal parts including fetal endothelium and stroma cells might be predominantly involved as a consequence of reduced placental function.

ENDOTHELIAL TO MESENCHYMAL TRANSITION (EndoMT) OF PREECLAMPTIC FETO-PLACENTAL ENDOTHELIAL CELLS (PE fpEC) IS GOVERNED BY ALK2-SNAIL PATHWAY

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Abstract

Introduction: Preeclampsia (PE) is a maternally derived inflammatory syndrome in pregnancy, characterized by immune alterations and endothelial dysfunction. Endothelial cells show remarkable phenotypic plasticity, including their ability to undergo EndoMT, a process, triggered by an inflammatory environment and leads to endothelial dysfunction. Generally, EndoMT is characterized by the loss of endothelial markers and gain of mesenchymal properties, during EndoMT, ECs develop a wide range of intermediate phenotypes, the phenotype switch can be either complete or partial and is also reflected in the function of the cells. TGFβ and BMP (bone morphogenetic protein) pathways have been implicated as a common underlying mechanism in almost all EndoMT associated disorders. Aim of this study is to identify EndoMT and its signaling in fpECs in the context of PE.

Methods: Primary CTR and PE macrovascular fpEC (n=5) were isolated from term placentae. RT-qPCR was used to determine basal expression of TGFβ superfamily receptors. EndoMT process was examined using qPCR, western blot, FACS and immunofluorescent staining. To elucidate the role of ALK2-Snail signaling, shRNA mediated silencing in PE fpECs was performed, pathway was triggered using BMP9 ligand. In addition to the ECIS assay, leakage assay on 2D-vessel on chip was performed to study the barrier function. *In vitro* migration assay was performed using a WoundMakerTM device and analyzed using IncuCyte instrument, respectively.

Results: Expression of ALK2, BMP type I receptor involved in promoting EndoMT, was significantly upregulated in PE fpECs. *In vitro*, morphological changes of cultured PE fpECs were observed. In addition to cobblestone-like forms, PE fpECs were prone to develop mesenchymal-like morphologies. Furthermore, mesenchymal properties of PE fpECs were reflected by significantly reduced basal expression of *vWF* and upregulation of *CDH2* and *TAGLN*, measured by RT-qPCR. Treatment with BMP9 ligand alone induced the protein expression of EndoMT transcription factor Snail, which was abrogated when *ALK2* was silenced. Furthermore, BMP9 enhanced the leakage and migration capability of ECs, which was consequently reflected on reduced endothelial barrier. Interestingly, silencing of *ALK2* resulted in PE fpEC exhibiting cobblestone morphology and mRNA and protein upregulation of endothelial markers: *Pecam1* and *CDH5*, whereas expression of mesenchymal markers; *FN1* and *CDH2* was reduced. In addition, silencing of *ALK2* affected the barrier function and reduced the leakage and the migration capability of fpECs.

Conclusions: We have identified ALK2-driven and ligand dependent EndoMT and its link to fpEC dysfunction in preeclampsia. A deeper understanding of BMP signaling may provide new insights into the dysfunction of the placental vasculature in PE.

EFFECT OF PENTAERYTHRITYLTETRANITRATE (PETN) ON THE DEVELOPMENT OF FETAL GROWTH RESTRICTION IN PREGNANCIES WITH IMPAIRED UTEROPLACENTAL PERFUSION AT MID GESTATION – A RANDOMISED CLINICAL TRIAL

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Abstract

*Early stage investigator - No

Background

Fetal growth restriction (FGR) and hypertensive pregnancy diseases including preeclampsia, the most important causes of perinatal mortality and morbidity, are strongly associated with impaired placentation and abnormal utero-placental blood flow. NO-donors like pentaerithrityltetranitrate (PETN) are strong vasodilators, lower the vascular resistance and protect the endothelium from stress. Based on data from a randomized controlled pilot study our aim was to confirm the effects of PETN on reduction of FGR and perinatal death, prematurity and as well as on pregnancy induced hypertension (secondary outcome) in pregnant women with placentation failure.

Methods: In a multicenter, randomized, double-blind, placebo controlled trial with two parallel groups pregnant women presenting with a mean uterine artery Pulsatility Index >95th percentile at 19+0 to 22+6 weeks of gestation were randomized to Pentalong® 50 mg or placebo twice daily. Randomization was performed block-wise with a fixed block length stratified by center and high- or low-risk group according to participants' medical history. The primary efficacy endpoint was the composite outcome of perinatal death or development of FGR. Secondary endpoints were neonatal and maternal outcomes. The trial was registered: EudraCT (2016-004396-51), DRKS (DRKS00011374) and clinicaltrials.gov (NCT03669185).

Results: Between August 2017 and March 2020 317 women were included in the study and 307 were analysed. The cumulative incidence of the primary outcome was 41.1 % in the PETN group and 45.5 % in the placebo group, (adjusted RR 0.90, 95 %CI 0.69-1.17; p=0.43). Secondary outcomes like preterm birth in 37.7 % vs. 51.9 % (adjusted RR 0.73, 95 %CI 0.56-0.94; p=0.01) and pregnancy induced hypertension in 19.4 % vs. 36.6 % (adjusted RR 0.45, 95 %CI 0.22-0.92; p=0.03) were significantly reduced. Kaplan-Meier analysis revealed that participants in the PETN group might have been less likely to be admitted to the hospital (HR 0.51, 95 % CI 0.30-0.85; p=0.01), had longer time to delivery and were less likely to develop elevated blood pressure. Additionally, angiogenic factors measured during pregnancy as the ratio of soluble Flt-1 and placental growth factor (PlGF) stayed significantly longer within the normal range in the PETN group.

Discussion: Although results could not confirm an effect of PETN on the development of FGR and perinatal death, the observed potential effect on maternal blood pressure further suggests PETN as a candidate to improve the outcome in pregnancies complicated by placental dysfunction. Experimental results of our group confirmed the protective effect of PETN on endothelial cells as well as trophoblastic cells exposed to oxidative stress.

PERI-CONCEPTIONAL STRESS SIGNALLING THROUGH O-GLCNACYLATION : EFFECTS ON FETAL/PLACENTAL DEVELOPMENT

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Abstract

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Introduction

Adverse events during critical points of pregnancy lead to impaired placental function, which may cause detrimental effects on fetal growth and long-term health issues after birth. The blueprint for placental formation is initiated very early in pregnancy and the periconceptional stage is exquisitely sensitive to the maternal environment. Post-translational modification of proteins by O-GlcNAcylation is involved in the regulation of placental development and is often related to stress-induced epigenetic changes. Here, we investigated the effects of elevated protein O-GlcNAcylation during peri-implantation on placental and fetal development.

Methods

Pregnant mice were injected intraperitoneally with thiamet G (TMG) to manipulate O-GlcNAc levels during the implantation window, i.e. E3.5-E7.5. Genes linked to trophoblast development in early pregnancy were assessed by qPCR. At term (E18.5), fetal and placental weights were taken and placental cell types and nutrient (System A amino acid) transport were assessed by immunohistochemistry and transfer of 14C-methyl-AIB respectively.

Results

As expected, protein O-GlcNAcylation in E7.5 implantation sites was increased by TMG treatment. Expression of two trophoblast lineage-specific transcription factors, eomes and stra13, was reduced at E9.5. At term, fetal weight was significantly decreased by 7% in TMG-treated mice, accompanied by reduced fetal:placental weight ratio. However, placental weight, gross morphology, total area of fetal vessels (immunostained with CD31) and area of trophoblast cells (immunostained with pan-cytokeratin) in labyrinth zone were unaffected at term, nor was System A amino acid transport altered.

Conclusions

Our data demonstrating decreased fetal weight and fetal:placental weight ratio suggest that increased protein O-GlcNAcylation during implantation compromises placental function, though identification of the underlying mechanism(s) requires further investigation.

PERINATAL, METABOLIC AND REPRODUCTIVE FEATURES IN PPARG-RELATED LIPODYSTROPHY

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Abstract

# Equally contributed

Objective: The adipogenic *PPARG*-encoded PPARγ transcription factor also display important placental functions. In deed, PPARγ control trophoblast invasion and fusion, two cellular processes indispensable for placental development and functions and consequently for pregnancy outcome. We evaluated metabolic, reproductive and perinatal features of patients with PPARG-related lipodystrophy.

Research Design and Methods: Current and retrospective data were collected in patients referred to a National Rare Diseases Reference Centre.

Results: 26 patients from 15 unrelated families were studied (18 women, median age 43 years). They carried monoallelic *PPARG* variants except a homozygote patient with congenital generalized lipodystrophy. Among heterozygote patients aged 16 or more (n=24), 92% had diabetes, 96% partial lipo-dystrophy (median age at diagnosis of 24 and 37 years, respectively), 78% hypertriglyceridemia, 71% liver steatosis and 58% hypertension. Mean BMI was 26. ± 5.0 kg/m2. Women (n=16) were frequently affected by acute pancreatitis (n=6) and/or polycystic ovary syndrome (n=12). Eleven women obtained one or several pregnancies, all complicated by diabetes (n=8), hypertension (n=4) and/or hypertriglyceridemia (n=10). We analysed perinatal data of patients according to the presence (n=8) or absence (n=9) of maternal dysmetabolic environment. The median gestational age at birth was low in both groups (37 and 36 weeks of amenorrhea, respectively). As expected, birth weight was higher in patients exposed to a foetal dysmetabolic environment. However, 85.7% of non-exposed patients (pathogenic variant carried by the father not by the mother) were small for gestational age, suggesting that placental expression of PPARG pathogenic variants carried by affected foetus impair prenatal growth and parturition.

Conclusion: Lipodystrophy-related *PPARG* variants induce early metabolic complications. Prenatal monitoring of offspring is needed in affected families. Further studies regarding the pathophysiological role of *PPARG* in human pregnancy are warranted.

THE ROLE OF THE ION CHANNELS PIEZO1 AND TRPV4 IN THE PLACENTAL ENDOTHELIUM - IMPLICATIONS FOR ENDOTHELIAL (DYS)FUNCITON

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Abstract

*Early stage investigator - No

Background: Preeclampsia (PE) is a leading cause of maternal and perinatal mortality. Insufficient placental function associated with inflammation and endothelial dysfunction are well described in PE. However, understanding of the underlying pathomechanisms as well as available therapeutic strategies are still limited. PIEZO1 is a mechanosensitive, Ca^{2+}-permeable ion channel expressed in various cell types. Recently, PIEZO1 was identified in the cell membrane of isolated placental endothelial cells (ECs) and shown to be involved in sensing shear stress and maintaining cell-to-cell contacts. However, its role in any pregnancy disorders has not been investigated yet. In neurons, a role for other ion channels in regulating PIEZO1, namely transient receptor potential (TRP) channels, has been shown, and inflammatory mediators can activate them. Thus, we hypothesize that PIEZO1 and TRPV4 mediate endothelial dysfunction in PE and aim to understand their regulation in normal and PE placentas.

Methods: *PIEZO1* and *TRPV4* expression was quantified by RT-qPCR in tissues of term, preterm, early-onset PE, and late-onset PE placentas (n=5-6/group). mRNA expression was localized with the RNAscope™ Multiplex Fluorescent V2 Assay and confocal microscopy on paraffin-embedded tissue slides and fixed primary feto-placental ECs (fpECs) isolated from chorionic arteries. Cytosolic Ca^{2+} uptake was measured in preterm and early-onset PE fpECs in response to ATP (100µM) inducing endoplasmic reticulum Ca^{2+} depletion, a PIEZO1- (10µM Yoda1) or TRPV4-agonist (50nM GSK1016790A). The fluorescent cytosolic Ca^{2+} dye Fura-2AM was used in these live-cell imaging experiments (n=3/group; 30-43 cells tracked/condition).

Results: While *PIEZO1* expression in placental tissue did not differ between the groups, *TRPV4* expression was significantly lower in preterm compared to term ($p<0.01$) and significantly higher in late-onset PE compared to early-onset PE ($p<0.01$). On placental tissue slides, RNA signals for both channels were predominantly located in the trophoblast layers and ECs. In fpECs, both channels were expressed in the cell membrane but *PIEZO1* signals were detected more frequently. Cytosolic Ca^{2+} uptake in early-onset PE fpECs was significantly reduced compared to preterm fpECs in response to ATP and both agonists ($p<0.05$).

Conclusion: PIEZO1 and TRPV4 are functionally expressed in the endothelium of normal and PE placentas. In early-onset PE fpECs, regulation of cytosolic Ca^{2+} levels is impaired, suggesting altered ion channel functionality and Ca^{2+} homeostasis. *TRPV4* but not *PIEZO1* expression levels differ between pathophysiological groups, implying that channel activation rather than expression is important for Ca^{2+} signaling in the placenta. As Ca^{2+} is an essential second messenger, reduced influx might lead to impairment of several downstream functions of the endothelium, e.g., barrier integrity or vasodilation. This will be investigated in functional studies in the future.

UNTANGLING THE ROLE OF THE UMBILICAL CORD IN PREGNANCY COMPLICATIONS

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Abstract

Umbilical cord (UC) coiling may relate to blood flow in umbilical vessels. Extremes of umbilical coiling index (UCI) are linked to adverse fetal outcomes; previous studies have poor consistency and heterogeneity in methods determining UCI and parameters defining UC pathology. UCI is not incorporated into clinical decision making currently. This study aimed to find the degree of similarity between structural and functional UC measurements *in vivo* (ultrasound) and *ex vivo* (laboratory) and determine if *in vivo* metrics of the UC can be replicated *ex vivo*, by perfusion of the umbilical vein.

Women underwent ultrasound examination to measure UC geometry and blood flow using Doppler prior to Caesarean delivery. UCs were collected, and geometry measured in the laboratory before and after *ex vivo* perfusion. Mathematical models were used to link UC geometry and blood flow *in vivo*.

UCI did not change between *in vivo* and *ex vivo* perfusion; coiling remains consistent despite UC collapse post-delivery. UCs underwent collapse to the width of 55.5% (mean) of their measured *in vivo* width. Average *ex vivo* width was brought within 72% (mean) of *in vivo* width after umbilical vein perfusion. No metric of UC morphology (width, coil pitch, vessel curvature, torsion) had a significant relationship with UC blood flow, so they may not be useful in determination of pregnancies at high risk of fetal complications due to UC pathology. Future studies should clarify any relationship between UC morphology and blood flow and identify interventions which help to reduce fetal mortality resulting from UC complications.

CELLULAR TARGETS AND UPTAKE MECHANISMS OF EXTRACELLULAR VESICLES FROM NORMAL AND PREECLAMPSIA PREGNANCIES

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Abstract

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Introduction | Preeclampsia (PE) remains one of the most common and severe pregnancy complications. Extracellular vesicles (EVs) filled with proteins, lipids, DNA and RNA play a crucial role in cell-cell-communication and can modulate immune responses. Secreted from trophoblasts and other fetal cells, EVs can reach maternal peripheral blood and influence many cellular effects therein. This study addresses the uptake of plasma EVs from normal (NP) and PE pregnancies by primary PBMCs and explores the underlying internalization mechanisms.

Methods | EVs were isolated from the plasma of pregnant women with normal pregnancies (NP-EVs) or complicated with preeclampsia (PE-EVs). EVs were characterized by cryo-transmission electron microscopy (cryo-TEM), nano tracking analysis (NTA), and western blot. EVs were quantified using both flow cytometry (FC) and microBCA. PBMCs were isolated from male-, female non-pregnant (FNP)- and pregnant- whole blood samples, and the percentage of monocytes and lymphocytes was measured by FC. For uptake detection, primary PBMCs and PMA-treated THP-1 cells were stimulated with PKH67- or PKH26-stained NP- or PE-EVs for 24h and fluorescent positive cells were identified and quantified using FC. Furthermore, uptake was confirmed by confocal microscopy. Uptake mechanisms were analyzed by detecting EV-internalization in PMA-treated THP-1 cells in presence of the inhibitors Wortmannin and Cytochalasin D. Released pro- and anti-inflammatory cytokines were evaluated by using the LEGENDplex assay.

Results | Cryo-TEM, NTA, flow cytometry, and microBCA show elevated EV concentration and protein in plasma of PE compared to normal pregnancies. A higher percentage of monocytes and a lower percentage of lymphocytes were observed in the isolated PBMCs from pregnant women compared to those from control groups (male and FNP-women). Among the PBMC populations, plasma EVs were mostly internalized by monocytes and only in a low proportion by lymphocytes. A higher internalization rate was observed after treatment with PE-EVs compared to NP-EVs in primary lymphocytes, monocytes, and THP-1 cells. After pharmacological inhibition of endocytic and phagocytic pathways, EV uptake in THP- 1 cells was significantly inhibited but not completely abolished. Cytokine analysis revealed both pro- and anti-inflammatory signals secreted by THP-1 cells after treatment with NP- and PE-EVs but no significant differences were observed between groups.

Conclusions | Pregnancy affects peripheral maternal immune cells. Monocytes are the major cellular targets of circulating EVs. Compared to those of normal pregnancies, EVs from PE patients are internalized at a higher rate in all tested models of monocytes and macrophages. The main mechanisms of this EV uptake are phagocytosis and endocytosis, but additional mechanisms could not be ruled out. The differences in the amount and internalization rate of circulating EVs strengthen their implication in thepathophysiology of PE.

ROLE OF HTRA1 IN PREECLAMPSIA : A POSSIBLE BIOMARKER?

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Abstract

*Early stage investigator - No

Introduction: The High temperature requirement A1 (HtrA1) is a multidomain secretory protein with serine-protease activity involved in different cellular processes. HtrA1 is more expressed in human first trimester placenta suggesting that this serine protease is tightly associated with early phases of human placenta development. In addition, HtrA1 expression was altered in both placental tissue and maternal plasma of pregnancies complicated by Preeclampsia (PE), a gestational syndrome that affects the 3-5% of the pregnancy worldwide characterized by new onset of maternal hypertension and proteinuria suggesting that HtrA1 could be considered a key molecule in the development of PE.

Methods: We analyzed plasma samples of women at first trimester of gestation by ELISA to evaluate HtrA1 levels. We also evaluated, by immunofluorescence, the cellular expression of HtrA1 in HTR8/SVneo, BeWo and HUVEC cell lines, three placental cell lines normally used as in vitro models to study human placental physiology. We overexpressed and silenced HtrA1 in HTR8/SVneo cells by specific plasmid and siRNA, respectively, and evaluated the migration and invasion ability of these cells. We also evaluated the HtrA1 expression levels in HTR8/SVneo cells exposed to oxidative stress by using H_2O_2.

Results: We found increased HtrA1 plasma levels in first trimester pregnancy which will develop PE later in pregnancy. HtrA1 was expressed in the cytoplasm of HTR8/SVneo, BeWo and HUVEC placental cell lines. Interestingly, HtrA1 expression was decreased when HTR8/SVneo cells were exposed to oxidative stress, a feature of PE. Silencing HtrA1 in HTR8/SVneo cells increased cells invasion and migration while HtrA1 overexpression significantly decreased the migration and invasiveness of these cells.

Conclusion: Our data showed that HtrA1 plays a key role in regulating trophoblast migration and invasion. Moreover, HtrA1 could be considered a useful early marker of PE onset allowing an early identification of patients at risk to develop PE during gestation and permitting a more appropriate and earlytreatment of these patients that may improve or avoid PE development.

CHOLESTEROL HOMEOSTASIS IS ALTERED IN A PREECLAMPSIA MODEL USING PRIMARY HUMAN CYTOTROPHOBLASTS

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Abstract

*Early stage investigator

Preeclampsia (PE) is a pregnancy-specific condition that can lead to increased cardiovascular risk for both the mother and the offspring later in life. Pregnancies with PE have a proatherogenic maternal lipid profile and exhibit an accumulation of cholesterol in the placenta. The ATP-binding cassette (ABC) transporters A1 and G1 (ABCA1, ABCG1), and the scavenger receptor type B, class I (SR-BI), play an important role in maternofetal cholesterol transport and homeostasis by mediating the efflux of cholesterol.

Aim: To determine the expression and function of genes involved in cholesterol efflux, transport and biosynthesis in parallel to intracellular cholesterol concentrations in hypoxic trophoblast models mimicking major features of PE.

Methods: The PE model was established in cytotrophoblast cells (CTB) isolated from healthy placentae by trypsin/DNAse digestions. CTB were placed either under normoxic (5% pCO2/21%pO2/24h), hypoxic (5% pCO2/1.5% pO2/24h) or hypoxia/reoxygenation (H/R, 6h interval each of normoxia and hypoxia until 24h) conditions. Total, esterified, and free intracellular cholesterol levels were assayed enzymatically. The mRNA expression of genes involved in cholesterol homeostasis (SREBP-1a, SREBP-2, LDLR, HMGCR, ACAT-1) and cholesterol transport (ABCA1, ABCG1 and SR-BI) was analysed by qPCR; protein expression of ABCA1, ABCG1 and SR-BI was determined by western blotting. Additionally, we determined the efflux of cholesterol to maternal and fetal serum (5% serum/6h/37°C) under normoxic, hypoxic and H/R conditions. Finally, we evaluated the protein secretion of apolipoproteins (ApoA-I and ApoE) and progesterone by ELISA.

Results: We observed that intracellular total and free cholesterol levels were elevated in hypoxia and H/R. To investigate the underlying factors responsible for the accumulation of cholesterol, we evaluated several genes controlling cholesterol homeostasis. We found that the mRNA expression of the transcription factors SREBP-2 and SREBP-1a, was increased in hypoxia together with HMGCR, the rate limiting gene involved in cholesterol biosynthesis. The mRNA abundance of ACAT1, an enzyme involved in the esterification of cholesterol, was lower in both hypoxia and H/R, explaining the increase in total cholesterol and the accumulation of free cholesterol in the cells.
To evaluate if the increase of intracellular cholesterol affects cholesterol efflux transporters, we determined the gene and protein expression of ABCA1, ABCG1 and SR-BI. The mRNA expression of ABCA1 and ABCG1 was increased in hypoxia; SR-BI mRNA levels were elevated in both hypoxia and H/R compared with normoxia. The protein abundance of ABCA1 was reduced in both hypoxia and H/R, while the protein expression of SR-BI was increased in hypoxia compared to normoxia. These results suggest that cholesterol transport is altered after the hypoxic insult. This was confirmed in functional experiments where cholesterol efflux to maternal serum was higher in hypoxia and H/R and efflux to fetal serum was increased in H/R compared to normoxic conditions. These findings suggest that in PE there is not only an increased cholesterol concentration in the placenta, but that both the mother and the fetus receive more cholesterol during pregnancy, which may result in an increased cardiovascular risk later in life.
To determine the underlying reasons and potential consequences of the altered cholesterol efflux, we measured the protein secretion of ApoA-I and ApoE (cholesterol acceptors) and progesterone (product of cholesterol metabolism). All these factors were unchanged after hypoxia and H/R.

Conclusion: Several pathways of cholesterol homeostasis as well as the function of ABCA1, ABCG1 and SRBI are altered in a primary trophoblast model mimicking the phenotype of PE. The increased cholesterol efflux and the observed dysregulation of cholesterol biosynthesis may have not only critical consequences for both the mother and the fetus during pregnancy but also later in life.

CHARACTERIZATION OF THE ROLE OF PREGNANCY ZONE PROTEIN (PZP) IN PREECLAMPSIA

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Abstract

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Preeclampsia (PE) is a complication affecting 3 to 5% of pregnancies. It is characterized by symptoms such as hypertension and proteinuria that usually appear after 20 weeks of gestation (wg). Currently, the diagnosis is mainly based on clinical symptoms and recently on the measurement of placental angiogenic and anti-angiogenic factors that are released by the placenta in response to the maintenance of a hypoxic environment beyond the physiological period of 10/12 wg. Unfortunately, these biomarkers are not deregulated early enough to predict PE occurrence by the end of first trimester. Our team conducted a clinical study called "EGEVE, NCT01490489" that included 148 pregnant women between 14-18 wg. Out of this cohort, 4 women developed PE later. Proteomic analysis of their plasma identified a glycoprotein called Pregnancy Zone Protein (PZP), whose circulating levels were significantly lower in PE patients.

The objective of this study was to characterize the role of PZP in placentas collected from the first trimester of pregnancy and to determine whether PZP expression is also deregulated in the placentas of PE women, during the third trimester of pregnancy. We used, human placentas (6 to13wg) that were collected from elective termination of pregnancy at Grenoble hospital (NCT05188066) to characterize the physiological expression profile of PZP. Placentas were also cultured in 3D system as placenta explants to determine the ontology of PZP expression and secretion throughout the first trimester of gestation and to determine the effect of hypoxia on its expression and secretion. Finally, 3rd trimester CTL and PE human placentas were used to compare PZP expression when the PE is established.

We demonstrated, using immunohistochemistry and western blot analysis, that PZP is expressed in different cell types of the human placenta, with specific expression in the most differentiated trophoblasts: syncytiotrophoblasts and extravillous trophoblasts. We also demonstrated a trend to an upregulation of the PZP protein under hypoxic conditions in the 3D culture system. These findings strongly suggest a positive regulation of PZP expression and or secretion by hypoxia. Finally, we demonstrated that PZP placental expression was higher in PE specimens compared to CTL. This finding strongly suggests a potential compensatory mechanism that occurs in the placenta to overcome the initial decrease that occurs during the first trimester in PE placenta. Ongoing experiments are in progress to validate these results. To conclude on a final role of PZP in PE, a larger cohort is needed, as it will allow better stratification of PE women. In fact, PZP levels are influenced by other factors such as, contraception or the age of the women.

In conclusion, we demonstrated for the first time that PZP protein may be considered as a biomarker of PE occurrence early-on during pregnancy and that this protein may contribute to placental development during early pregnancy.

THE POTENTIAL OF S-(2-BORONOETHYL)-L-CYSTEINE (BEC) AS A THERAPY TO ENHANCE NITRIC OXIDE PRODUCTION BY THE FETOPLACENTAL ENDOTHELIUM

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Abstract

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Background: Nitric oxide (NO), produced by endothelial NO synthase (eNOS), has a key role in reducing vascular tone in the fetoplacental circulation. Shear stress is the most effective stimulator of NO production in this vascular bed. Pregnancies complicated by fetal growth restriction (FGR) have raised vascular resistance and a different eNOS profile in this circulation, with higher expression and activity compared to normal pregnancy. In umbilical arteries from healthy pregnancies, arginase-2 competes with eNOS for L-arginine and prevents the NOS-dependent relaxation. Here we test the hypothesis that therapeutic intervention aimed at arginase inhibition by BEC, alone and with tetrahydrobiopterin (BH4), an eNOS co-factor with anti-oxidant properties, would augment NO production by the placenta, with potential future therapeutic benefit in FGR.

Methods Human placental arterial endothelial cells (HPAECs) from normal pregnancy (n=6) were grown to confluency in 0.4 Luer T/C treated micro-chambers (Ibidi BioDiagnostics). Cells were subjected to unidirectional laminar shear stress (20 dynes/cm2) for 48 hours. Four cell flow systems operated in parallel and each cell line was exposed to (i) PBS control, (ii) 25 μM BEC; (iii) 20 μM BH4 or (iv) BEC + BH4. At 48 hours, the conditioned medium was tested for nitrite levels (NO stable breakdown product) using the Griess reaction, following nitrate to nitrite conversion. The concentration dependency of BEC (0125-1250 μM) and BH4 (0.2-2000μM) on cell growth was assessed via a metabolism (MTT assay, N=6); and a cell stress protein array was performed on static cultured HPAECs (12.5 μM BEC; 20 μM BH4 n=3).

Results BEC did not affect MTT at any concentration used and BH4 was without effect at 200μM (Wilcoxon vs control 100%: NS). BEC (25μM) did not increase nitrite levels in conditioned medium from normal pregnancy compared to the PBS control (14.5±7.6μM and 17.1±4.7 μM, mean±SE, respectively), but BH4 (20μM) neared significance in increasing nitrite levels (31.3±5.7 μM; P=0.06, Wilcoxon). Experiments with BEC+BH4 had reduced numbers (n=3) due to technical problems and data were not statistically tested. BEC reduced 17 of 26 human cell stress related proteins in normal HPAECs; and notably included a 61% reduction in expression of the antioxidant SOD2 and a 28% reduction in HIF-1alpha, which dysregulates NOS expression.

Conclusion The arginase inhibitor BEC did not enhance NO production by the HPAECs in normal pregnancy. This result might arise if increasing L-arginine availability alone is not enough to promote NO production. Alternatively, BEC could have off target actions to elevate oxidative stress and it might be possible to counteract this by concomitant treatment with the eNOS co-factor BH4. Further work is required to assess the potential of BEC, and BEC+BH4, as therapeutic strategy to enhance the capacity of placental endothelial cells to generate NO in FGR.

DIFFERENTIAL EXPRESSION OF GLUCOSE TRANSPORTER PROTEINS GLUT-1, GLUT-3, GLUT-8 AND GLUT-12 IN THE PLACENTA OF MACROSOMIC, SMALL-FOR-GESTATIONAL-AGE AND GROWTH-RESTRICTED FETUSES

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Abstract

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Aim: Placental transfer of glucose constitutes one of the major determinants of the intrauterine fetal growth. The objective of the present study was to evaluate the expression of glucose transporter proteins GLUT-1, GLUT-3, GLUT-8 and GLUT-12 in the placenta of macrosomic, small-for-gestational-age (SGA) and growth-restricted fetuses (FGR).

Material and methods: A total of 70 placental tissue samples were collected from women who delivered macrosomic ≥4000g (n=26), SGA (n=11), growth-restricted (n=13) and healthy control neonates (n=20). Computer-assisted quantitative morphometry of stained placental sections was performed to determine the expression of selected GLUT proteins.

Results: Immunohistochemical staining identified the presence of all glucose transporters in the placental tissue. Quantitative morphometric analysis performed for the vascular density-matched placental samples revealed a significant decrease in GLUT-1 and increase in GLUT-3 protein expression in pregnancies complicated by FGR as compared to other groups ($p<.05$). In addition, expression of GLUT-8 was significantly decreased among SGA fetuses ($p<.05$). No significant differences in GLUTs expression were observed in women delivering macrosomic neonates. In the SGA group fetal birth-weight (FBW) was negatively correlated with GLUT-3 (rho=-.59, $p<.05$) and positively with GLUT-12 (rho=.616, $p<.05$) placental expression. In addition, a positive correlation between FBW and GLUT-12 expression in the control group (rho=.536, $p<.05$) was noted.

Conclusions: In placentas derived from FGR-complicated pregnancies the expression of two major glucose transporters GLUT-1 and GLUT-3 is altered. On the contrary, idiopathic fetal macrosomia is not associated with changes in the placental expression of GLUT-1, GLUT-3, GLUT-8 and GLUT-12 proteins.

GOT1 AND GOT2 ARE IMPORTANT PLAYERS IN ENERGY MAINTENANCE OF THE PLACENTA IN RESPONSE TO PHYSIOLOGICAL SHEAR STRESS

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Abstract

*Early stage investigator - Yes

Introduction: Adequate placental development is crucial for the wellbeing of the developing fetus. Glucose plays a key role, by being the main energy source for the growing baby. To ensure energy supply to the fetus, also the placenta and its own glucose consumption/production is involved in this calculation. Various changes in the environment may be the trigger for altered glucose uptake in the placenta and its delivery to the fetus. One important factor can be the fluidic flow and the resulting shear stress, acting on the developing placenta, and in particular on the syncytiotrophoblast. We aimed to characterize changes in syncytiotrophoblast development, focusing on metabolic pathways in response to physiological flow rates.

Methods: Flow culture experiments were performed using differentiated BeWo cells and placental villi (first trimester and term) subjected to flow chambers. They were cultured under physiological shear stress values, ranging from 0,3-1dyne/cm^2 up to 6dyne/cm^2 for pathological conditions, and low oxygen concentration. The expression of RNA was investigated with RNA Sequencing and was confirmed with qPCR. Protein levels were analyzed using Immunofluorescence and Western Blot. All results from cells exposed to shear stress were compared to static conditions to identify important metabolic players under physiological flow conditions. Mitochondrial activity was measured by IF. Glucose consumption of the cells was investigated after silencing the main glucose transporter (*SLC2A1*) GLUT1 and under different glucose concentrations. To gain insights about the glucose transport under (patho)-physiological conditions, we silenced the main glucose transporter GLUT1 in an artificial placental barrier and observed GOT1 and GOT2 (Glutamic-oxaloacetic transaminase) expression as well as the glucose uptake in different cell layers.

Results: With RNA Sequencing we were able to observe lower expression of genes involved in the glycolytic pathway, beginning with the main glucose transporter itself, GLUT1. Genes, which encode for key enzymes in this pathway are downregulated (*HK2*, *PFKP)*. The main product of the glycolytic pathway, pyruvate and further lactate, were increased on cellular level as well as in the supernatant, under flow conditions. Mitochondria in cells exposed to shear stress show lower glucose dependency to hold their membrane potential. Upregulation of genes involved in the energy metabolism could be observed for amino acid pathways. GOT1 and GOT2 were significantly upregulated in cells exposed to shear stress. These two enzymes are also altered in IUGR, as well as in glucose uptake studies, by silencing the glucose transporter GLUT1.

Conclusion: We suggest, that there is a shift of the energy metabolism from the glycolytic to the amino acid pathway in placenta with physiological shear rates. The use of amino acids to gain energy in the placenta can be important, to support the glycolysis and to force glucose net transport to the fetus.

MODELLING OF A 3D *IN VITRO* HUMAN PLACENTA-ON-CHIP MODEL USING CHORION CARCINOMA-DERIVED TROPHOBLAST CELL LINE (BeWo B30) AND IMMORTALISED UMBILICAL VEIN CELL LINE (EA.hy-926)

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Abstract

* Presentiong author; * early stage investigator - Yes

Background: The placenta is an intricate organ that supports fetal development, performs vital functions for the fetus and acts as a physical and immunological barrier to pathogens. Investigating the human placenta remains a challenge due to ethical issues related to pregnancy, interspecies differences, and lack of physiologically relevant in vitro models. Here we used the Mimetas 3-line OrganoPlate® platform to create a novel 3D placental model that closely mimics the physiological and anatomical architecture of the human placenta.

Methods: The OrganoPlate® contains 40 chips made up of 3 channels (Ch) each: Ch1 was seeded with the BeWoB30 human trophoblastic cell line, Ch3 with human umbilical vein immortalised cells (EA.hy-926), and Ch2 held collagen type I thus creating an extracellular matrix interface. The system was optimised for cell attachment, tubule formation and leak-tight barrier functions. Forskolin was used to promote the fusion of BeWoB30 into a syncytium. Immunofluorescence staining was used to confirm the formation of a functional and syncytial placental barrier. Cell viability was visualised using Live/Dead Assay. Imaging was performed using the Andor Dragonfly spinning disk microscope.

Results: Immunostaining of trophoblast (occludin, ezrin) and endothelial cell markers (actin, VE-cadherin) showed the formation of a physiologically relevant human placental phenotype while the expression of GLUT-1 presented an intensity gradient consistent with its *in vivo* expression. Staining of the syncytialization marker SPTAN-1 allowed visualisation of a clear cell border on the edges of a polynuclear syncytium. Production of human chorionic gonadotropin confirmed the functionality of our placental barrier model.

Conclusions: We made significant advances toward establishing a novel human placenta-on-a-chip model. Our results demonstrate a vital component of placental function and suggest our model has the potential for modelling placental development. This will allow imaging of the cross-placental transfer of both maternal antibodies and TORCH pathogens.

PLATELET-DERIVED FACTORS DEREGULATE THE TRANSCRIPTOME OF THE HUMAN TROPHOBLAST

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Abstract

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Introduction: During first trimester of human pregnancy the spiral arteries of the human placenta are occluded by accumulations of extravillous trophoblasts. This prevents maternal blood cells from entering the intervillous space. Recently, however, studies have shown by methods of immunohistochemistry and transmission electron microscopy that maternal platelets are already present in the intervillous space even in early pregnancy. It is suggested that due to their small size, platelets find alternative routes through intercellular clefts into the intervillous space and thus are in direct contact with trophoblasts at a very early stage.
In this study, we tested whether platelets and their factors have an effect on the gene expression of the syncytiotrophoblast and the milieu of the human placenta and therefore the development of the fetus.

Methods: The trophoblast cell line BeWo was pre-differentiated with forskolin to simulate the syncytiotrophoblast by fusion of the cells. Cells were then incubated for 24h with freshly isolated human platelets from healthy donors. RNA from three individual experiments was isolated and subjected to RNA sequencing, which was subsequently analyzed using DAVID software. Supernatants of these experiments were tested for progesterone expression via ELISA. Furthermore, first trimester villous explants were treated with different platelet products at 37°C, 2,5% O2 and 5% CO2 for 24h. RNA and protein from four individual experiments was isolated and subjected to qPCR and Western Blot experiments.

Results: RNA sequencing showed that 1462 genes were significantly deregulated when differentiated BeWo cells were incubated with human platelets. Of these genes, 385 genes were downregulated and 1077 genes were upregulated. DAVID software identified several signaling pathways that were affected by co-incubation (e.g., inflammatory responses, immune responses, and neutrophil chemotaxis). Some genes that are involved in the steroid hormone synthesis showed significant deregulations and progesterone release into the media was also decreased upon treatment. qPCR and Western Blot confirmed findings of RNA-Sequencing data in first trimester placental explants.

Conclusion: Platelet activation at the maternal-fetal interface may strongly influence the transcriptome of the fetal trophoblast, initiating signaling pathways such as thromboinflammation. These could subsequently lead to activation of maternal immune cells. Additional experiments are needed to gain deeper insight into this promising topic and decipher the role of maternal platelets in human placenta, pregnancy and pregnancy complications.

PLACENTAL SPHINGOSINE-1-PHOSPHATE RECEPTORS ARE DIFFERENTIALLY EXPRESSED ACROSS GESTATION

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Abstract

Introduction: Sphingosine-1-phosphate (S1P) is an essential and bioactive sphingolipid, which exerts its functions through five different G-protein coupled receptors (S1PR1-5). Platelets can produce and store S1P in large amounts and will eventually release it during platelet activation. S1P has various functions on female reproductive organs before and during pregnancy, and hence is increasingly becoming the focus of pregnancy research. S1P signaling pathways are dysregulated in pregnancy pathologies such as preeclampsia, which has been associated with aberrant trophoblast differentiation as well as turbulent blood flow in the intervillous space and increased wall shear stress at the villous syncytiotrophoblast. Here, we aimed to analyze expression dynamics of placental S1P receptors in human first trimester, pre-term and term cases, and characterize their expression pattern in different primary placental cell types and on differentially treated BeWo cells and first trimester placental explants.

Methods: The expression of the five S1P receptors was analyzed by qPCR in first trimester placental samples, preterm and healthy third trimester placentas. In addition, the expression behavior of these receptors was measured in the BeWo cell line and placental explant cultures cultured under specific flow conditions. Immunohistochemistry and Western blot were used to characterize S1P receptors in specific primary cell types of placental tissue. Moreover, we investigated whether or not the placental S1PR subtype repertoire is deregulated in BeWo cells as well as first trimester placental explants, upon treatment with platelet-derived factors and different culture conditions including hypoxia and different flow rates.

Results: qPCR analysis revealed that S1PR2 is the predominant placental S1P receptor in first trimester and vanishes towards term. All other S1P receptors increased from first trimester towards term. RNA and Protein data as well as immunohistochemical stainings confirmed that S1PR1 is predominantly found in the endothelium of placental villi, while S1PR2 and S1PR3 are mainly located in trophoblasts. Flow culture of the BeWo cell line showed no effect on the expression level of S1PR2, while platelet-derived factors decreased the expression of some receptors significantly.

In conclusion, our study suggests that the placental S1PR repertoire is differentially expressed across gestation. S1P receptor expression pattern in trophoblasts depends on its differentiation status and may be deregulated in response to hemodynamic forces and the presence of platelet-derived factors. Additional experiments are needed to further decipher the role of S1P receptors in the placenta over the course of human pregnancy and in pregnancy complications.

ASSESSMENT OF ANTI-INFLAMMATORY AGENTS TO TREAT PLACENTAL DYSFUNCTION

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Abstract

*Early stage investigator - Yes

Background: There is increasing evidence that sterile inflammation plays a role in the placental dysfunction observed in fetal growth restriction. The NOD-like receptor, pyrin domain-containing protein 3 (NLRP3) inflammasome, which is expressed in the placenta, is activated in response to inflammation. Aspirin (ASA) is a non-steroidal anti-inflammatory drug (NSAID), currently prescribed to high-risk women to improve pregnancy outcomes, but the exact mechanism underpinning its benefit remains unknown. Flufenamic acid (FA) and mefenamic acid (MA) are also NSAIDs which inhibit the NLRP3 inflammasome, but their anti-inflammatory effects have never been studied in placenta. This study aims to assess the safety and efficacy of ASA, FA and MA for treating and preventing placental inflammation.

Methods: Fresh term placental explants were obtained from healthy pregnancies. An in vitro model of inflammation was established using LPS (0.1µg/ml). Placental explants (n=6) were incubated with vehicle controls or LPS+/-ASA (50, 100µM), FA (10, 50µM) or MA (10, 50µM) for 24 or 48 hours. To assess the effects of pre-treatment, placental explants (n=7) were incubated with ASA, FA or MA at 2 concentrations for 24 hours, then incubated with LPS for another 48 hours. Culture medium was collected to assess the release of pro- and anti-inflammatory cytokines (IL-1β, IL-6, IL-10, IL-1ra) downstream of NLRP3 activation. HCG release was measured to assess placental endocrine function. Explants were also collected for immunohistochemistry to detect cleaved caspase 3 (CC3) to assess induction of apoptosis.

Results: LPS treatment significantly increased IL-10 secretion at 24 and 48 hours ($p=0.02$, $p=0.01$) and IL-1ra secretion at 24 hours ($p=0.05$), compared to controls. Co-treatment with ASA (100µM) significantly reduced IL-10 secretion compared to the LPS group after 24 and 48 hours ($p=0.006$, $p=0.005$). Co-treatment with FA acid increased median pro-inflammatory IL-6 levels at 24 and 48 hours. Pre-treatment with MA (10, 50µM) significantly decreased LPS-induced release of IL-1β ($p=0.003$, $p<0.0001$), IL-10 ($p=0.0073$, $p=0.0068$) and IL-1ra ($p<0.0001$, $p<0.0001$). HCG secretion was unchanged by all treatments, indicating intact placental endocrine function. A low number of CC3-positive cells was observed in all explants, suggesting that none of the treatments induced cell death at the concentrations tested.

Conclusion: ASA and MA reduced LPS-induced placental inflammation in healthy placental explants, without altering endocrine function or causing cytotoxicity. FA may increase inflammation in some placentas. This study suggests that drugs known to target the NLRP3 inflammasome can alter cytokine release from placental tissue, thus merit further investigation to determine whether this approach could reduce sterile inflammation. Mefenamic acid is licenced in the UK for dysmenorrhoea and menorrhagia (0.5g three times a day), which may facilitate further studies on placental inflammation.

THE ECTONUCLEOTIDASES CD39 AND CD73 IN THE HUMAN PLACENTA

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Abstract

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Introduction

Pro- and anticoagulant mechanisms of the placenta play an important role during pregnancy. One of these mechanisms involves the hydrolysis of extracellular adenosine triphosphate (ATP) via adenosine di- and monophosphate (ADP, AMP) to adenosine by the ectonucleotidases CD39 and CD73. ATP can be released from cells into the extracellular space in response to metabolic disturbances and tissue insults. ATP and ADP are also released by platelets into their environment upon activation, which in turn can lead to inflammatory processes and cell damage at elevated concentrations. Dysregulations in this process can thus lead to the development of pregnancy pathologies, such as preeclampsia. In this study, we aimed to investigate the role of the ectonucleotidases CD39 and CD73 in the human placenta.

Methods

The expression of CD39 and CD73 was investigated in placental tissue from the first and third trimester of healthy and preeclamptic pregnancies as well as in trophoblast cell lines at protein and gene expression levels. Placental explant cultures were incubated in presence of either platelet releasate or isolated activated platelets from autologous donors for 24h. Total RNA and protein were subjected to qPCRs and Western blots, whereas FFPE placental tissue was analysed by immunohistochemistry.

Results

Analysis of placental tissue from first and third trimester revealed an increase of CD39 towards term on protein as well as on gene expression level, whereas the expression of CD73 decreases towards the end of pregnancy. Furthermore, CD39 expression was shown to be decreased in preeclamptic tissue compared to healthy controls.

Conclusion

Decreased placental expression of CD39 could result in reduced hydrolysis of extracellular ATP, which may contribute to a pro-inflammatory microenvironment in the intervillous space in preeclampsia.

EFFECT OF PRAVASTATIN ON PLACENTAL EXPRESSION OF EGFL7 IN PE AND IUGR : A NEW POTENTIAL THERAPEUTIC APPROACH

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Abstract

Maternal systemic manifestations of Preeclampsia (PE) are mainly due to the placental release of anti-angiogenic factors. Epidermal Growth Factor Like Domain 7 (Egfl7) is an angiogenic factor, highly expressed in proliferating endothelial cells during embryogenesis and in physiologic and pathologic angiogenesis discovered by our group simultaneously with other authors. Recently the trophoblast as a novel source of EGFL7 in the human placenta was also identified. In PE-complicated pregnancies, a significant EGFL7 downregulation in placental tissues is on the other hand accompanied by a significant increasein the maternal plasma. Moreover, circulating EGFL7 levels can discriminate between women with early- and late-onset PE (e-PE and l-PE) and isolated intrauterine growth restriction (IUGR).

Pravastatin (PRA) is a lipid-lowering drug with hydrophilic activity widely taken to reduce the risk of cardiovascular events. In the last years, the potential of PRA in the prevention and treatment of PE was highlighted. Aim of our pilot case-control study is to evaluate the ability of PRA to modulate EGFL7 expression in human chorionic villous explant culture from uncomplicated pregnancies and from women with PE and IUGR.

Methods: 19 women were enrolled for this study: 10 healthy controls, 4 e-PE, 3 l-PE and 2 IUGR. In all groups, placenta was collected after caesarean delivery with standard biopsy procedure. Explants of chorionic villi were prepared as described by Miller et al and chorionic villous explants were cultured for 24 hours with or without 10 μM PRA. Gene and protein expression of EGFL7 and other angiogenic factors was quantified by qRT-PCR and Western Blot analysis on RNA and protein extracts from chorionic villous cultures, respectively.

Results: PRA significantly increased EGFL7 gene expression in villous explant cultures obtained from healthy, l-PE and IUGR pregnancies ($p < 0.001$, $p = 0.006$, and $p = 0.014$, respectively), while its levels decreased in e-PE villi after PRA treatment ($p = 0.025$). This trend was confirmed at protein level, although in a not-significant way. Differently from the other groups, in samples obtained from l-PE patients all NOTCH target genes were significantly upregulated by PRA treatment. NOTCH1 transmembrane subunit (NOTCH1 NTM) protein levels were higher in villous cultures obtained from treated compared to untreated l- and e-PE patients; no changes were observed for healthy controls.

Conclusion: PRA is able to modulate the expression of EGFL7 in human placenta; the differences observed between groups and in particular between l-PE and e-PE underlines the well-known different nature of the two forms of PE and isolated IUGR; from a clinical point of view, the effect of PRA on villi from e-PE encourages the use of this molecule in prevention rather than as treatment when the disease is already established and the severe damaged placental villi may not be able anymore to correctly answer to PRA treatment.

BIOLOGICAL EFFECTS OF BENZO-[A]-PYRENE AND CERIUM NANOPARTICLES ON THE HUMAN PLACENTAL BARRIER

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Abstract

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Background: The human placenta is a multifunctional organ essential for fetal development and growth, whose function can be disrupted by xenobiotics in maternal blood. Benzo-[a]-pyrene (B[a]P) is a carcinogenic, mutagenic and reprotoxic pollutant as well as an endocrine disruptor that can be internalized in the human body by respiratory and/or oral exposure. Cerium dioxide nanoparticles (CeO_2 NPs) have been introduced into our environment, mainly for their catalytic properties. These nanoparticles currently share the same emission sources as B[a]P (e.g. cigarette smoke, diesel engine exhaust). CeO_2 NPs are also being studied for their antioxidant and pro-oxidant properties for therapeutic applications. The study of these nanoparticles must take into account their ability to coat themselves with surrounding molecules such as B[a]P from common emission sources. In order to understand their impact on human health, in 2010 the OECD included CeO_2 NPs in the priority list of nanomaterials requiring urgent assessment. The aim of our study is to determine the impact of these two atmospheric pollutants on the human placental barrier in concomitant exposure, in order to get closer to the environmental reality.

Methods: The chosen concentrations ratio used in this study was 10 µg/cm2 NPs CeO_2 for 1 µM B[a]P, which is the quantity of B[a]P necessary to cover the entire surface of the NPs when they are encountered together. Sections of chorionic villi from human placentas at term of pregnancy exposed in vitro to the pollutants were observed by confocal and Raman microscopy. Villous cytotrophoblasts (VCT) isolated from human placentas at term of pregnancy were cultured and exposed *in vitro* to these pollutants. Cytotoxicity was assessed using metabolic activity of mitochondrial dehydrogenase (WST-1 test) and extracellular lactate dehydrogenase assay. Placental endocrine activity was assessed by ELISA. The signaling pathways impacted by exposure to these pollutants have been studied by Western Blot and RT-qPCR. The production of reactive oxygen species (ROS) was assessed by the DCF probe.

Results: We demonstrated that CeO_2 NPs coated with B[a]P can be internalized within the chorionic villi. The two pollutants used at realistic exposure doses do not induce toxicity nor disturbance of hCG production on VCT *in vitro*. However, B[a]P alone or in co-exposure with CeO_2 NPs activate the metabolic pathway of the aryl hydrocarbon receptor (AhR), causing DNA damage. B[a]P also stabilizes the stress transcription factor p53 and its transcriptional target p21. At the studied doses and for short exposure times, CeO_2 NPs alone lead to an increase in ROS production, unlike B[a]P.

Conclusion: Although these two pollutants do not cause major toxicity on term human trophoblasts after *in vitro* exposures, cellular stress markers are induced.