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Abstract Outline - IFPA 2025

Elsevier Trophoblast Research Award Lecture ELSTR

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ELSTR.
ACTIVIN-FOLLISTATIN-LIKE 3 DIALOG AT THE UTERINE-PLACENTAL INTERFACE IMPACTS DEEP HEMOCHORIAL PLACENTATION

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Objectives: During pregnancy, maternal and extraembryonic cells interact at the uterine-placental interface (**UPI**) to enable necessary changes for fetal growth and development. Trophoblast stem (**TS**) cells can differentiate into invasive trophoblast cells, which contribute to uterine blood vessel remodeling. Within the UPI, activin affects the proliferation and differentiation of trophoblast cells. FSTL3 acts to antagonize the actions of activin by limiting access to their receptors. The goal of this study is to examine the roles of activin and FSTL3 in regulating trophoblast cell development and the process of placentation.
Methods: We investigated the actions of activin with or without FSTL3 on human TS cell differentiation into extravillous trophoblast (**EVT**) cells. FSTL3 was depleted in human TS cells using shRNAs. In vivo roles for FSTL3 on placentation were investigated in a genome edited rat model.
Results: FSTL3 was expressed in EVT cell columns of first-trimester human placentas. In vitro, human TS cells showed a robust induction of FSTL3 expression following differentiation into EVT cells. Activin inhibited TS cell differentiation, which was attenuated by FSTL3. In the rat, FSTL3 is prominently expressed in invasive trophoblast cells of the UPI. Disruption of FSTL3 resulted in large cyst-like structures in the junctional zone and was compatible with the initial phases of invasive trophoblast cell differentiation within the junctional zone, but not with intrauterine trophoblast cell invasion. Trophoblast cells failed to enter the UPI or exhibited asymmetric distributions within the UPI.
Conclusion: Activin signaling prevents trophoblast cell differentiation. FSTL3 can facilitate trophoblast cell differentiation by suppressing the actions of activin, permitting acquisition of invasive properties essential for establishment of the UPI and deep hemochorial placentation.
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NIH.
PLASMA EXRNA PROFILING FOR PREGNANCY MONITORING AND NON-INVASIVE DIAGNOSTICS OF PREECLAMPSIA

Klaas E.A. Max, Pavel Morozov, Stephanie Morgan, Hemant Suryawanshi, Vivian Wei, Zev Williams, Thomas Tuschl.

Liquid biopsy approaches using extracellular RNAs (exRNAs) are a promising avenue for non-invasive diagnostics, offering insights into gene expression, tissue lineage, and disease states. However, exRNAs in plasma are present in low quantities, are highly fragmented, and are dominated by abundant non-coding RNAs including ribosomal RNAs (rRNAs). We developed an RNA sequencing workflow that employs custom-oligodeoxynucleotide-based depletion of rRNAs, 7SL RNAs, and hemoglobin A and B mRNAs followed by cDNA library preparation including SPRI bead size selection to enrich for longer nucleic acid fragments. We applied this workflow to plasma samples from 130 individuals (199 samples total), including healthy pregnancies (n=159 samples) and cases with complications such as fetal growth restriction (n=10) and preeclampsia (n=18). Using differential gene expression analysis of ex-mRNAs, we identified over 100 genes with significant changes during pregnancy, including those encoding placenta-specific peptide hormones (CSH1 and 2, PSG1 to 11, CGA and CGB, LEP, and CRH), and key metabolic enzymes linked to fetal growth and development (PAPPA1 and 2, HSD17B1). Our RNA-seq approach was deep enough to detect the male sex of an embryo capturing Y-chromosome specific mRNAs (DDX3Y, RPS4Y). The gene set was further reduced to transcripts only detected in pregnant women. The inherent changes in this subset reflect gestational development and age and adverse pregnancy outcomes. These selective transcripts and their normalized abundance values may serve as biomarker for preeclampsia. Gene network analysis of ex-mRNA exploiting the variability of ex-mRNAs across the entire sample population uncovered contributions from diverse tissue and immune cell sources, notably the liver and myeloid lineages, reflecting individual-specific variations in metabolic and immune activity. In addition, we detected pegivirus infections and COVID-19 mRNA vaccination. Our study establishes an advanced exRNA sequencing workflow that enables high-resolution molecular profiling of plasma and offers new opportunities for biomarker discovery in pregnancy monitoring, infectious diseases, and other clinical applications. We are currently optimizing multiplexed digital PCR assays for faster detection of our biomarker candidates directly from DNase-treated plasma exRNAs.

PL1.
DEVELOPMENT OF AN ARTIFICIAL PLACENTA SYSTEM: A CLINICAL AND A TECHNICAL PERSPECTIVE

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Preterm birth remains the leading cause of mortality among neonates. Despite improvements in neonatal intensive care over the years, current treatments for lung and kidney failure are highly invasive, associated with lifelong disability, and limit family integration. Research on artificial womb and artificial placenta technologies promise to offer alternatives by providing more tailored and less invasive neonatal care. Although these technologies share some similarities, artificial womb and artificial placenta technologies differ significantly in terms of treatment initiation, treatment environment, and the potential to support family-centered care. Moreover, even though acute kidney injury is common in neonatal extracorporeal membrane oxygenation (ECMO) patients, most artificial placenta and artificial womb devices currently under development lack renal support functionality. Additionally, most artificial womb and artificial placenta studies focus on the technical feasibility of these technologies based on in-vivo animal tests. However, translation toward envisioned use of these devices in preterm neonates remains mostly underexposed. A comprehensive stakeholder analysis and a close cooperation of engineers, neonatologists, caregivers, parent representatives, basic scientists, etc. is crucial for the development of socially acceptable artificial placenta and artificial womb systems. We will provide an overview of conventional neonatal lung and kidney treatments, explain the differences between artificial womb and placenta technologies, and address the technological and ethical challenges in advancing these technologies toward potential clinical implementation.

PL2.
GENETIC HISTORY OF EUROPE: ADAPTATION AND MIGRATION IN PREHISTORY

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Ancient DNA can reveal prehistoric events that are difficult to discern through the study of archaeological remains and modern genetic variation alone. Over the past decade, the newly emerging field of archaeogenetics analyzed more than 5,000 ancient human genomes spanning the last 10,000 years of western Eurasian prehistory. We have uncovered at least two major genetic turnover events at the beginning and at the end of the Neolithic period that dramatically changed the genetic landscape of Europe. These changes were likely caused by at least two major migration events, first by early farmers who spread from Anatolia beginning about 8,000 years ago, bringing agriculture and domesticated animals to Europe. Following their arrival, early farmers genetically mixed with indigenous Europeans over the next 3,000 years. At the end of the Neolithic period, about 5,000 years ago, we can find the first genetic evidence of another major migration event of groups from the eastern European Pontic steppe, north of the Black Sea, into the European heartland. The newcomers were herders, practiced pastoralism, and were highly mobile. Besides

introducing new cultural practices, they may have been responsible for the spread of Indo-European languages.
Thus, we find that all modern European populations today are a genetic mixture of these steppe herders, early Anatolian farmers, and indigenous European hunter-gatherers in varying proportions. Over the past 10,000 years, we observe major changes in human phenotypes such as eye and skin color, and the ability to digest lactose, which can be attributed to genetic mixing and local biological adaptation.

PL4.1.
TRANSPLACENTAL TRANSFER OF ANTIBODIES IS MODULATED BY IGG1 ALLOTYPES

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Objectives: The transplacental transfer of maternal immunoglobulin (Ig) G to the fetus protects the neonate from infections during the initial months of life. Using samples from healthy mothers and their neonates across subsequent pregnancies, we identified significant variability in the transfer rates of pathogen-specific IgG antibodies among different mothers, but not within multiple pregnancies of the same mother. To elucidate the underlying causes of this interpersonal variability, we aimed to identify specific single nucleotide polymorphisms (SNPs) within the gene encoding the constant region of the heavy chain of IgG1 (IGHG1), which give rise to distinct maternal IgG1 allotypes.
Methods: We performed our analysis in a subset of mothers and their neonates enrolled in the prospective, population-based pregnancy study 'PRINCE' (Prenatal Identification of Children's Health). We quantified the transplacental antibody transfer rate (TPTR%) for each vaccine-specific antibody and computed the z-scores corresponding to the TPTR for each mother-neonate pair. Exome sequencing of the IGHG1 gene utilizing DNA isolated from the participants was performed to detect
Results: Through both ex vivo and in vitro evaluations of the mechanisms governing transplacental IgG transfer, we established that distinct IgG1 allotypes significantly determine the IgG transfer rate across the placenta. Our findings further highlight a higher risk for respiratory infections among infants born to mothers possessing IgG allotypes associated with diminished IgG transfer rates.
Conclusion: Our study underscores the importance of considering maternal IgG allotypes with regard to vaccination strategies during reproductive years and in the context of prenatal vaccination programs, with the aim of optimizing neonatal protection against infectious diseases.

PL4.3.
HOW WELL DO WE UNDERSTAND THE PLACENTAL BARRIER?

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Objectives: The placental barrier is a critical interface that enables the selective transfer of nutrients and wastes while protecting the fetus from harmful substances. Recent advances in ultrastructural 3D imaging and single-nucleus sequencing provide the opportunity to address key questions about the placental barrier's structure and function. Here, I will explore what we know and what remains uncertain about the placental barrier, primarily focusing on the human placenta.
The syncytiotrophoblast, a continuous multinucleated layer in direct contact with maternal blood, is the most studied component of the human placental barrier. It expresses a wide array of transport proteins with polarised expression, which has been the focus of much research on placental transfer. Yet recent discoveries such as trans-syncytial nanopores highlight data showing the passive transfer of hydrophilic solutes across the placenta.
In contrast to the syncytiotrophoblast, the fetal capillary endothelium has received much less attention. Key questions remain about the balance of paracellular diffusion and directional transcellular transport in mediated transfer across the endothelium.
The extent to which cytotrophoblast, fibroblasts, macrophages or pericytes affect placental transfer through physical or metabolic means remains unclear.
Despite data showing the placenta's apparent passive permeability, it can maintain higher fetal concentrations of certain substances than those in maternal blood. Understanding how this is achieved is essential for clarifying the true nature of placental barrier function.
It is important to note that the structure of the placental barrier in humans is very different from that of most other mammals. The fact that the placental barrier is subject to such selective pressures sufficient to drive this diversity demonstrates its importance for reproductive success in humans and other mammals.
In summary, the placental barrier is an essential determinant of pregnancy biology. New technologies offer insight to resolve longstanding questions about structure, function, and evolution.

NI1.1.
LEVERAGING A PLACENTAL MICROPHYSIOLOGICAL SYSTEM FOR PLACENTAL TOXICOLOGY STUDIES

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Objectives: Traditional choriocarcinoma-derived placental cell models offer limited physiological relevance, motivating the need for improved trophoblast lines and microphysiological systems (MPS). We immortalized primary trophoblast cells, developed a placental MPS, and investigated the impact of endocrine-disrupting compounds (EDCs) on placental and fetal brain functions.
Methods: Primary human placental trophoblasts were isolated, immortalized ($hPTC^{CTB}$), and differentiated into syncytiotrophoblasts ($hPTC^{STB}$). A placenta MPS (2TPLA-OOC) incorporating decidual, endothelial, trophoblast, and stromal cells was fabricated via photo- and soft-lithography. Materno-fetal blood flow was replicated via pressure-gradient culture media loading. We then exposed the chip to classic EDCs (BPA, BPS, PBDE-47, and -99) at 150 ng/mL, assessing viability, antioxidant capacity, apoptosis/necrosis, cytokine/hormone profiles, immune cell migration, and glucose transport. To evaluate PBDE-mediated neural effects, a novel fetal blood-brain barrier organ-on-chip (FB-OOC) with endothelial cells, pericytes, and neuronal tricultures was employed.
Results: Immortalized trophoblasts ($hPTC^{CTB}$) maintained transcriptomic and functional similarity to primary trophoblasts, surpassing BeWo cells in physiological relevance. In 2TPLA-OOC, EDC exposure elicited cell type-specific responses yet did not disrupt overall glucose transport. Meanwhile, the FB-OOC retained barrier integrity and viability under perfusion; PBDEs, including 2T-PLA-OOC-derived metabolites, induced elevated glutamate levels in neuronal chambers without triggering significant neuroinflammation. However, direct 2D culture of neuronal cells showed pronounced inflammatory responses.
Conclusion: We demonstrated the utility of combining physiologically relevant trophoblast lines with microphysiological systems that maintain intercellular interactions, unlike 2D systems, to investigate EDC-driven reproductive and neurotoxic effects. The 2T-PLA-OOC exhibited adaptability to EDC exposure and highlighted how placental exposure may modulate fetal neural outcomes, supporting its application in future reproductive toxicology studies.

NI1.2.
SARS-COV-2 INFECTION DURING PREGNANCY INDUCES FERROPTOSIS AND LONG-TERM CHANGES IN PLACENTAL IRON STORAGE

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Objectives: SARS-CoV-2 infection is associated with adverse pregnancy outcomes, particularly preeclampsia, but underlying mechanisms remain unclear. Ferroptosis, a form of iron-dependent cell death characterized by

reduced redox surveillance and increased lipid peroxidation, has been implicated in pregnancy complications including preeclampsia. Studies suggest SARS-CoV-2 changes cellular iron distribution and induces ferroptosis, but whether this occurs in the placenta is unclear. Thus, our objective was to determine the impact of SARS-CoV-2 on placental iron dynamics and ferroptosis.

Methods: Term villous placental tissue and umbilical cord blood were obtained from individuals who contracted SARS-CoV-2 during pregnancy and compared to controls (n=10/group). Redox surveillance (GPX4), iron transport, and lipid peroxidation markers were assessed in placental tissue via immunofluorescence and western blotting. Iron localization was examined with Prussian Blue. Ferritin levels in umbilical cord blood were quantified using Luminex. To examine active infection, Jeg-3 cells were infected with SARS-CoV-2 Delta variant for 48 hours, after which iron transport proteins and ferroptosis markers were analyzed using western blotting.

Results: SARS-CoV-2-exposed placentas displayed mislocalization of the iron efflux transporter, ferroportin (FPN), and storage protein, ferritin, along with iron accumulation. Ferritin levels in cord blood were decreased in these pregnancies. Expression of GPX4 was markedly reduced in SARS-CoV-2-exposed placentas, though lipid peroxidation markers were not significantly altered. Infection of Jeg-3 cells with live SARS-CoV-2 Delta variant significantly induced lipid peroxidation and reduced expression of FPN and GPX4, supporting the activation of ferroptosis.

Conclusion: SARS-CoV-2 inhibits iron efflux and induces trophoblast ferroptosis during active infection. Long-term changes in placental iron transport and storage persist, likely compromising fetal access to iron. Combined with evidence linking ferroptosis to pregnancy complications and the importance of iron for fetal development, this mechanism provides a new avenue through which SARS-CoV-2 may impact pregnancy outcomes. Further investigation into this mechanism could identify diagnostic or therapeutic targets.

NI1.3.
TWO CIRCULATIONS ONE GOAL: IMPAIRED OXYGEN TRANSFER IN PLACENTAE WITH COMPROMISED MATERNAL VENOUS RETURN IN PREGNANCIES WITH HIGH RISK FOR STILLBIRTH.

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Objectives: Stillbirth, the loss of a fetus after 24 weeks of gestation, has been linked to risk factors, such as fetal growth restriction (FGR) and high BMI. The aim of this study is to elucidate the role of maternal venous return from maternal lacunae (ML) and its significance for oxygen transfer from maternal blood to the fetal circulation.

Methods: Employing the well-established ex-utero human placental dual perfusion system, we examined in-flow real-time oxygen transfer from ML to the umbilical vein in normal pregnancies (low-risk for stillbirth, LRS) and those complicated by high BMI or FGR (high-risk group, HRS), (n=10). We characterized the presence and role of maternal septal veins through EphB4+ immunohistochemistry and Evan's blue (EB; 960 g/mol) tracer exit.

Results: Significantly longer oxygen transfer time was found from the ML to the umbilical vein in HRS pregnancies (8.2 ± 3.3 min) compared to LRS cases (1.8 ± 1.3 min); $p<0.05$. Venous exit pathways were identified to septal veins in all LRS placentae, bar one. HRS placentae did not have septal veins ($p<0.05$). EB confirmed the veins as venous exit pathways. Principal component regression a significant positive correlation (R^2 = 0.8, p=0.01) between birthweight centiles and the presence of septal veins, and a negative correlation with oxygen transfer time (R^2 = 0.8, p=0.009). BMI was positively correlated with oxygen transfer time (R^2=0.8, p=0.04).

Conclusion: The significantly longer oxygen transfer times in two distinct complicated pregnancy groups and the absence of septal veins therein, emphasises the important role of maternal venous return in the dynamics of materno-fetal oxygen transfer.

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NI1.4.
TARGETED LIPID NANOPARTICLE DELIVERY OF SHORT INTERFERING RNA TO TREAT PREECLAMPSIA

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Objectives: Preeclampsia, is a severe pregnancy complication resulting in the deaths of >76,000 mothers and >500,000 neonates annually. Central to its pathogenesis is placental dysfunction, and excess circulating maternal antiangiogenic factors. The only cure is delivery of the placenta, which can have profound consequences in cases of early-onset preeclampsia. Lipid nanoparticles (LNPs) offer a promising option to deliver gene-silencing nucleic acid therapies to the placenta to halt pathogenesis, mitigating risk due to their biocompatibility and reduced off-target effects. We aim to target LNPs to the placenta, delivering short interfering RNA (siRNA) to silence pathogenic drivers of preeclampsia.

Methods: Cyanine 5-labeled non-functional siRNA (siRNA-Cy5) encapsulated within LNPs was decorated with epidermal growth factor receptor (EGFR) antibodies (abundantly expressed on the placental surface) via thiol-maleimide chemistry. CytoFLEX Nano Flow Cytometry was used to validate antibody-LNP conjugation. Targeting efficiency was assessed by incubating human cytotrophoblasts with targeted and untargeted LNPs (500ng siRNA/well) for 1 hour, followed by flow cytometry and confocal imaging. In vivo; pregnant mice (D14.5) were injected with PBS (control), untargeted, or targeted LNPs (0.3mg/kg) via tail vein. After 24 hours (D15.5), maternal organs, fetuses and placenta were imaged using In Vivo Imaging System to detect siRNA-Cy5 uptake.

Results: CytoFLEX revealed highly efficient antibody conjugation, >97% antibody attachment, with no detectable signal in controls. Flow cytometry identified significantly higher fluorescence in cytotrophoblasts treated with targeted LNPs compared to untargeted and control groups ($p<0.0001$), further validated by confocal microscopy. In vivo experimentation demonstrated significantly higher Cy5 radiant efficiency in mouse placenta treated with targeted LNP compared to untargeted (P=0.0008).

Conclusion: These findings establish the feasibility of targeted siRNA delivery to the placenta, paving the way for therapeutic application of LNPs in pregnancy. This demonstrates strong potential to revolutionise placental drug delivery, representing a significant advancement for maternal-fetal medicine.

NI1.5.
PLACENTAL SMALL EXTRACELLULAR VESICLES ALTER GENE EXPRESSION AND CELLULAR FUNCTION OF PERICYTES IN NORMOTENSIVE PREGNANCY AND PREECLAMPSIA

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Objectives: Placental small extracellular vesicles (psEVs) released by syncytiotrophoblasts into maternal circulation contribute to preeclampsia (PE) pathogenesis by communicating placental stress to distant systemic organs and blood vessels. In microcirculation, pericytes are known to interact with endothelial cells to maintain vascular integrity, blood flow, and angiogenesis, yet their involvement in preeclampsia remains poorly understood. Therefore, we investigated the effects of psEVs from normotensive and PE pregnancy placentae on pericytes, comparing their contractility, gene expression, and angiogenic proteome profile.

Methods: psEVs were isolated by dual-lobe perfusion of six normotensive pregnancy and six early-onset preeclampsia placentae. psEVs were characterised using western blot, nanoparticle tracking analysis and electron microscopy. Using flow cytometry and confocal imaging, internalization of psEVs by human brain vascular pericytes (HBVP) was then investigated. Functional effects on pericyte contractility were assessed using the iCelligence electrical impedance assay, while gene and protein expressions were compared using RT-qPCR and angiogenesis proteome array, respectively. mRNAs from the psEV-treated pericytes were then sent for sequencing.
Results: Pericytes internalised psEVs in a time- and dose-dependent manner, with PE psEVs showing higher rate of uptake. psEVs from both groups induced more sustained contractions compared to controls, particularly at 4–12 hours ($p<0.0001$). Furthermore, PE psEVs significantly lowered the expression of pericyte marker PDGFRβ and angiogenic factors (VEGF-A, VEGF-B, VEGF-C, Angiopoietin-1, and TGF-β), and downregulated 13 proteins in the angiogenesis proteome profiler, compared to normotensive psEVs. Additionally, RNA-seq revealed 358 upregulated and 411 downregulated genes in PE psEV-treated pericytes, and highlighted ribosome and oxidative phosphorylation amongst the most highly enriched pathways. Further investigations into these affected pathways are underway.
Conclusion: Overall, these findings suggest that psEVs induce alterations in pericyte gene expression and functions, potentially disrupting their communication with endothelial cells. This could potentially provide evidence of the involvement of pericytes in vascular dysfunction associated with preeclampsia pathogenesis, mediated by psEVs.

NI1.6.
DISRUPTED LYMPHATIC MIMICRY IMPAIRS SPIRAL ARTERY REMODELING AND RESULTS IN REDUCED UTERINE NK CELL INFILTRATION IN PREECLAMPTIC MICE

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Objectives: Preeclampsia (PE) is a hypertensive pregnancy disorder characterized by placental dysfunction and often fetal growth restriction (FGR). The anti-angiogenic soluble fms-like tyrosine kinase 1 (sFLT1) is elevated in serum and placentas of PE patients and is associated with impaired spiral artery (SpA) remodeling and dysregulated lymphatic mimicry.
Methods: We used a human sFLT1 (hsFLT1)-induced PE/FGR mouse model to investigate the effect of systemic hsFLT1 overexpression on early (12.5 dpc) and mid-gestational (14.5 dpc) SpA remodeling. Expression levels of lymphangiogenic, hypoxic, and inflammatory markers were evaluated on mRNA and protein level in the mesometrial triangle (MT) tissue of the sFLT1-expressing PE compared to the control group. Placentas were stained with Masson-Goldner-Trichrome (MGT) to quantify the vessel wall thickness of SpAs. In addition, SpAs were characterized by transmission electron microscopy, and immune cell infiltration was studied by DBA-lectin staining to detect uterine natural killer cells (uNKs).
Results: Two days after induction of systemic hsFLT1 overexpression, hsFLT1 protein levels were significantly increased in maternal serum. Further, fetal weight and placental efficiency were significantly reduced in the PE group. Impaired SpA remodeling was associated with decreased expression of lymphangiogenic markers PDPN, PROX1, NRP1, NRP2, and LYVE1 in the MT of the PE dams. Sex-specific responses were observed in the expression of hypoxia-related markers (HIF1α, HIF2α, HO1) and in the proinflammatory cytokine TNFα, whereas CCL21 expression was reduced in both sexes. Electron microscopy of SpAs revealed uNK accumulation around SpAs at 12.5 dpc. Previous data showed significantly reduced uNK cell infiltration in the PE group at 14.5 dpc.
Conclusion: Here we show the early effects of the antiangiogenic molecule sFLT1 on the SpA remodeling process, decoding the important and dysregulating role of sFLT1 during lymphatic mimicry and immune cell infiltration in preeclampsia. Further analysis of the detailed mechanism is in progress.

NI2.1.
DYNAMIC MORPHOGENESIS OF TROPHOBLASTS AND VILLOUS CORE CELLS IN THE DEVELOPING HUMAN PLACENTA

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Objectives: Coordinated interactions between trophoblasts (TB) and villous core cells (VCC) are critical for the morphological and functional maturation of the early human placenta. However, little is known about their characteristics and networks before and after the onset of maternal blood flow into the intervillous space. Here, we delineated the structural and molecular architectures of 6/7th (early) and 10/11th (late) week placentae and established 3D in vitro co-culture models to elucidate cell interactions regulating VCCs fate.
Methods: We performed single-cell RNA sequencing (scRNA-seq) on placental cells ($n=9$), and single-cell proteomics on selected placental cell types to construct comprehensive cellular profiles of first-trimester placentae. We used multi-color flow cytometry, whole tissue immunofluorescence (IF), and spinning disk confocal microscopy to validate our findings. Furthermore, we developed 3D Matrigel-based models of VCCs to investigate placental vascular network formation in vitro.
Results: scRNA-seq and IF analysis revealed gestational age-dependent changes across placental cell types. In late weeks, villous cytotrophoblasts exhibited reduced proliferation, loss of polarity markers, and disrupted epithelial integrity. Early week syncytiotrophoblast and extravillous trophoblasts already expressed differentiation markers indicating early lineage commitment. In contrast, VCCs showed pronounced temporal differences: early villous cores (VC) were loosely organized, with immature endothelial networks, sparse perivascular cells (PVC) and fibroblasts (FIB), while late VCs displayed organized vascular structures with PVCs and endothelial cells (EC) embedded in dense FIB and Hofbauer cell networks. In 3D co-cultures, we supported ECs, PVCs, and FIBs under conditions propagating either a network of immature ECs or larger EC structures with PVC and FIB, possibly recapitulating vascular network formation in vitro.
Conclusion: In summary, trophoblasts matured by the 6th week of gestation, while villous core cells showed delayed development, revealing distinct timelines. Our 3D models offer a novel, valuable system to study early placental vascular development and cell interactions in first-trimester placentation.

NI2.2.
TROPHOBLAST RECEPTOR LANDSCAPE REVEALS A PARACRINE ROLE FOR SYNCYTIOTROPHOBLAST-DERIVED ACTIVIN A IN EARLY PLACENTAL DEVELOPMENT

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Objectives: Activin A, a TGF-β superfamily cytokine, is elevated in maternal blood and the senescent placental secretome in preeclampsia. We found increased Activin A levels in first-trimester serum from women who later developed preeclampsia, suggesting a role in early disease pathogenesis. Although Activin A is known to promote extravillous trophoblast (EVT) invasion, its placental source and its role in trophoblast lineage specification remain unclear.
This study aims to identify the cellular source of Activin A in early pregnancy and define how receptor expression patterns across trophoblast subtypes shape its signaling and potential role in preeclampsia pathogenesis.
Methods: We analyzed single-nucleus RNA-sequencing (snRNA-seq) data from 13 first-trimester placentas (5–11 weeks' gestation) to map the expression of TGF-β family type I and II receptors across trophoblast subtypes. Pseudotime analysis using STREAM2 was used to assess dynamic expression of receptors and downstream targets. In parallel, human trophoblast stem cells (hTSCs) were differentiated toward the syncytiotrophoblast (STB) lineage to evaluate Activin A secretion (ELISA, luciferase reporter assays) and receptor expression.
Results: ELISA and luciferase assays showed increased Activin A secretion during STB differentiation. snRNA-seq data revealed that Activin A receptors (ALK4 and ACVR2A/B) are strongly expressed in EVTs and in cytotrophoblasts (CTBs), but not in STBs. STBs instead co-express ALK2, ACVR2A/B, and BMPR2, components of a non-signaling complex that can bind Activin A but does not mediate downstream signaling. BMP7, a known ALK2 ligand, was enriched in juvenile STBs and CTBs. During in vitro STB differentiation, ALK4 expression decreased while ALK2 and ACVR2B remained stable.
Conclusion: Our findings suggest a model of paracrine Activin A signaling from STBs to receptor-expressing CTBs and EVTs. Activin A competes with BMP7 for type II receptors, thereby modulating BMP-SMAD1/5 signaling. This regulatory mechanism may fine-tune trophoblast differentiation in early pregnancy and become dysregulated in preeclampsia.

NI2.3.
LOSS OF HAPLN3 IMPAIRS TROPHOBLAST DIFFERENTIATION AND INVASION

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Objectives: Human placentation relies on tightly regulated trophoblast differentiation and invasion, with extravillous trophoblasts (EVTs) playing a central role in remodeling the maternal uterine vasculature. These processes depend critically on extracellular matrix (ECM) remodeling. We hypothesize that HAPLN3, a hyaluronan and proteoglycan link protein, is essential for maintaining ECM integrity and facilitating proper trophoblast lineage specification.
Methods: HAPLN3 expression was analyzed in mouse and human trophoblast stem cells (mTSCs and hTSCs) during differentiation. CRISPR/Cas9 technology was used to generate Hapln3 knockout (KO) mTSCs and HAPLN3 knockdown (KD) hTSCs. Differentiation was evaluated in 2D and 3D culture systems (trophospheres and organoids) using qPCR, RNA sequencing, immunofluorescence, and Western blotting. Changes in ECM composition and associated signaling pathways were also assessed.
Results: Hapln3 KO mTSCs showed impaired differentiation, with reduced Tpbpa expression and abnormal trophosphere architecture. In hTSCs, HAPLN3 knockdown led to decreased HLA-G expression and reduced fibronectin levels in EVTs, suggesting defective ECM remodeling and impaired invasive capacity.
Conclusion: HAPLN3 plays a conserved and critical role in EVT differentiation by regulating ECM components and downstream signaling. These findings underscore its importance in placental development and suggest potential implications in pregnancy complications characterized by impaired trophoblast invasion.

NI2.4.
THE MYOMIRS, MIR-1-3P AND MIR-133A-3P REGULATE THE DIFFERENTIATION POTENTIAL OF PLACENTAL MESENCHYMAL STROMAL CELLS TOWARDS A VASCULAR SMOOTH MUSCLE CELL LINEAGE

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Objectives: Gestational diabetes (GDM) placentae display abnormal vascularisation which may contribute to short and long-term complications associated with GDM. However, the mechanisms responsible are unclear. We have previously shown that vascular regulatory, muscle-specific microRNAs (myomiRs), including miR-1-3p and miR-133a-3p, are altered in both maternal plasma extracellular vesicles (EVs) and placental tissue in GDM. Here we utilised primary placental mesenchymal stromal cells (PMSCs) to assess whether myomiRs influence placental vascularisation in GDM pregnancies.
Methods: PMSCs were isolated from uncomplicated term placentae (n=8) and phenotype was confirmed by flow cytometry, immunocytochemistry (ICC) and trilineage (adipogenic, chondrogenic and osteogenic) differentiation. PMSCs were cultured in endothelial growth medium (EGM-2) containing VEGF-A (50 ng/mL) for up to 25 days or in DMEM containing TGF-β1 (5 ng/mL) and ascorbic acid (300 μM) in collagen coated plates for up to 14 days to differentiate cells towards an endothelial cell (EC) or vascular smooth muscle cell (VSMC) lineage, respectively. PMSCs undergoing VSMC differentiation were transfected on day 7 with control, miR-1-3p or miR-133a-3p anti-miRs to mimic levels in the GDM placenta. RT-qPCR and ICC were used to assess levels of PMSC, EC and VSMC markers.
Results: Whilst there was limited differentiation of PMSCs towards an EC lineage even at 25 days, increased expression of VSMC markers MYH11 and ACTA2/αSMA ($p<0.05$) were observed by 14 days, demonstrating that PMSCs were capable of VSMC lineage differentiation. VSMC differentiation was accompanied by increased levels of miR-1-3p and miR-133a-3p. Inhibition of miR-1-3p and miR-133a-3p, to mimic levels in the GDM placenta, resulted in reduced expression of the VSMC markers MYH11 and CALD1 ($p<0.05$).
Conclusion: We demonstrate that PMSCs can be used to model placental VSMC differentiation, which is regulated by miR-1-3p and miR-133a-3p. This suggests that trafficking of myomiRs to the placenta via EVs may contribute to altered placental vascularisation in GDM.

NI2.5.
CROSSTALK BETWEEN EXTRAVILLOUS TROPHOBLASTS AND DECIDUAL GLANDS IN EARLY HUMAN PREGNANCY

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Objectives: During early pregnancy extravillous cytotrophoblasts (EVT) invade the maternal uterine tissue thereby interacting with various maternal cell types, including immune cells and decidual glandular epithelial cells (DG). It is hypothesized that the interaction between EVTs and DGs stimulates glandular secretions which is essential for early pregnancy support. However, the cellular and molecular mechanisms underlying EVT-DG interaction remain poorly understood. This study aims

to stepwise investigate this interaction by creating a single cell atlas and establishing a 3D co-culture system between EVTs and DG.
Methods: Human first trimester placental and decidual tissues (decidua basalis (dec-bas) and parietalis (dec-par); n=4) were collected for single-cell RNA sequencing to create a single cell atlas. To study EVT-DG interaction, glandular epithelial organoids (DG-O) were generated from dec-par and co-cultured with placental EVTs (pEVT) under various conditions. Morphological and molecular changes in co-cultures were assessed using descriptive analyses, including immunofluorescence, Western blot, and RT-qPCR. Additionally, donor-matched dec-par and dec-bas tissues were analyzed histologically to evaluate DG morphology.
Results: Histological analysis revealed structural differences in DGs showing cylindrical, intact glands in the non-invaded dec-par and disrupted gland morphology in the EVT-invaded dec-bas, suggesting EVT-induced or -supported erosion which may facilitate glandular secretion. In co-cultures, EVTs attached to or integrated into the DG-O epithelium, mimicking the vivo interaction. Notably, DG-O developed convoluted morphologies and showed reduced expression of the stemness marker SOX9 in the presence of EVTs, indicating EVT-driven glandular differentiation.
Conclusion: Our findings highlight EVT-dependent differences in DG structure and demonstrate a direct interaction between EVTs and DG. The co-culture model suggests an EVT-specific remodeling and differentiation of DG-O. Our results offer new insights into EVT-mediated modulation of DGs and emphasize the functional significance of EVT-DG interaction in early gestation.
This project is supported by the Austrian Science Fund (FWF) under the project number P36159-B.

NI2.6.
DECODING IRON–OXYGEN REGULATION OF EXTRAVILLOUS TROPHOBLAST MIGRATION AND INVASION THROUGH TROPHOBLAST ORGANOIDS

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Objectives: Successful pregnancy requires the precise migration and invasion of extravillous trophoblasts (EVTs) into maternal spiral arteries—a process partially orchestrated by oxygen during placentation. Iron, essential for fetal and placental development, modulates oxygen-sensing pathways. **To date, the integrated roles of oxygen and iron in regulating EVT migration/invasion during placentation remain poorly defined.** Iron homeostasis relies on glutathione peroxidase 4 (GPx4), a key antioxidant enzyme that prevents iron-induced lipid peroxidation and consequent cell damage. **We hypothesize that GPx4 governs EVT migration/invasion in an iron/oxygen-dependent manner during early placentation.**
Methods: 5–8-week (~15–20mmHg O_2, physiological hypoxia) and 10–13-week (~60mmHg O_2, normoxia) human placentae (n=12/group) were collected with informed consent at Sinai Health System. In-vitro, trophoblast organoids derived from 5–7-week placentae (n=3) were differentiated into EVTs under varying oxygen (normoxia vs. hypoxia) in the presence/absence of iron (3,10μM). GPx4 and EVT markers (HLA-G, MMPs, integrins) were assessed by Western blotting and immunofluorescence. GPx activity, iron and lipid peroxidation contents were measured by colorimetric assays. EVT migration and invasion were evaluated using 3D invasion assays.
Results: Mirroring the rise in placental oxygen, iron assays revealed augmented iron levels between 5–8 and 10–13 weeks($p<0.001$). This coincided with elevated GPx4 protein/activity($p<0.05$), and its progressive enrichment along the proximal-to-distal axis of EVT columns as confirmed by immunofluorescence. In organoids, differentiation under normoxia upregulated GPx4 in HLA-G^+ EVTs, which was markedly suppressed under hypoxia. Under normoxia, low-dose iron (3μM, mimicking physiological serum levels) enhanced GPx4 and EVT migration/invasion, while excess iron (10μM) attenuated these responses and induced lipid peroxidation. Under hypoxia, iron further diminished GPx4 and EVT markers, impaired migration/invasion, and exacerbated lipid peroxidation.
Conclusion: We uncover a critical iron–oxygen–GPx4 axis governing EVT migration and invasion, whose disruption may promote placental insufficiency. (Supported by CIHR).
the prevention of miscarriage and PTB.

SYM1.1.
UNDERSTANDING THE DISEASE SIGNATURE OF THE PLACENTA WITH SINGLE-NUCLEI RESOLUTION

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Objectives: Maternal obesity involves chronic inflammation, hyperinsulinemia, and disrupted lipid metabolism, which alter the intrauterine environment. These changes raise the risk of pregnancy complications, including frequent delivery of large-for-gestational-age (LGA) infants—a key risk factor for birth trauma, childhood obesity, and long-term cardiometabolic and neurological disorders. Understanding placental responses at a single-cell level, rather than whole-tissue or cell culture models, may uncover cell-type-specific mechanisms behind AGA and LGA outcomes and guide the development of targeted interventions.
Methods: We performed single-nucleus RNA sequencing on term placentas from a Chilean cohort of women with obesity, who more frequently delivered large-for-gestational-age (LGA) infants but had no other complications. Placentas were grouped as Control (normal BMI, AGA infants), O-A (obesity, AGA), and O-L (obesity, LGA). We identified transcriptomic changes, either unique or shared between O-A and O-L groups, reflecting maternal obesity or fetal overgrowth responses. Ligand-receptor analysis revealed key cell types mediating these effects. A microfluidic co-culture of adipose spheroids and trophoblast organoids partially replicated these changes, offering an in vitro model to study obesity's impact on placental function.
Results: In maternal obesity, regardless of fetal growth, syncytiotrophoblasts showed upregulated hypoxia and TNF-α signaling, while cytotrophoblasts exhibited downregulated receptor tyrosine kinase signaling. Only in LGA placentas did villous non-trophoblasts display upregulated TNF-α signaling and inflammatory responses. Notably, Hofbauer cells in LGA placentas showed transcriptional changes in immunometabolism-related genes and acted as key signaling senders via SPP1. Importantly, we recapitulated syncytiotrophoblast responses to maternal obesity using a novel microfluidic organoids-on-a-chip co-culture.
Conclusion: Our study reveals a complex cell-type-specific regulatory network in the placenta, driven by hypoxia, nutrient transport, inflammation, and TNF-α signaling in maternal obesity and fetal overgrowth. With rising global obesity rates, these insights enhance understanding of placental-fetal interplay. Precisely targeting these pathways may help reduce pregnancy complications and long-term risk of adverse outcomes in offspring.

SYM1.4.
SEX, ALCOHOL, MENTAL HEALTH, AND THE PLACENTA

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Objectives: Previous research has identified that early exposure of the placenta to hypercortisolaemia leads to epigenetic modifications of the fetal placental unit, altered fetal growth and programming of offspring health. Furthermore, several studies have identified these programming effects induced by hypercortisolaemia vary based on the sex of the fetus. From the Queensland Family Cohort Study, a longitudinal birth cohort study of the general population in Brisbane Australia, we have identified that maternal asthma, maternal mental health and periconceptional alcohol consumption are the most common health issues that affect pregnancy and the placenta. Interestingly the downstream drivers of placental alterations in presence of these common complications are hypercortisolemia and inflammation.
Methods: Data associated with placental RNA seq and Nanopore sequencing will be discussed

Results: Our team has focussed on the impact of these common complications of pregnancy on placental function and fetal outcomes. In the presence of maternal stress, anxiety and depression placental glucocorticoid regulated pathways upregulate inflammation via a new and novel receptor glucocorticoid receptor α D1 (GRα D1) and we will report on the downstream gene changes induced by the upregulation of GRα D1 expression. Both stress and alcohol consumption have been reported to alter methylation in the placental genome. We have been examining the placental methylome using nanopore sequencing in pregnancies complicated by periconceptional alcohol consumption with and without an abnormal cerebroplacental ratio, and with respect to fetal sex.
Conclusion: Nanopore sequencing in combination with RNA seq data permits a more comprehensive view of genome-wide methylation, allele-specific methylation and downstream gene alterations in placental studies which will be discussed in the symposium.

SYM1.5.
ENDOTHELIAL-TO-MESENCHYMAL TRANSITION IN THE FETOPLACENTAL MACROVASCULATURE AND MICROVASCULATURE IN PREGNANCIES COMPLICATED BY GESTATIONAL DIABETES

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Objectives: Increased rates of accelerated fetal growth and long-term cardiometabolic complications observed in pregnancies complicated by gestational diabetes (GDM) are associated with alterations in the feto-placental micro- and macro-vasculature. Here we assessed whether alterations in the fetoplacental vasculature could be attributed to endothelial-to-mesenchymal transition (EndMT), the transdifferentiation of endothelial cells towards a mesenchymal phenotype.
Methods: Human placentae were collected at term from GDM (n=18) and non-GDM (n=17) pregnancies. Human umbilical vein macrovascular endothelial cells (HUVECs; n=5-6) were isolated from umbilical cords and the endothelial phenotype was confirmed using flow cytometry. HUVECs were exposed to known EndMT inducers, TGF-β2 (10 ng/mL) + IL-1β (10 ng/mL), for 6 days. Cultured HUVECs and placental villous tissue were fixed or processed for RNA. Immunocytochemistry, immunohistochemistry and RT-qPCR were performed to assess levels of endothelial (CD31, Vwf, VE-Cadherin), mesenchymal (αSMA, Transgelin, CD73) and EndMT markers, including key transcriptional regulators, Slug and Snail.
Results: Non-GDM and GDM HUVECs co-expressed CD31 and VE-Cadherin (98.57±0.40% and 99.59±0.24%, respectively). TGF-β2+IL-1β induced morphological and molecular changes consistent with EndMT in GDM and non-GDM HUVECs. The ability of TGF-β2+IL-1β to alter expression of *VWF*, *TGFBR1*, *IL1B*, and *IL1R1* was diminished in GDM HUVECs, however, all other hallmarks of EndMT were similar. In both non-GDM and GDM human placental tissue, Slug and Snail were detected in the villous stroma. In GDM placenta there was a reduction in endothelial genes and in some EndMT regulators (*SNAI2*, *TGFB2*, *TGFB3* and *TGFBR2*), however, there was no change in mesenchymal markers or other EndMT regulators.
Conclusion: Our data suggests that there are limited changes in EndMT regulatory molecules in the fetoplacental macrovasculature in GDM. Several EndMT regulatory molecules were altered in placental villous tissue in GDM, suggesting changes in EndMT in the microvasculature, however further work is needed to explore this.

SYM1.6.
DISSECTING THE MECHANISMS OF SYNCYTIOTROPHOBLAST REGENERATION USING HUMAN PLACENTAL ORGANOIDS

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Objectives: The syncytiotrophoblast (STB) serves as the maternal-fetal interface, carrying out many critical functions for a successful pregnancy including defense against pathogens, exchange of nutrients and waste between the mother and the fetus, and hormones synthesis. The syncytiotrophoblast, like other epithelial tissues, is constantly shed in the maternal blood in healthy pregnancies, a process essential for removing damaged or old syncytial cells, and ensuring syncytial homeostasis. However, defects in syncytiotrophoblast regeneration can compromise the placenta's integrity and barrier function. How the syncytiotrophoblast turnover is regulated, and which one are the molecular mechanisms triggering its regeneration?
Methods: To shed light on the mechanisms of syncytiotrophoblast regeneration, we use first trimester human-derived placental organoids. A major limitation of tissue-derived organoid models grown in Matrigel is their reversed polarity, with the syncytiotrophoblast not accessible. To overcome this limitation, we developed apical-out placental organoids derived from the human first-trimester placenta. By combining microscopy, RNA sequencing, and secretome analysis we aim to uncover the key players involved in STB regeneration.
Results: We show that first trimester apical-out placental organoids maintain the expression of canonical trophoblast markers, exhibit the presence of microvilli on their surface, and maintain their endocrine function. To mimic syncytiotrophoblast shedding, we induced syncytiotrophoblast damage by mechanically disrupting the apical-out placental organoids. We observed an increase of syncytiotrophoblast markers over time upon damage. This suggests that syncytiotrophoblast regeneration occurs spontaneously, making this model suitable for investigating the mechanisms triggering its regeneration. We are now performing RNA sequencing analysis to identify the molecular players that emerge upon damage and their contribution to syncytiotrophoblast regeneration.
Conclusion: While syncytiotrophoblast turnover is necessary for placental function, defects in syncytiotrophoblast regeneration can compromise nutrient transfer and increase susceptibility to infections. Our work will identify new regulatory mechanisms required for syncytiotrophoblast regeneration and represent an important resource for understanding pregnancy complications

SYM2.2.
EXTRACELLULAR VESICLES FROM M1 AND M2 MACROPHAGES ELICIT SEXUALLY DIMORPHIC RESPONSES IN THE HUMAN PLACENTA

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Objectives: We previously demonstrated that extracellular vesicle (EV) messaging at the maternal-fetal interface is bidirectional, with EVs actively internalised by the placenta. This mechanism of communication opens the possibility for functional messaging between the maternal immune system and placental trophoblast. Here we investigated first trimester placental responses to EVs from pro-resolution M2 macrophages associated with normal pregnancy, and from M1 pro-inflammatory macrophages, as a model of inflammation at the maternal-fetal interface.
Methods: M1 and M2 macrophages were polarised from healthy female monocytes, EVs isolated by size exclusion chromatography, and characterised by NTA ELISA, WB and TEM. miRNA cargo and protein cargo were examined by RNAseq and quantitative proteomics. EVs were applied to male and female first trimester explants. Female responses were measured by RNAseq, and qPCR of transcripts of interest were further examined in male and female placentae by qPCR. Pathway analysis of placental responses was performed and overlaid with EV cargo information.
Results: We identified miRNA and protein cargo of M1 and M2 macrophage EVs, with differential abundance of 72 and 132 molecules, respectively. EV protein cargo related to cellular source, with M1 EV cargo associated with pro-inflammatory and antiviral functions, while M2 EV cargo associated with immune-regulation and tissue repair. We identified

distinct differentially expressed genes in female placentae in response to M1 and M2 EVs, including genes involved in immune response/inflammation, as well as general cellular processes. Many of these were sexually dimorphic. Bioinformatic analysis identified links between EV cargo and placental responses, including a potential positive feedback loop involving metallothioneins.

Conclusion: The placenta exhibits differential responses to M1 and M2 EVs, which are sex-dependent. EVs may therefore play a role in initiating or promoting placental responses to changed macrophage phenotypes in pregnancy pathologies associated with inflammation, and contribute to observed sex-dependent differences in placental responses to infection/inflammation.

SYM2.3.
MEMBRANE-BOUND MITOCHONDRIAL DNA: A POTENTIAL SENESCENCE SIGNAL IN PLACENTA TISSUE

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Objectives: The mitochondrial function tends to decline with age in mammals leading to mitochondrial dysfunction. Stress signals and defective mitochondrial function generate increased production of reactive oxygen species (ROS), which in turn causes further mitochondrial deterioration and global cellular damage. However, dysfunctional mitochondria can contribute to cell aging independently of ROS. This could happen through several mechanisms, for example, by releasing mitochondrial DNA (mtDNA) to the extracellular milieu encapsulated in vesicular structures, which is defined as membrane-bound mtDNA. In this study, we characterize the mitochondrial DNA content in stress-derived EVs released by trophoblast cells and its potential role on senescence expansion.

Methods: BeWo cells were cultured in F12K supplemented with 10 % FBS and 1% penicillin/streptomycin in presence or absence of forskolin (50 nM) and 5 nM H_2O_2. Cytotrophoblast cells (CTBs) from term placenta were isolated and cultivated in DMEM supplemented with 20% FBS exosome-depleted and 1% penicillin/streptomycin. Supernatants were collected and EVs were isolated by ultracentrifugation. The size and concentration distribution of EVs was measured by nanotracking analysis. EVs were visualized by transmission electron microscopy (TEM). Total DNA was isolated from EVs and the presence of mtDNA genes was assessed by qPCR.

Results: The induction of stress by H_2O_2 increased the amount of EV released by BeWo cells and CTBs. Stress-derived EVs contained higher amounts of total DNA. mtDNA genes including CO2, ND2, ND4, CYB, ATP8, RND2 were detected in EVs isolated from BeWo cells and CTBs. We found higher levels of CO2, CYB, RND2 and ND4 in stress-derived EVs but no difference in EV release and mtDNA content from BeWo cells treated with forskolin.

Conclusion: Stressed trophoblasts (CTBs but not syncitialized cells) release large quantities of EVs enriched in mtDNA, potentially serving as a signal of mitochondrial damage propagating stress to healthy cells. This might drive senescence within the placenta.

SYM2.4.
DO PLACENTAL EXTRACELLULAR VESICLES INFLUENCE FETAL HEART DEVELOPMENT?

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Objectives: Proper placental function is essential for fetal growth and development. We previously showed that the transcription factor OVO-like 2 (OVOL2) is expressed in the placenta and regulates trophoblast differentiation in mice. Notably, OVOL2-deficient mice exhibit severe heart defects and die at midgestation, despite an absence of detectable OVOL2 expression in the heart at that stage. This observation supports the emerging concept of a developmental interplay between the placenta and embryonic heart. The mechanisms underlying this relationship remain poorly understood. Extracellular vesicles (EVs) are lipid-bound particles containing bioactive cargo, and are known mediators of intercellular communication. We hypothesize that EVs released by trophoblasts promote fetal heart development.

Methods: Ovol2$^{+/-}$ male and female mice were bred to generate Ovol2$^{+/+}$, Ovol2$^{+/-}$ and Ovol2$^{-/-}$ embryos. Placentas, fetal hearts, and plasma were collected at various embryonic stages. Ovol2$^{+/+}$ and Ovol2$^{-/-}$ trophoblast stem cells (TSCs) were maintained in undifferentiated states, or differentiated into junctional zone or labyrinth zone lineages. RNA sequencing comparing Ovol2$^{+/+}$ and Ovol2$^{-/-}$ TSCs under differentiating conditions was performed to determine transcriptomic differences between lines. Media were collected from cells and ultracentrifuged to enrich for EV-containing fractions. To profile EVs, nanoflow cytometry and electron microscopy were performed.

Results: Ovol2 was expressed in the placenta and localized primarily to labyrinth zone lineages. Ovol2$^{-/-}$ TSCs were unable to differentiate effectively and failed to maintain epithelial identity. Fetal hearts exposed to media conditioned by placental tissue or differentiated Ovol2$^{+/+}$ TSCs showed increased heart rate and cell outgrowth. This effect was recapitulated when hearts were exposed to EV-enriched fractions but was not observed when hearts were exposed to media or EV fractions from undifferentiated cells or Ovol2$^{-/-}$ TSCs. Both maternal and fetal plasma contained abundant quantities of placental EVs.

Conclusion: Placentas and differentiated TSCs release EVs that support cardiac morphogenesis, indicating that placenta-derived EVs may contribute to nascent heart development.

SYM2.5.
EXTRACELLULAR VESICLES: WHAT ARE WE ACTUALLY STUDYING?

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Objectives: Extracellular vesicles (EVs) have emerged as powerful mediators of intercellular communication, yet their study is often challenged by inconsistent terminology, misclassification, and methodological limitations. A major issue in the field is the persistent confusion surrounding EV subtypes, particularly exosomes and ectosomes, due to the lack of definitive size- or marker-based criteria.

Methods: Common techniques such as nanoparticle tracking analysis (NTA) and protein profiling cannot reliably isolate pure EV subpopulations, and most preparations contain heterogeneous mixtures, including non-vesicular particles. Furthermore, the biological functions attributed to EVs remain poorly defined, with overlapping and often unverified claims that undermine reproducibility and hinder clinical translation.

Results: In pregnancy, placental cells release EVs in response to changes in oxygen tension and maternal metabolic status. These EVs influence placental adaptation and exert systemic effects on maternal endothelial cells, skeletal muscle, and adipose tissue. In gestational diabetes mellitus (GDM), EV secretion from both placental and adipose tissue is increased and biologically active, affecting fetal growth and maternal metabolism through pathways such as mTOR, OXPHOS, and sirtuin signaling. Placenta-derived EVs are detectable from as early as six weeks of gestation, with concentration and cargo changes linked to complications including GDM, preeclampsia, preterm birth, and fetal growth restriction.

Conclusion: EV-based diagnostics hold promise for non-invasive prenatal testing, pregnancy monitoring, and newborn screening, offering new opportunities for proactive, personalized care.

SYM2.8.
SMALL EXTRACELLULAR VESICLES FROM PREECLAMPTIC WOMEN AND HYPOXIC PLACENTAS DISRUPT BLOOD-BRAIN BARRIER INTEGRITY VIA CLDN5 REDUCTION

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Objectives: To determine whether small extracellular vesicles (sEVs) derived from the plasma of women with preeclampsia or from hypoxia-exposed placental explants impair blood-brain barrier (BBB) integrity by reducing the expression of the tight junction protein claudin-5 (CLDN5), and to investigate the involvement of vascular endothelial growth factor (VEGF) and its receptor KDR in this process.
Methods: sEVs were isolated from plasma of women with normal pregnancies (sEVs-NP, n=9) or preeclampsia (sEVs-PE, n=9), and from placental explants cultured in normoxia (5% O2, 18 h, sEVs-Nor, n=10) or hypoxia (1% O2, 18 h, sEVs-Hyp, n=10). BBB function was assessed in vitro using human and murine brain endothelial cells, and in vivo using non-pregnant C57BL/6J mice (4–5 months old, n=13) injected with sEVs-Hyp. Protein levels of CLDN5 and VEGF, as well as KDR activation, were measured by Western blot.
Results: sEVs-PE and sEVs-Hyp reduced total and membrane-bound CLDN5 levels ($p<0.05$), while sonicated vesicles lost this effect. In vivo, sEVs-Hyp caused neurological deficits and localized BBB disruption in the posterior brain, associated with vesicle uptake, extravasation, and reduced CLDN5 in brain tissue. VEGF content was higher in sEVs-PE and sEVs-Hyp than in controls. sEVs-PE decreased KDR activation in endothelial cells. While KDR inhibition enhanced CLDN5 reduction in cells treated with sEVs-Hyp.
Conclusion: sEVs from preeclamptic plasma and hypoxic placentas impair BBB integrity by downregulating CLDN5, associated with VEGF enrichment, but independently of KDR activation. These findings offer mechanistic insight into cerebrovascular vulnerability in preeclampsia.
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SYM3.3.
BMAL1 REGULATES THE INITIAL STEP OF EMBRYO IMPLANTATION BY MODULATING ENDOMETRIAL ADHESION AND MIGRATION

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Objectives: Embryo implantation is a critical and tightly regulated process essential for successful human reproduction. Although the role of endometrial receptivity is well established, the molecular mechanisms governing this process remain incompletely understood. Circadian rhythm regulators, particularly brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein 1 (*BMAL1*), have emerged as key modulators in reproductive biology. In this study, we explored the role of *BMAL1* in regulation of endometrial receptivity and embryo implantation.
Methods: Ishikawa cells were transfected with *BMAL1*-specific or control siRNA, followed by analyses including RT-qPCR, Western blotting, and immunofluorescence. Functional assays such as migration, co-culture with JEG-3 spheroids, cell viability, and cytotoxicity were conducted.
Results: *BMAL1* knockdown in Ishikawa cells downregulated adhesion-related genes, including *ITGAV*, *ITGB3*, and *CD44*, and led to reduced protein expression of integrins αV and β3, as well as impaired cell migration. A co-culture model using JEG-3 spheroids demonstrated a significantly decreased embryo attachment following *BMAL1* suppression.
Conclusion: These findings indicate that *BMAL1* is a critical regulator of integrin-mediated adhesion and endometrial receptivity, underscoring its potential as a therapeutic target in implantation failure.

SYM3.4.
CIRCADIAN TIMING OF MATERNAL HYPOXIA INFLUENCES FETAL CARDIAC TRANSCRIPTOMIC RESPONSE

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Objectives: Prenatal hypoxia alters cardiac development and increases offspring's cardiovascular risk. However, it remains unclear whether the fetal heart responds similarly to hypoxia at different times of the day. Since previous studies have demonstrated that hypoxia elicits distinct transcriptional responses depending on exposure time, this study investigated how the timing of hypoxia (light vs dark phase) in late pregnancy affects the fetal heart transcriptome.
Methods: Pregnant Wistar rats were divided into four groups: control light, hypoxia light, control dark, and hypoxia dark ($n = 5$/group). On gestational day (GD) 20, dams from hypoxia groups were exposed to 10.5% O_2 for 12 hours, either during the light or dark phase. On GD21, two male fetal hearts per litter were collected, pooled and snap-frozen. RNA was isolated (RNAzol® RT), sequenced (NovaSeq X Plus, PE150) and differential gene expression was analyzed (DESeq2).
Results: Light-phase hypoxia upregulated two genes (0.15 and 0.03 log2 fold change) and downregulated one gene (–0.10 log2 fold change; $P < 0.0001$). In contrast, dark-phase hypoxia induced broader transcriptomic changes, with 18 genes upregulated (0.25 to 0.10 log2 fold change, $P < 0.001$); and 14 downregulated (–0.14 to –0.09 log2 fold change, $P < 0.05$). These genes are implicated in extracellular matrix organization, angiogenesis, cell cycle, and mitochondrial function. *Calcrl* (receptor for vasodilator adrenomedullin) was the only gene upregulated in both phases.
Conclusion: Dark-phase hypoxia induced more transcriptomic changes than light-phase hypoxia, suggesting a time-dependent sensitivity to oxygen deprivation. Whether this heightened response indicates better adaptability or increased vulnerability remains unclear. Nevertheless, our findings underscore the importance of considering circadian timing in studies of prenatal hypoxia and in designing interventions for pregnancy complications.
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SYM3.6.
UTERINE ISCHEMIA/REPERFUSION INJURY (IRI) INCREASES OXIDATIVE STRESS AND PATHOLOGY IN THE MACAQUE PLACENTA

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Objectives: Placental insufficiency appears to be the major contributor to nearly all adverse pregnancy outcomes (APOs). The etiology behind this insufficiency is often unknown, although pathologies observed suggest increased oxidative stress and inflammatory injury at the placenta. An ischemia/reperfusion injury (IRI) occurs when ischemic tissues are rapidly refilled with oxygen-rich blood – resulting in a paradoxical increase in injury. We hypothesized that a uterine IRI at mid-gestation would result in

placental pathologies commonly observed in APOs of placental insufficiency.

Methods: To this end, we utilized pregnant macaques for an IRI time-series investigation. Macaque uteri were surgically exposed at gestational day 110 and ligated to occlude uterine arteries for one (n=1) or two (n=1) hours. Ligations were then relieved, allowing uterine blood to reperfuse the placenta. A sham control (n=1) received an identical surgery, without ligation. Maternal blood chemistries and fetal heart rate were monitored during and after the procedure. Twenty-four hours following reperfusion, placentae were collected, isolated by cotyledon (n=39), sectioned, stained for 3-nitrotyrosine (protein oxidation) and H&E, and digitally annotated for pathology.

Results: Ligation led to uterine blanching, reduced fetal heart rate, and maternal blood chemistries indicating successful uterine ischemia. Following reperfusion, the two-hour ligated dam showed increased D-dimers, white blood cells, and platelets compared to control, suggesting additional damage with reperfusion. At the placenta, a two-hour ligation resulted in increased cotyledon protein oxidation, inflammatory injury (p=0.0181), villous agglutination (p=0.0088), and mineralization (p=0.0068), and total pathology (p=0.0193) compared to controls.

Conclusion: Here, we show evidence that the placenta is susceptible to an IRI *in vivo*, through placental oxidative stress and pathologies following ligation. As transient placental ischemia has been reported *in vivo*, an IRI may represent an etiological basis for a subset of insufficiency pathologies observed clinically. Future studies will aim to determine if these IRI-induced pathologies result in APOs at term.

SYM3.6.
FETAL GROWTH RESTRICTION INDUCES DYNAMIC, SEX-SPECIFIC CHANGES TO MOLECULAR SIGNATURES IN THE PLACENTA-HEART AXIS OF THE NEAR-TERM SHEEP

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Objectives: The interconnected relationship between placenta and heart is critical for establishing lifelong cardiovascular health. Intriguingly, sex differences in the placenta-heart axis in response to pregnancy complications are known; male placentae have impaired adaptability and reserve capacity compared with females, which may exacerbate heart development perturbations. Despite this, the underlying mechanisms that contribute to these responses remain unclear. Therefore, the current study aimed to characterise sex-specific molecular signatures in the placenta-heart axis of the near-term sheep model of fetal growth restriction (FGR).

Methods: Left ventricle (LV) and placenta tissue was collected from control and FGR fetuses (n=4/sex/group) at 140d gestation (term=150d) for RNA-seq. Differences in gene expression between FGR and control for each sex were determined using DESeq2 (padj<0.05, logFC±1.5), and pathway enrichment analysis was performed using pre-ranked gene set enrichment analysis (GSEA).

Results: There were minimal changes in male placenta pathway analysis, with only the epithelial mesenchymal transition gene set downregulated in response to FGR; this coincided with upregulation of pathways involved in oxidative phosphorylation, cell growth (i.e. MYC targets), and damage (i.e. reactive oxygen species pathway; DNA repair) in the LV. In contrast, the female placenta-heart axis revealed multiple changes to pathways involved in proliferation, metabolism, immune signalling and stress response, indicative of dynamic, adaptive responses.

Conclusion: Minimal placental adaptations in FGR males may place the heart under greater stress to maintain cardiac function, thereby programming lifelong cardiovascular disease risk and burden. In contrast, heightened molecular responses in female placentae may provide greater cardioprotection in response to FGR.

SYM4.5.
TIME-RESOLVED CYTOKINE SECRETION PROFILE IN AN EX VIVO DUAL-COMPARTMENT MODEL OF HUMAN FETAL MEMBRANES FOLLOWING INFLAMMATORY STIMULATION

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Objectives: Fetal membranes play a key role in the initiation and regulation of inflammation-associated preterm birth. Ascending bacterial infections are known to trigger pro-inflammatory cytokine responses, contributing to membrane weakening and labor induction. This study aimed to investigate time-dependent cytokine secretion following bacterial stimulation in an ex vivo dual-compartment model of human fetal membranes.

Methods: Fetal membranes from term cesarean sections (n = 4) were cultured in an in house established ex vivo two-compartment system, maintaining the anatomical separation between amnion and choriodecidua. Lipopolysaccharide (LPS, 10,000 ng/ml) was applied to the amniotic side. Interleukin (IL)-1β, IL-6, and IL-10 levels were measured in the choriodecidual compartment at 1, 6, and 24 h and compared to baseline.

Results: Cytokine secretion (pg/ml) patterns showed time-dependent changes under both conditions: baseline vs. LPS treated group. For IL-1β, no significant differences were found at 1 h (515.0 ± 41.2 vs. 513.2 ± 37.2) or 6 h (527.4 ± 8.0 vs. 538.7 ± 12.8), but a significant increase was observed at 24 h in the LPS group (599.8 ± 23.4 vs. 1038 ± 285.2, p = 0.0007). IL-6 levels showed no significant differences at 1h (143.8 ± 87.0 vs. 133.0 ± 77.2) and 24h (55626 ± 32370 vs. 75849 ± 5566) but were significantly higher at 6 h in LPS-treated samples (1811 ± 267.3 vs. 3984 ± 1901, p = 0.0080). IL-10 concentrations remained stable across all time points with no significant differences between baseline and LPS-treated groups (1h: 60.72 ± 5.939 vs. 54.38 ± 6.434; 6h: 107.9 ± 24.57 vs. 99.67 ± 16.77; 24h: 138 ± 39.36 vs. 152.1 ± 54.61).

Conclusion: This study highlights the potential of the ex vivo fetal membrane model to generate time-resolved cytokine profiles following inflammatory stimulation. Understanding temporal dynamics of key cytokines is essential for designing future in vitro pharmacodynamic studies and may help define optimal intervention windows for therapies targeting inflammation-related signaling pathways in infection-associated preterm birth.

SYM4.6.
FROM PLACENTAL SYNCYTIUM-ON-CHIP (PSOC) TO TROPHOBLAST INVASION-ON-CHIP (TIOC): A PUMPLESS MICROFLUIDIC PLATFORM TO STUDY TROPHOBLAST MIGRATION AND ENDOTHELIAL INTERACTIONS IN PLACENTAL BIOLOGY

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Objectives:

- Fine tune shear stimulation to induce trophoblasts syncytialization without using drug
- Compare chemical and mechanical trophoblasts syncytialization with or without endothelial co-culture
- Fine tune extracellular matrix (ECM) to induce trophoblasts migration and extravillous differentiation with endothelial co-culture

- Develop an endometrial vasculature on-chip to study trophoblast vascular remodeling function

Methods: Here we use a trophoblastic BeWo cell line to systematically compare the effect of forskolin treatment in static culture with WSS stimulation in a pumpless and recirculating organ-on-chip. We measured hormones levels, assessed cell-cell fusion, differentiation and barrier function. We also included HUVECs endothelium in the Placental Syncytium-on-chip (PSoC). Next, trophoblasts were cultured against different ECMs with HUVECs co-culture in the Trophoblast Invasion-on-Chip (TIoC). We investigated their migration and phenotype distribution. We also produced an engineered endometrial vasculature in the TIoC.
Results: We show that BeWo cells undergo a comparable differentiation under WSS exposure as under forskolin treatment. 0.1 dyn cm^{-2} WSS lead to cadherin loss, cell fusion, polarization, barrier functions, chorionic gonadotropin secretion, and increased expression of key transporters. Moreover, WSS alone induced favorable changes in the levels of FMS-like tyrosine kinase-1 and Placental Growth Factor suggesting the development of a physiologically relevant PSoC. We furthermore expanded the platform to a syncytiotrophoblast/endothelial co-culture showing physiological endocrine functions. In the TIoC, we found that BeWo cells were more migratory in fibrin-based gel mixtures and showed upregulation in extravillous phenotype markers. We correlated this behavior to ECMs physical characterization. Finally, promoting trophoblast migration in a factor or cell dependent manner helped elucidating the drivers of vascular remodeling on-chip.
Conclusion: The scalable, pumpless design of our microfluidic platform facilitates the establishment of a physiological relevant drug free-PSoC and real-time monitoring of trophoblast invasion and endometrial vascular remodeling. This model also provides a versatile tool for studying placental biology and finding therapeutic strategies for pregnancy-related disorders.

SYM5.1.
PLACENTAL ADAPTATION AFTER ENVIRONMENTAL EXPOSURE – A TALE OF TWO PARTICLES

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Objectives: Environmental pollutants, including the particulate matter found in air pollution, have been identified within the placenta, fetal, and offspring tissues, highlighting direct cellular-particle interactions. Exposure to these particles during pregnancy often leads to restricted fetal growth, but the mechanisms remain unclear. The purpose of these studies was to assess uterine vascular function and placental morphology after maternal inhalation of two contaminants, to assess nutrient delivery and exchange after gestational exposure.
Methods: Pregnant Sprague-Dawley rats were exposed to either nanotitanium dioxide ($nTiO_2$; surrogate for ultrafine particulate matter) or polyamide-12 (nylon representative of micro- and nanoplastic particles (MNP)) aerosols via whole body inhalation from gestational day (GD) 5 to GD 20. Control animals did not enter the inhalation facility. At GD 20, the uterine horn was removed and rinsed. Uterine artery and radial arteriole segments were removed and assessed by either wire or pressure myography, respectively, with (10^{-9}M-10^{-4}M) of methacholine, sodium nitroprusside, or phenylephrine. Placenta from male and female fetuses were fixed in 10% formalin, trimmed, sectioned, and stained with hematoxylin and eosin or smooth muscle actin using immunohistochemistry. Maternal/fetal blood-space area, intrahemal distance and trophoblast invasion were calculated.
Results: Maternal uterine artery reactivity was unaffected by either exposure. Radial arteriolar endothelium dependent dilation was significantly reduced after both exposures, but more severely after MNP inhalation, indicative of reduced vascular function and placental perfusion. Exposure to $nTiO_2$ significantly reduced the labyrinth and decidua zone areas, with no change after exposure to MNP. Maternal surface area significantly increased after $nTiO_2$ exposure, but significantly decreased after MNP. There was no change to fetal surface area.
Conclusion: Overall, uterine vascular function and placental morphology are impacted by gestational exposure to inhaled particulate. However, these outcomes are not uniform. Identification of the unique particle responses is crucial to understand their impact on human health.

SYM5.2.
EARLY LIFE IMPACT OF DIESEL EXHAUST PARTICLES: PLACENTAL ACCUMULATION, FETAL TRANSFER, AND MATERNAL-FETAL CROSSTALK DISRUPTION

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Prenatal air pollution exposure, in particular to traffic-related combustion particles, is associated with adverse pregnancy and fetal outcomes but the mechanisms underlying the observed developmental toxicity remain elusive. In particular, the impact of combustion-derived particles on the placenta, as central mediator of maternal-fetal crosstalk, is largely unexplored. This talk will present our latest findings on the placental uptake, localization and translocation of diesel exhaust particles (DEPs) and how their accumulation can disrupt the secretion of placental signaling factors leading to indirect fetotoxicity even in the absence of direct maternal-fetal particle transfer. These results underscore the importance of investigating placental responses to environmental pollutants and supports the need for preventative strategies to mitigate early-life exposure risks. As part of the newly launched EU project UPRISE, we will expand on these findings by investigating how air pollution—particularly ultrafine particles and micro-/nanoplastics—affects fetal development and birth outcomes, through a combination of targeted experimental models, environmental monitoring, and clinical pregnancy cohort studies.

SYM5.3.
UPTAKE AND TOXICITY OF MICRO- AND NANOPLASTICS IN THE HUMAN PLACENTA: EMERGING CONCERNS FOR MATERNAL-FETAL HEALTH

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Micro- and nanoplastics (MNPs) are emerging environmental contaminants increasingly detected in human tissues, including blood and placenta. Their presence during pregnancy raises concerns about potential developmental toxicity, either through transplacental transfer to the fetus or via disruption of placental function, with possible consequences for maternal-fetal health. Drawing insights from in vitro placental cell models, ex vivo placental perfusions, and in vivo studies, we will present the current state of research on in utero MNP exposure, including key challenges in exposure characterisation and hazard assessment. We also present new findings from the H2020 AURORA and Dutch ZonMw MOMENTUM projects, which employ diverse in vitro placental models to investigate MNP uptake, translocation, functional alterations, and immune-inflammatory responses. Together, these data help build the

evidence base needed to evaluate how MNPs might impact placental physiology and maternal-fetal well-being.

SYM5.4.

PRECONCEPTION AND GESTATIONAL MATERNAL EXPOSURE TO A MIXTURE OF SHORT-HALF-LIFE FOOD CHEMICALS ALTERED FETOPLACENTAL DEVELOPMENT AND PLACENTAL FUNCTION IN A RABBIT MODEL, BASED ON EXPOSURE DATA FROM THE SEPAGES MOTHER-CHILD COHORT.

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Objectives: Pregnant women are exposed to chemical pollutants that can affect offspring's health. Based on a recent cohort with improved exposure assessment, several phenols, parabens, and phthalate (PPP) metabolites measured in urines from pregnant women were associated with placental and child growth indicators. This study aimed to determine whether the defined PPP mixture responsible for human offspring outcomes exhibited similar effects on offspring health in a rabbit model exposed to a dose equivalent to that of pregnant women. Using SEPAGES cohort, a mixture of BPA, BPS, triclosan, butyl-paraben, DEHP, BBzP, DnBP, and DiNP was defined.
Methods: From preconception to 28 days postconception (dpc), female rabbits were exposed daily orally to this mixture (PPP group, n=16) or excipient (C group, n=16) at the doses estimated from the maximum urinary concentrations measured in SEPAGES. Doppler 2/3D measurements were performed at 14, 21, and 27 dpc to explore fetoplacental development. At 28 dpc, fetoplacental units were collected. Placental function was investigated by RNA-seq to identify differentially expressed genes (DEG), associated with gene set enrichment analysis (GSEA).
Results: At 14 dpc, ultrasound measurements were not affected by PPP exposure. At 21 dpc, only body width increased significantly in PPP *vs* C. At 27dpc, placental vascular resistance and umbilical artery pulsatility index increased significantly in PPP *vs* C. MFAs of the ultrasound data, at each stage, showed a good separation between groups. Placental DEG identified 299 deregulated genes in PPP *vs* C, some of them were sex-specific. The GSEA has also identified several deregulated pathways between both groups, according to fetal sex, among them vasculogenesis, angiogenesis and xenobiotic metabolism.
Conclusion: Exposure to a PPP mixture based on a human mother-child cohort affected fetoplacental development and placenta function in a rabbit model, suggesting that this mixture could induce fetal malprogramming with long-term effects.

SYM5.5.

PFOS EXPOSURE IMPAIRS CAMP-MEDIATED VASCULAR FUNCTION IN PLACENTAL CHORIONIC PLATE ARTERIES, ELEVATING PREECLAMPSIA RISK

Sathish Kumar, Pankaj Yadav. *University of Wisconsin, Madison, USA*

Objectives:
Background: Pregnancy induces adaptive vascular changes, including enhanced vasodilation, decreased blood pressure, and increased uterine artery blood flow. Preeclampsia, a serious pregnancy complication characterized by hypertension and vascular dysfunction, has unclear etiology. Epidemiological studies associate elevated perfluoroalkyl substance (PFAS) levels in maternal plasma with hypertensive disorders of pregnancy.
Objective: This study aimed to identify predominant PFAS linked to preeclampsia and investigate whether these PFAS compounds impair gestational vascular function via altered vasoconstriction/dilation in human placental chorionic plate arteries.
Methods: A case-control study included 40 preeclampsia cases and 40 normotensive controls. Maternal serum PFAS levels (38 compounds) and angiogenic biomarkers (sFLT1, PLGF) were analyzed. Vascular reactivity of chorionic plate arteries was assessed post-PFAS exposure, with evaluation of mitoquinone (MitoQ) protection.
Results: PFOS and PFHpS concentrations were significantly higher in preeclampsia cases. Each IQR increase in log-transformed PFOS and PFHpS levels elevated preeclampsia odds by 7.18-fold (95% CI: 2.24–23.0) and 5.40-fold (95% CI: 1.81–16.1), respectively. Both PFAS correlated with increased sFLT1 and sFLT1/PLGF ratios.
PFOS (10 μM; the PFAS with the highest odds ratio) enhanced contractile responses to K^+ depolarization and ET-1, while reducing isoproterenol- and forskolin-induced vasodilation. Intracellular ATP and cAMP levels decreased under PFOS exposure, but cGMP remained unaffected. MitoQ restored ATP/cAMP levels and rescued vascular dysfunction.
Conclusion: PFOS and PFHpS exposure elevates preeclampsia risk, with PFOS directly impairing vascular function via cAMP-mediated pathways. Mitochondrial-targeted antioxidants (e.g., MitoQ) may mitigate PFAS-induced placental dysfunction, offering therapeutic insights.

SYM6.5.

DETECTION AND CHARACTERIZATION OF MATERNAL MICROCHIMERIC CELLS IN UMBILICAL CORD BLOOD BY HLA-TARGETED SPECTRAL FLOW CYTOMETRY CELL SORTING

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Objectives: Maternal microchimerism (mMC) is the transmission of small quantities of maternal cells to the fetus. By homing to fetal lymphoid organs they may influence development of the fetal immune system. We aim to identify mMC in umbilical cord blood (UCB) and target unique maternal and fetal HLA molecules by use of human monoclonal HLA antibodies for cell sorting, cell expansion, and functional characterization of these cells.
Methods: Thirty UCB and maternal PBMC dyads from uncomplicated pregnancies were HLA typed. Maternal microchimerism was detected in UCB by real-time qPCR, targeting HLA molecules unique on allele level to the mother. To optimize antibody titration and test for potential cross-reactivity, artificial mixtures of maternal PBMC were spiked into a bulk background of UCB in ratios of 50%, 10%, 1%, and 0.1%. Using a multicolor antibody panel for spectral flow sorting, maternal CD3+ T cells were sorted from UCB by using human monoclonal HLA-specific antibodies unique to the mother into a 96-well plate. These were cultured in vitro for further expansion and characterization.
Results: We developed a flow cytometry panel combining cell lineage surface markers with human recombinant- and hybridoma derived HLA-A and HLA-B-specific monoclonal antibodies of the IgG isotype. An initial survey showed that a panel of 13 different antibodies, covering 7 HLA-A and 11 HLA-B antigens, is potentially able to distinguish maternal cells from fetal background in 16/30 (53.3%) of pregnancies. In the mixtures of maternal PBMC and UCB combinations, the minor cell populations were detected by the HLA antibodies at the level in which they were spiked.
Conclusion: HLA-targeted flow cytometry is a suitable tool to enrich and characterize mMC cells in fetal cord blood. Understanding the type and functional features of maternal cells that traffic to the fetus is expected to provide insight into their effect on fetal immunity.

SYM6.6.
IMAGING-BASED SNP DETECTION FOR THE SPATIAL DETECTION OF MICROCHIMERISM AND MATERNAL-FETAL INTERACTIONS IN THE PLACENTA

Emiel Slaats [1], Katharina Schuch [1], Bernadette Bramreiter [1], Katja Sallinger [1], Julia Schönberger [1], Jacqueline Anholts [2], Jos Drabbels [2], Daniel Jacobsen [3,4], Anne Staff [3,4], Amin El-Heliebi [1], Michael Eikmans [2], Thomas Kroneis [5]. [1] *Medical university of Graz, Graz, Austria;* [2] *Leiden university medical centre, Leiden, Netherlands;* [3] *Oslo University Hospital, Oslo, Norway;* [4] *University of Oslo, Oslo, Norway;* [5] *Medical university of Graz, Graz, Norway*

Objectives: Classically, the investigation of microchimerism is dominated by PCR based methods. However, while those methods allow for sensitive detection of microchimeric sequences in various DNA extracts, they do not provide spatial information. This makes them unsuitable to investigate the processes behind the trafficking of microchimeric cells at the maternal-fetal interface. Our objective was to develop a sex-unbiased detection method for the discrimination between maternal and fetal haplo-identical cells within the spatial context of the placenta.
Methods: Padlock probes targeting common (frequency within the population above 20%) single nucleotide polymorphisms (SNPs) present in ubiquitously expressed transcripts were designed to be amplified in situ by rolling circle amplification. This generates discrete signals for each targeted transcript within a cell allowing image-based detection. In this way, it is possible to discriminate between individuals based on the expressed SNP phenotype.
Results: We developed a panel of assays targeting twelve biallelic SNPs and three assays against HLA-A alleles. Combinatorial use of the assays allowed the detection of up to 50 discrete signals per cell. The method was validated in spike experiments, evaluating its sensitivity and specificity. Furthermore, proof of concept was obtained in placental samples, showing the methods capacity to discriminate cells from mother and child.
Conclusion: We developed a new sex-unbiased methodology to detect and visualize (micro)chimeric cells within host tissues and investigate maternal-fetal interactions within the context of the maternal-fetal interface.

SYM7.4.
HYPERGLYCEMIA-INDUCED PLACENTAL FIBROSIS IN TYPE-1-DIABETES: POTENTIAL ROLE OF TGF-β SIGNALING

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Objectives: Pregnant women with Type 1 Diabetes Mellitus (T1DM) face a more than threefold increased risk for perinatal mortality compared to healthy pregnancies, potentially resulting in intrauterine fetal death near term. This increased mortality is hypothesized to stem from placental insufficiency, although the molecular mechanisms remain poorly understood. While diabetes-related organ dysfunction is well-characterized in other systems, with fibrosis, Advanced Glycation Endproducts (AGEs), Transforming Growth Factor-β (TGF-β) and Epithelial-to-Mesenchymal Transition (EMT) identified as key contributors, placental failure mechanisms in T1DM remain understudied at the molecular level.
Our study investigates the occurrence of fibrosis in placentas from diabetic pregnancies and examines the potential involvement of AGEs, TGF-β and other components of profibrotic pathways in diabetic placental dysfunction.
Methods: Formalin-fixed paraffin-embedded placentas from T1DM patients and matched controls were analyzed for fibrosis via Picrosirius-red staining (n=89 per group), Carboxymethyl-lysine (CML) via immunohistochemistry and epithelial (e-cadherin) and mesenchymal (vimentin) markers via multiplex immunofluorescence (n=50 per group). Western blot analysis of placental lysates (n=9 per group) assessed epithelial-mesenchymal markers, CML, collagen, and profibrotic pathway components (SNAIL, β-catenin). In vitro, choriocarcinoma-trophoblast hybrid cells (AC1-M32) exposed to varying glucose concentrations (5-30 mM) for 72h or 7d were examined for the identified relevant proteins via Western Blot and TGF-β secretion via ELISA.
Results: T1DM Placenta-Sections showed significantly increased fibrosis, increased formation of AGEs, as indicated by CML, and increased vimentin expression. β-catenin was significantly elevated in T1DM placenta lysates as well as in lysates from trophoblastic cells after 7 days of exposure to hyperglycemic conditions. SNAIL and TGF-β were significantly increased in vitro under hyperglycemic conditions after 7 days.
Conclusion: Hyperglycemia in T1DM pregnant women induces significant fibrosis, AGE formation, and increased expression of mesenchyme in placentas. We identified key contributors to profibrotic signalling in T1DM placental lysates and hyperglycemia-treated trophoblastic cells, with β-catenin, SNAIL, and TGF-β emerging as central mediators.

SYM8.1.
BEYOND THE BRAIN: THE PLACENTA AS AN INTERFACE FOR MONOAMINE SYNTHESIS, TRANSPORT, AND METABOLISM

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Objectives: Monoamines – serotonin, norepinephrine, and dopamine – are critical regulators of fetal neurodevelopment, placental function, and maternal-fetal signaling. Despite its non-neuronal nature, recent work highlights the placenta's active role in monoamine homeostasis. We aimed to comprehensively characterize placental monoamine synthesis, transport, and metabolism across species, gestational stages, and pathological contexts, and to investigate their vulnerability to pharmacological and metabolic disruptions.
Methods: We combined data from a series of systematic studies using complementary models and a multi-level physiological, pathophysiological, pharmacological, and toxicological approach. This included human and rat placenta perfusion, membrane vesicle transport assays, primary human trophoblasts and placental cell lines, and whole-organ and cellular expression profiling. We assessed the effects of clinically relevant antidepressants and metformin, alongside the impact of pathological conditions such as preterm birth. Chronic in vivo paroxetine exposure in pregnant rats was used to model long-term pharmacological effects.
Results: Our findings establish that the placenta expresses dynamic, developmentally regulated monoamine handling systems. It synthesizes serotonin early in gestation, while dopamine and norepinephrine synthesis is limited. Monoamine clearance is mediated by a polarized transporter network: SERT and NET in the maternal-facing membrane and OCT3 in the fetal-facing membrane, complemented by robust MAO-A-mediated metabolism. OCT3 emerges as a key regulator of fetal monoamine exposure. Antidepressants and metformin potently inhibit SERT and OCT3, disrupting placental monoamine clearance and fetal monoamine profiles. Chronic paroxetine exposure in rats induces marked disturbances in placental monoamine systems, vascular resistance, and fetal growth. Lastly, preterm birth induces marked alterations in placental monoamine transporter expression and metabolism.
Conclusion: Monoamine synthesis, transport, and metabolism in the placenta represent an essential interface between maternal and fetal physiology. Our integrated findings highlight that this system is highly vulnerable to pharmacological agents and pathological states, with key

implications for medication safety and the understanding of placental contributions to fetal neurodevelopment.

SYM8.2.
GENETICS, PARITY, AND MORE: UNRAVELING THE REGULATION OF PLACENTAL TRANSPORTERS IN A HEALTHY BIRTH COHORT

Lauren Aleksunes. *Rutgers University, Piscataway, USA*

Objectives: The placenta expresses a number of membrane transporters including uptake/bidirectional carriers (OATP2A1, 2B1, and OCT3) and efflux pumps (BCRP and PGP) that regulate the maternal-fetal transfer and placental accumulation of endogenous and exogenous chemicals. In this study, we evaluated relationships between placental OATP2A1, 2B1, OCT3, BCRP, and PGP proteins in a healthy U.S. cohort and 1) maternal factors (e.g., gestational weight gain, early pregnancy body mass index, smoking, parity, pregnancy complications, age) and 2) infant factors (e.g., birth weight, race, gestational age, and transporter genetics where available). A select number of additional variables were considered as regulators including environmental chemical exposures and hormone concentrations.
Methods: Term placentas were collected from healthy participants in the Understanding Pregnancy Signals and Infant Development Study (UPSIDE) birth cohort (Rochester, NY, n=237). Proteins were quantified in membrane preparations from frozen villous placenta tissues using quantitative targeted absolute proteomic mass spectrometry in the Integrated Transporter Elucidation Center (InTEC). We examined determinants of placental protein concentrations through bivariate analyses and multivariable linear regression models.
Results: Parity is an important determinant in regulating placental concentrations of BCRP, PGP, OCT3 and OATP2B1, but not OATP2A1. Reduced function variants in ABCG2 (rs2231142) and ABCB1 (rs1045642) were associated with lower levels of BCRP and PGP. Smoking was associated with lower PGP protein concentrations. Additional analyses are underway for OATPs and OCT3.
Conclusion: Identifying maternal and infant factors that contribute to the regulation of membrane transporter levels in healthy, term placentas is the first step in dissecting the impact of disease and environment on the integrity of the placental barrier. Supported by R01ES029275, P30ES005022, R01HD083369, UC2HD113039, UG3OD023349, and UH3OD023349.

SYM8.3.
DOES PLACENTAL VITAMIN D TRANSPORT DETERMINE MATERNAL AND FETAL VITAMIN D STATUS?

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The placenta may mediate the effects of changes to the maternal environmental during gestation on fetal development and both fetal and maternal lifelong health. We investigate the effects of exposures during pregnancy on human placental function, gene expression and epigenetics and the subsequent associations with fetal development. We use ex-vivo systems including placental perfusion, villous and trophoblast culture, multi-scale imaging, epigenetic, proteomic and transcriptome analysis as well as placental samples from our Southampton cohort studies.
Findings include, maternal vitamin D levels, smoking and poor diet during pregnancy associate with placental gene expression of key nutrient transporters. These placental transporters relate to fetal and neonatal growth and body composition as well as maternal body composition. We investigate the role of the placenta in regulating the relationships between maternal vitamin D and fetal physiology. We demonstrate active placental uptake and placental metabolism, with subsequent release of these metabolites into both the maternal and fetal circulations. Vitamin D induces rapid effects on the placental transcriptome, epigenome and proteome that effect placental function and thereby fetal development, independent of vitamin D transfer. These data demonstrate a complex interplay between vitamin D and the placenta and will inform future interventions using vitamin D to support fetal development and maternal adaptations to pregnancy.

SYM8.4.
EFFICIENT IRON TRANSPORT TO THE FETUS REQUIRES CELL TYPE SPECIFIC ADAPTATION OF HUMAN PLACENTAL CELLS

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Objectives: The elevated iron demands during pregnancy put pregnant women at high risk for developing iron deficiency (ID), or even iron deficiency anemia (IDA). ID(A) is a major global health problem associated with various adverse pregnancy outcomes. Surprisingly little is known about placental iron transfer in humans. Our aim was to comparatively analyze the expression patterns of important iron transport and storage proteins of human primary placental cells (trophoblast cells (hTCs) and placental endothelial cells (PLECs)).
Methods: hTCs and PLECs were isolated from human term placentas and subjected to ID conditions, mimicked by treatment with the common iron chelator Desferoxamine (DFO). Changes of expression patterns of major iron metabolizing proteins (Transferrin Receptor 1 (TFR1), Ferroportin 1 (FPN1), and ferritin light (FTL1) and heavy (FTH1) chain)), were analysed by qPCR (FPN1), immunoblot (TFR1, FTL1, FTH1) or ELISA (FTL1, FTH1).
Results: DFO treated hTCs did not seem to compensate potential iron deficits by upregulation of TFR1 or degradation of their iron stores (ferritin), but continuously secreted ferritin into the medium. We analysed whether ferritin could serve as a potential iron source for PLECs and observed endosomal enrichment of ferritin upon DFO treatment. Furthermore, PLECs treated with DFO were in a state of increased iron transfer capacity (i.e., increased TFR1 protein and FPN1 mRNA levels).
Conclusion: We conclude that hTCs and PLECs respond differently to ID to ensure proper iron supply to the fetus. Iron-loaded ferritin is constantly secreted from trophoblasts into stroma and during iron deficient conditions PLECs upregulate their TFR1 expression to take up this ferritin, degrade it, and transport the iron via FPN1 to the fetal circulation.

SYM8.5.
A NOVEL PHYSIOLOGICALLY-RELEVANT MODEL OF THE SYNCYTIOTROPHOBLAST BARRIER FOR THE STUDY OF TRANSPLACENTAL ANTIBODY TRANSFER

Alice Hawkins [1], Paschalia Pantazi [1], Liheng Yang [2], Carolyn Coyne [2], Jose Lemme-Dumit [3], Sara Barnett [1], Marcela Pasetti [3], Margaret Ackerman [4], Beth Holder [1]. [1] *Imperial College London, London, United Kingdom;* [2] *Duke University School of Medicine, Durham, USA;* [3] *University of Maryland School of Medicine, Baltimore, USA;* [4] *Dartmouth College, Hanover, USA*

Objectives: Transfer of maternal immunoglobulin G (IgG) across the placenta to the fetus involves selective mechanisms that remain poorly understood, largely due to limitations of existing models. Maternal antibody transfer is highly species specific, with rodents transferring most of their antibodies postnatally via milk. Non-human primates exhibit similar anatomy to the human placenta, however, there are ethical limitations in using such animals. Existing in vitro models have been limited by the poor relevance of trophoblast cell lines, the short life-span of primary trophoblast cells, and the complexity and low throughput of placental perfusion. We thus sought to develop a novel syncytiotrophoblast barrier model utilising primary placental trophoblast cells.
Methods: Cytotrophoblasts were isolated from term placenta, through enzymatic digestion and gradient separation, and expanded into proliferative trophoblast cells using stem cell-promoting supplements. Cells

were differentiated into syncytiotrophoblast barriers on transwells and an organ-on-a-chip (OOAC) system by addition of forskolin. Barrier integrity was measured by trans-epithelial electrical resistance (TEER) and FITC-dextran permeability assays. Maternal to fetal transfer of IgG subclasses, IgM and IgG from applied maternal serum was measured by Luminex assay.
Results: Long-term proliferative trophoblast cultures were established from term placentas. These cells could syncytialise, with a ~3-fold increase in expression of syncytialisation markers and human-chorionic gonadotropin, forming intact barriers on transwells and OOAC which were impermeable to FITC dextran. Unlike BeWos, these cells express the IgG transporter FcRn. We have demonstrated selective passage of IgG1-4, with a transfer hierarchy that recapitulates that seen in in vivo maternal-cord blood samples, whilst IgM and IgA were not transferred.
Conclusion: This novel in vitro model demonstrates a physiologically relevant, high-throughput and tractable platform for studying the selective molecular mechanisms of trans-placental IgG transfer, without the use of animal models.

SYM8.6.
EFFECT OF THE TIMING OF MATERNAL HYPOXIA AND FETAL SEX ON THE RAT PLACENTAL TRANSCRIPTOME

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Objectives: Prenatal hypoxia is associated with long-term offspring disease and affects placental function depending on the onset, duration, and severity of hypoxia. This study investigated whether placental transcriptomics is differentially affected by the timing of hypoxia and fetal sex.
Methods: Pregnant Wistar rats were assigned to four groups: control light (CL), hypoxia light (HL), control dark (CD), and hypoxia dark (HD), ($n = 5$/group). On gestational day (GD) 20, dams from hypoxia groups were exposed to 10.5% O_2 for 12 hours, either during the light or dark phase. On GD21, one male and one female placenta per dam were collected for RNA isolation. Following RNA library preparation and sequencing, differential expression analysis was performed.
Results: Four genes were differentially expressed between HL and CL placentas of male fetuses (adjusted p values < 0.05): Prl2c1 (3.3 log2 fold change), Elf5 (1.0), Cldn11 (-1.8), and Slc6a9 (-1.6). In the female group, only Evpl (1.1) was upregulated in response to light-phase hypoxia. When comparing HD with CD placentas of male fetuses, six genes (e.g., Slc38a8; 1.3) were upregulated and six genes (e.g., Cldn22; -2.6) downregulated, while only one gene (Alox15; 1.3) was upregulated in placentas of female fetuses. Gene set enrichment analysis revealed that the timing of hypoxia and fetal sex affected different biological processes; in three of the four groups, oxidative phosphorylation was impaired.
Conclusion: Hypoxia during the dark phase induced more transcriptomic changes than during the light phase, suggesting a phase-dependent sensitivity to oxygen deprivation. Furthermore, gene expression showed greater changes in male than in female placentas. Our findings highlight the importance of considering circadian timing and fetal sex in prenatal hypoxia research.
Funded by the VEGA 1/0327/24, EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V05-00012, UK/3106/2024, APVV-22-0061 and APVV-21-0223.

SYM9.1.
PITFALLS USING SCRNA SEQ AND OUTLOOK TO NEW TECHNOLOGIES LOOKING AT THE HUMAN PLACENTA

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Objectives:
Introduction: Single cell RNA sequencing (scRNA seq) opened the door to identify cellular subtypes, and changes in temporal expression and between healthy and diseased states. However, such technologies need respective knowledge of the tissue of interest.
Advantages: scRNA-seq resulted in an updated understanding of the complexity of placental cells and tissues. Respective studies revealed developmental changes and cell type specific differences in e.g. gestational diabetes, fetal growth restriction and preeclampsia.
Methods: Disadvantages and pitfalls: In the human placenta, scRNA seq reaches its limits quite soon since the syncytiotrophoblast mostly escapes cell dissociation and thus is underrepresented in subsequent analyses. Single nucleus RNA sequencing (snRNA seq) may help, while adding other disadvantages, including absence of matured cytoplasmic transcripts. Additionally, both sequencing techniques suffer from the lack of information on the original cellular context of the tissues.
Besides these methodological limitations, there are limitations regarding the authors' appreciation of the placental structure and morphology. An example: In a recent study, scRNA seq was used to identify changes in the placenta/decidua in preeclampsia. Extravillous trophoblasts (EVTs) were identified as the major cell type (about 50%) in the villous part of the placenta, while endothelial cells were absent in the placenta and EVTs were absent in the decidua.
Results: Outlook to new techniques: The single cell techniques disclosed the urgent need for a better understanding of the spatial resolution of placental tissues. Hence, spatial histology, including spatial transcriptomics and spatial proteomics on the single cells level in tissues sections, has evolved as emerging technology. In addition, lightsheet microscopy has shown its power in detecting new structures and interactions using intact 3D visualization of tissues.
Conclusion: The use of new technologies needs to go hand-in-hand with a proper understanding of the limits of such technologies plus a reasonable understanding of tissues to avoid inappropriately conducted studies.

SYM9.2.
MULTI-OMICS OF CHORIONIC VILLUS SAMPLES REVEALS EARLY PREGNANCY PLACENTAL DYSFUNCTION ASSOCIATED WITH PRETERM AND TERM PREECLAMPSIA.

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Objectives: Preeclampsia, a severe pregnancy-induced disorder unique to humans, is a leading cause of maternal and fetal morbidity and mortality. Strong evidence supports placental dysfunction as central to preeclampsia, because the condition arises only in the presence of a placenta or shortly after its delivery. However, there is inadequate understanding of the precise pathogenesis of preeclampsia.
Methods: To address this knowledge gap, we conducted the very first comprehensive multi-omics analysis of early pregnancy placental biopsies (chorionic villus samples; CVS; n=4-6/group; 11-14 weeks gestation) from pregnancies that later developed preterm preeclampsia, term preeclampsia or remained normotensive. Recognizing that a single omics approach is insufficient to fully characterize complex disease states we assessed multiple regulatory levels (mRNA, non-coding RNA and protein) to gain a holistic understanding of the molecular mechanisms driving

preeclampsia. We employed DIABLO (Data Integration Analysis for Biomarker discovery using Latent cOmponents), a supervised multivariate method, to integrate and analyze the multi-omics data.
Results: Integration of 6 molecular layers (mRNA, lncRNA, miRNA, snoRNA, tRNA, proteomics) effectively distinguished samples across the three clinical groups. We uncovered distinct molecular signatures associated with preterm preeclampsia: dysregulated lipoprotein metabolism; and term preeclampsia: inflammatory pathways, notch signaling and ribosome assembly. These molecular changes were identified in placental samples collected months before the onset of maternal preeclampsia symptoms, suggesting causal rather than consequential roles in the disease process. To validate our findings, we investigated the function of Melanophilin (MLPH), which was significantly downregulated in CVS from term preeclampsia. MLPH loss disrupted syncytiotrophoblast fusion in vitro, triggering the production of anti-angiogenic factors known to drive preeclampsia.
Conclusion: Our study provides critical insights into the early pregnancy aberrations underlying preterm and term preeclampsia. This work represents a significant step towards unravelling the complex etiology of preeclampsia and paves the way for earlier detection and more effective management of this critical pregnancy complication.

SYM9.3.
DECODING HOW MATERNAL SIGNALS REGULATE TROPHOBLAST INVASION DURING HUMAN PLACENTAL DEVELOPMENT USING A HIGH-CONTENT IMAGING SCREEN

Elisa Magistrati, Sabine Reither, Hans-Rudolf Hotz, Tim-Oliver Buchholz, Jan Eglinger, Karolina Guja-Jarosz, Lhéanna Klaeylé, Margherita Y. Turco. *Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland*

Objectives: A key event in early placental development is the differentiation and invasion of extravillous trophoblast (EVT) into the maternal uterine lining. As EVT invade, they interact with numerous maternal cells that influence this process through the secretion of a variety of ligands. These maternal-fetal interactions are established during the first weeks of pregnancy and are critical for placental development, but a comprehensive knowledge is still lacking. The objective of this study is to perform a systematic analysis to identify which and how maternal signals influence EVT differentiation and invasion.
Methods: To this goal, we devised a high-content imaging screen. First, we used transcriptomics data of the first trimester maternal-fetal interface to perform a ligand-receptor interaction analysis and identify maternal signal that can potentially regulate EVT invasion. Then, we set up a 3D invasion assay using human trophoblast organoids in a 384-well format that is suitable for an image-based screen. Upon induction of EVT differentiation, the organoids are treated with our library of maternal secreted ligands, and their effects on the differentiation and invasive behavior of EVT are evaluated using image analysis tools.
Results: We identified 164 ligands secreted by maternal cells that may influence EVT differentiation and invasion, and compiled a library of 132 ligands for functional analysis. Using our 3D model of EVT differentiation, we assessed the effects of individual ligands and observed striking changes on the extent and pattern of EVT differentiation and invasion. Several ligands modulated key features of invasion, including the maintenance of cell-cell contacts, suggesting their role in shaping invasive properties of the trophoblast within the tissue context.
Conclusion: With our systematic and unbiased approach, we reveal the mechanisms by which maternal cells regulate trophoblast invasion, elucidating the first steps of placental morphogenesis. This study advances our understanding of the key maternal-fetal interactions essential for human placental development.

SYM9.4.
BENCHMARKING WORKFLOWS FOR EXTRACELLULAR RNA BIOMARKER DISCOVERY IN PREGNANCY

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Objectives: Pregnancy complications such as preeclampsia (PE) are a significant cause of maternal morbidity and mortality worldwide. Extracellular RNA (exRNA) biomarkers, including those of placental origin, have the potential to non-invasively identify women at risk of developing PE. Robust and cost-effective high-throughput methods for acquisition and analysis of exRNA data are needed for efficient discovery and validation of such biomarkers.
Methods: We tested a variety of manual and automated exRNA isolation methods from biofluids including plasma, serum, and urine using commercial kits. Small RNA-Seq libraries were prepared using the NEB Next Small RNA Library Prep kit. Different methods for long RNA-Seq library prep were tested including Takara's SMARTSeq v4 Ultra Low Input kit (urine), and the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian kit (plasma, serum). All library preparation methods were conducted at $1/5^{th}$ the manufacturer's recommended volume. The resulting data were normalized (CPM, TMM, or DESeq2) and assessed for variability and detection of differentially expressed features.
Results: We have developed an efficient and reproducible automated, miniaturized, high-throughput workflow for exRNA isolation, RNA-Seq library preparation, and data analysis. These methods utilize starting sample volumes of 250-500mL and substantially reduce the cost associated with RNA-Seq library preparation. Applying stringent quality control standards, our methods yield complex RNA libraries and high-quality RNA-Seq data, which can be mined using an optimized bioinformatics pipeline to identify RNA biomarkers for PE and other pregnancy complications.
Conclusion: Using these scalable methods, we have identified bivariate miRNA biomarkers of PE in maternal serum at the time of triage, including those of likely placental origin. Identification and stratification of women at risk of developing PE based on exRNA biomarkers in biofluids obtained using minimally invasive/non-invasive methods will enable better management of these pregnancies using a personalized medicine approach and greatly reduce the economic burden of disorders such as PE.

SYM9.5.
MAPPING THE HUMAN PLACENTA ACROSS GESTATION USING ADVANCED GENOMIC AND SPATIAL TECHNOLOGIES

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Objectives: As a component of the NIH Common Fund's Human BioMolecular Atlas Program (HuBMAP), our tissue mapping center is building a high-resolution, single-cell atlas of the human placenta across gestation. Using a suite of advanced genomic and spatial technologies, we aim to construct an integrated, three-dimensional, multiomic map that illuminates placental structure, development, and function throughout pregnancy.
Methods: We enrolled healthy pregnancies, and collected placental samples spanning early to term gestation, including both labored and non-labored term deliveries. For a subset of matched cases, we performed bulk RNA-seq and ATAC-seq, single-nucleus multiome profiling (10x Genomics), and spatial transcriptomics using the GeoMx Whole

Transcriptome Assay (Bruker Spatial Biology). Insights from these multimodal datasets guided the development of two custom placenta-focused panels: a 300-gene panel for targeted spatial transcriptomics using the Xenium v1 platform (10x Genomics), and a 28-antibody panel for spatial proteomics via imaging mass cytometry (Standard BioTools). Together, these tools enabled us to generate high-resolution, cell-type-specific spatial maps of gene and protein expression across gestation.

Results: Our multimodal strategy allowed us to track placental cell types and subtypes with high precision, revealing dynamic changes in their abundance and molecular signatures throughout gestation, along with insights into the cellular neighborhoods they occupy. We identified gestation-dependent, differentially expressed genes within trophoblast subtypes (Figure 1), and further characterized novel subclusters within each lineage. We also uncovered labor-associated gene expression patterns, mapping these shifts to specific cell types along with their spatial contexts.

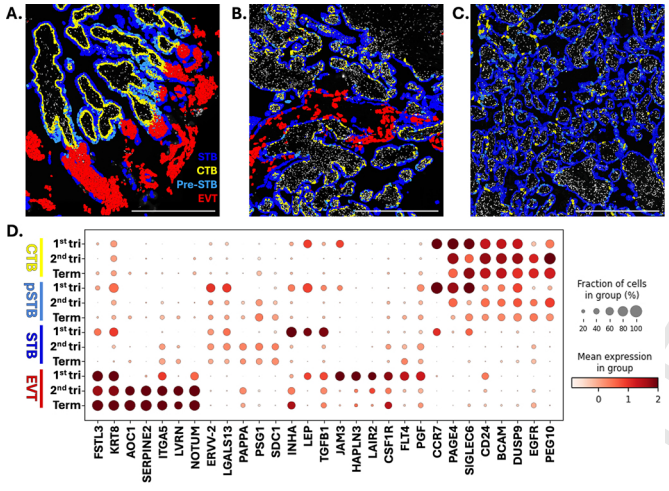

Figure 1. Xenium v1 spatial transcriptomics data generated with a custom 300-plex placenta-focused panel. **A-C.** Placental villi from a representative first trimester (**A**), second trimester (**B**), and term (**C**) placenta. Trophoblast are colored according to their subtype (STB, syncytiotrophoblast; CTB, cytotrophoblast; Pre-STB, pre-syncytiotrophoblast; EVT, extravillous trophoblast); nuclei are colored white. Scale bar represents 500 μm. **D.** DotPlot highlighting genes that are differentially expressed between trophoblast subtypes and across gestation (trimester indicated on y-axis).

Conclusion: We present a comprehensive, spatiotemporally resolved, and multimodal molecular atlas of the human placenta. This resource provides critical insights into the cellular and molecular mechanisms of placental development and function, offering a foundational reference for understanding normal pregnancy and identifying potential pathways implicated in pregnancy complications.

SYM9.6.
THE PLACENTAL TRANSCRIPTOME MEDIATES THE RELATIONSHIP BETWEEN PRENATAL PHTHALATE EXPOSURE AND PLACENTAL EFFICIENCY

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Objectives: The placental transcriptome can reveal how the maternal environment alters fetal and placental growth. Phthalates are pervasive plasticizer chemicals for which prenatal exposure has been linked to alterations in placental development as well as low birthweight and preterm birth. Our objective was to generate transcriptomic signatures of prenatal phthalate exposure and fetal growth and integrate these signatures to study the placentas role as a mediator and reveal shared biological mechanisms of toxicity.

Methods: This study was conducted within the PATHWAYS Global Alliance to Prevent Prematurity and Stillbirth cohort, part of the ECHO PATHWAYS consortium. Transcriptomic signatures were derived from individual genes and co-expressed gene modules generated from weighted gene correlation analysis. Linear models were fitted to estimate associations between placental gene expression at birth and maternal urinary phthalate metabolites as well as Birthweight/Placental weight (BW/PW) ratio, and birthweight adjusted for placental weight (BW_{adj}). All models were adjusted for confounding variables, and genes were considered differentially expressed at a false discovery rate <0.05. We performed high-dimensional mediation analysis to identify mediator genes and gene modules.

Results: We identified 1,652 genes and 22 gene modules whose expression was associated with metabolites of several different phthalates. $Birthweight_{adj}$ was associated with 515 genes and 11 gene modules, and BW/PW ratio was associated with 9 genes and 7 gene modules. Seven gene modules and 47 genes were associated with both prenatal phthalate exposure and $Birthweight_{adj.}$ 34 of these genes were significant mediators of indirect effects. These modules were enriched for genes involved in metabolic, gap junction, focal adhesion, and extra-cellular matrix communication pathways.

Conclusion: This study reveals mechanisms by which phthalates may disrupt fetal growth and establish the placental transcriptome as a mediator of this process. We identified perturbations in processes essential to the placenta's role in nutrient supply, hormone production, and detoxification.

P1.1.
CAN WE APPLY AI MODELS TO DIGITAL PLACENTAL PHOTOGRAPHS TO AUTOMATE AND IMPROVE MORPHOLOGICAL ASSESSMENTS?

Alison Gernand [1], Rachel Walker [1], Yimu Pan [1], Manas Mehta [1], Gwen Sincerbeaux [1], Kelly Gallagher [1], Lisa Bebell [2,3], Joseph Ngonzi [4,5], Drucilla Roberts [2,3], Janet Catov [6,7], Lauren Skvarca [8], Jeffery Goldstein [9,10], James Wang [1]. [1] *The Pennsylvania State University, State College, USA;* [2] *Massachusetts General Hospital, Boston, USA;* [3] *Harvard Medical School, Boston, USA;* [4] *Mbarara University of Science and Technology, Mbarara, Uganda;* [5] *Mbarara Regional Referral Hospital, Mbarara, Uganda;* [6] *Magee-Womens Research Institute, Pittsburgh, USA;* [7] *University of Pittsburgh, Pittsburgh, USA;* [8] *University of Pittsburgh Medical Center, Pittsburgh, USA;* [9] *Northwestern Memorial Hospital, Chicago, USA;* [10] *Northwestern University, Chicago, USA*

Objectives: Measuring placental size after delivery should be easy but current methods are time-consuming and error-prone. We developed PlacentaVision using artificial intelligence(AI)-based models, to automatically, accurately, and precisely measure placentas from a digital photograph. Here we aimed to compare placental morphology between human measurements using gross pathology examination and automated PlacentaVision measurements.

Methods: PlacentaVision is a multi-site study to assess placental morphology, features, and pathologies from digital photographs. We built a large dataset of digital placenta photographs and clinical data from singleton births at three large hospitals: Northwestern Memorial (Chicago; n=28,149), Magee Womens (Pittsburgh; n=1383) and Mbarara Regional Referral (Uganda, n=1672). Photographs and pathology reports were from the medical record for Northwestern, part of the MOMI biobank study for Magee, and from our prospective cohort studies for Mbarara. We divided data into training and testing sets to build PlacentaVision models. We defined long and short axis length by Amsterdam criteria. We compared measurements from the gross exam and PlacentaVision by calculating the difference and using Bland-Altman; we stratified by regular or irregular disc shape.

Results: Mean (SD) disc length was 19.6 (3.6) and 18.6 (3.2)cm from PlacentaVision and human measurement, respectively, with a difference of 0.96 (2.98)cm. Disc width was 16.4 (2.6)cm and 16.0 (2.5) cm from PlacentaVision and human measurement, respectively, with a difference of 0.39 (2.24)cm. Human measurements were lumped near whole cm. The difference in length was lower for discs with regular (0.64 [2.51] cm) vs. irregular shape (2.73 [4.45] cm); results were similar by shape for width. Only 42% of length and 48% of width measurements were within 1cm, comparing humans and PlacentaVision. Bland-Altman limits of agreement were -4.89-6.79cm for length and -4.00-4.78cm for width.

Conclusion: Human measurements were slightly smaller overall and were farther from "true" PlacentaVision AI-measurements when shape was irregular.

P1.2.
3D VIRTUAL HISTOLOGY OF PLACENTAL TISSUE – WHOLE ORGAN TO BIOPSIES: ANALYSING VASCULATURE WITH AN NNU-NET TO EXAMINE PRE-ECLAMPTIC PLACENTAS

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Objectives: Disruptions to the placental vasculature are central to conditions like pre-eclampsia, yet conventional two-dimensional (2D) histology fails to capture the full complexity of vascular architecture as important three-dimensional (3D) factors like blood flow, vascular branching and connectivity. This study aims to apply 3D virtual histology to placental tissue using phase contrast tomography and deep learning-based segmentation to investigate vascular morphology in both whole placentas and biopsies. Our goal is to identify structural biomarkers associated with pre-eclampsia by enabling volumetric analysis of placental vasculature.
Methods: Placental tissue samples from both healthy and pre-eclamptic pregnancies were imaged using high resolution 3D phase contrast tomography in laboratory μCT scanner, at the Synchrotron at the P10 beamline GINIX end station (DESY, Hamburg) and at BM18 (ESRF, Grenoble). The resulting data sets represent the tissue structure. This allows to investigate the morphology of the vascular system. In order to segment the vascular structure we manually annotated datasets and trained a neural network (nnU-Net). Quantitative metrics—including vascular density, branching, and connectivity—were derived from the segmented 3D datasets for comparative analysis between healthy and pathological samples.
Results: The nnU-Net model successfully segmented vasculature in biopsies with high accuracy, demonstrating the potential of deep learning for automated analysis of complex tissue structures. 3D visualization of the segmented vasculature allows for a detailed exploration of spatial patterns and variations and indicates that 3D virtual histology, in combination with deep learning-based segmentation, can offer valuable insights into the pathophysiology.
Conclusion: This study demonstrates the effectiveness of combining 3D phase contrast tomography and nnU-Net deep learning segmentation for the analysis of placental vasculature. The ability to visualize and analyse vascular patterns in 3D opens up new avenues for understanding placental diseases and may lead to the development of improved diagnostic tools for pre-eclampsia and could be used to identify diagnostic biomarkers.

P1.3.
DEVELOPMENT OF AN AUTOMATED SAMPLER OF HUMAN PLACENTA EXPLANTS

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Objectives: Placenta ex vivo explants serve as an excellent model for human toxicology studies. However, their pharmacological application remains limited due to the time-consuming nature of manual sample collection and the required specialized knowledge. The Optobiopsy project aims to develop an automated biopsy system to standardize and expedite this process.
Methods: A placenta sampling chamber was engineered in collaboration with biomedical scientists, engineers, and software developers. Biopsy modules were tested on fresh placentas using modified forceps and needles, simulating robotic movement directions manually. A build-in camera captured high-resolution images of whole placentas from fixed distance under different exposure settings. Manual labeling of surface morphology from both, the fetal and maternal side was performed using open source image annotation software. By machine learning different tissue structures should be identified for automated explant sampling.
Results: Manual testing revealed that fetal-side sampling is particularly challenging due to amnion and chorion membranes, requiring angled, stronger, or faster motions. Both, sampling chamber and annotation software were optimized for their usability during pilot trials. So far, more than 150 placentas—including healthy, fetal growth restriction, twin placentas, and those with abnormal morphology—have been imaged and added to the database. Labelled datasets were successfully used to train a machine learning model for automated recognition of placental surface structures. Training the model on 100 maternally labeled images enabled the system to achieve an 89% precision and 94% recall rate in recognizing cotyledons.
Conclusion: A comprehensive image catalogue of over 150 placentas was established to train a machine learning model for placental surface morphology recognition. The manually tested biopsy module will be integrated into an automated system to enhance efficiency and yield. Multispectral imaging is under evaluation for improved tissue differentiation. This approach supports standardized explant collection and advances the placenta's role as a pharmacological model system.
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P1.4.
3D BIOPRINTING USING PEG-BASED EXTRACELLULAR MATRIX DRIVES EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION IN ORGANOIDS

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Objectives: Studying physiological and pathological placentation is challenging due to the lack of reliable models of human pregnancy. Placental organoids, three-dimensional cultures derived from stem cells, offer a new experimental model of placental development. However, most organoid cultures rely on Matrigel which varies between batches and cannot be tuned for composition and stiffness. We aimed to comprehensively assess the impact of extracellular matrix on trophoblast organoids by comparing those generated by Matrigel-embedding and bioprinting within a biocompatible synthetic matrix.
Methods: ACH-3P trophoblast cells were embedded in Matrigel or bioprinted in a polyethylene glycol (PEG)-based matrix using a RASTRUM platform (Inventia Life Science) for up to 12 days. Organoid formation and growth were captured by live cell imaging and viability analysed by Alamar Blue assay. Organoids were harvested for analysis of trophoblast differentiation by confocal microscopy, single cell RNA sequencing and proteomics using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Both Matrigel- and bioprinting PEG-based organoid methodologies were validated using the trophoblast stem cell line, CT29.
Results: Trophoblasts encapsulated within the Matrigel and bioprinted PEG matrix self-formed organoids within 2-3 days, demonstrating invasive properties within the matrix. The presence of key trophoblast subtypes was confirmed by immunofluorescence labelling for markers E-cadherin, HLA-G and β-hCG. Single cell RNA sequencing revealed a greater proportion of extravillous trophoblasts and comparatively fewer syncytiotrophoblasts within bioprinted organoids compared to Matrigel-derived. This was confirmed by proteomics, with bioprinted organoids displaying significantly increased levels of extravillous trophoblast markers, HLA-G and ITGA5.
Conclusion: Here, we present a novel approach to placental organoid generation with highly tunable and reproducible synthetic PEG-based hydrogels using 3D bioprinting. This study highlights an increased

capacity to study trophoblast responses to their environment and differentiation pathways. This platform shows promise for high-throughput drug and biomarker screening for placental dysfunction disorders.

P1.5.
THE IMPACT OF FETAL SEX ON PLACENTAL VASCULAR STRUCTURE AND FUNCTION IN FETAL GROWTH RESTRICTION

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Objectives: Whilst female fetuses prioritise placental reserve capacity, males prioritise fetal growth, limiting feto-placental adaptability and placing them at greater risk for adversity in pregnancy. However, sex-specific differences in placental vascular structure and function in normal pregnancy or Fetal Growth Restriction (FGR) remain unclear. Here we used computational models to investigate how sex-specific placental vascular anatomy influences placental haemodynamics and contributes to the pathophysiology of FGR.
Methods: Population-level geometric models of normal male and female placental vasculature were initially parameterised from literature data. Blood flow simulations and sensitivity analysis for changes in capillary density was performed. Fetal aspects from normal (female n=20, male n=22) and FGR (female n=3, male n=6) placentae were imaged, and chorionic plate areas and arteries mapped. Maps were skeletonised for branching-analysis and further model refinement. Blood flow was simulated in each model.
Results: Initial models predicted that normal male placentae required reduced driving pressures to achieve equivalent umbilical artery flow to normal female placentae. There were no sex-specific differences in model sensitivity to capillary density. Anatomically, branching-analysis revealed reduced chorionic artery diameters and branching density in male (but not female) FGR placentae. Whilst, female FGR placentae had significantly reduced weights (P=0.0259) and chorionic surface areas (P=0.0289), male FGR placentae had significantly reduced weights (p<0.0001), but no change in chorionic surface area indicating a thinner placenta. Refined models including anatomical data predicted 2.6-3 fold greater vascular resistance in male FGR placentae compared to female FGR or controls of either sex, with increased driving pressures required to achieve equivalent umbilical artery flow compared to all other groups.
Conclusion: Structural differences in male FGR placentae are predicted to have greater haemodynamic impact, potentially increasing male fetal vulnerability to severe FGR outcomes. Further data collection and model refinement is needed to parameterise sex-specific differences in meso- and microvasculature, to understand their role in FGR.

P1.6.
IMPACT OF PLACENTAL EXTRACELLULAR VESICLES FROM SEVERE PREECLAMPSIA ON RENAL CELL FUNCTION IN A 3D KIDNEY-CHIP MODEL

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Objectives: Preeclampsia (PE) is the most common cause of pregnancy-related acute kidney injury. The placenta releases extracellular vesicles (EVs) into the maternal circulation throughout pregnancy, with increased numbers in PE. Placental EVs can be trafficked to distant maternal organs including the kidney, but little is known about their impact on proximal tubules and their contribution to the pathophysiology of PE. We investigated the impact of placental EVs from severe PE (sPE) on the transcriptome of renal proximal tubule cell types using a 3D microfluidic human kidney-chip model (Emulate, inc.).
Methods: Placental EVs were isolated using size exclusion chromatography following placental explant culture from normal or sPE pregnancies. Primary human renal proximal tubular epithelial cells (hRPTECs) and human renal glomerular microvascular endothelial cells (hRMVECs) were seeded in the top and bottom channels respectively of polydimethylsiloxane chips, and treated with normal (n=2, 4 replicates) or sPE (n=2, 2 replicates) placental EVs at a dose of $1x10^{10}$ EVs/mL for 72h. RNA-Seq was conducted on placental EV-treated hRPTECs and hRMVECs, data were analyzed using established pipelines, and differential expression analysis was conducted using DESeq2 (FDR<0.01).
Results: Placental EVs from sPE altered the expression of >1000 transcripts in hRPTECs and hRMVECs compared to normal placental EVs. Molecular pathways analysis (gProfiler) showed that these transcripts were significantly enriched (FDR<0.01) for genes involved in the complement cascade in hRPTECs, and interleukin signaling and apoptosis in hRPTECs and hRMVECs. In hRPTECs, sPE placental EVs upregulated >20 transcripts involved in complement and coagulation, including fibrinogen (FGA, FGB, FGG). Fibrinogen release from hRPTECs was measured in media effluents from kidney-chips using a commercial immunoassay, and was significantly increased in response to sPE EVs (p<0.01).
Conclusion: Placental EVs from sPE altered pathways involved in inflammation and apoptosis in renal proximal tubule cell types, potentially contributing to proximal tubular damage in sPE.

P1.7.
COMPARING THE HAEMODYNAMICS OF PLACENTAL VASCULAR MODELS GENERATED USING 2D IMAGES AND MICROCT

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Objectives: Computational models of the placental vasculature can be used to predict the haemodynamic consequences of pathology. Structural characteristics of placentae can be obtained ex-vivo by high-resolution 3D imaging, but 2D photographs of the chorionic plate vasculature are far more accessible. This study aims to determine if predicted haemodynamics in placental vasculature models derived from photographs of the chorionic plate are comparable to those obtained from microCT of the same placenta.
Methods: Two placentas with differing cord insertions was perfused with contrast agent, photographed and microCT imaged. Visible arteries and placental outlines were extracted from each image type and a volume filling branching algorithm used to create a synthetic vascular network. A computational blood flow model was used to simulate haemodynamic function in the synthetic vascular trees, and flow at terminal vessels and insertion, and tree resistance were compared. To test robustness of the photograph-derived model, an analysis on response to vessel occlusion was conducted on umbilical artery flow and vessel resistance.
Results: The chorionic vasculature segmented from photographs (central and eccentric) compared well to micro-CT, with correspondence in identifiable vessels from each modality. Computational simulations of vascular resistance were similar between micro-CT and photographs (<0.3 Pa/mm3 difference). A 10% increase in umbilical artery flow rate resulted in a 6.7% (micro-CT) and 7.2% (photograph) increase in umbilical arterial pressure. Progressively obstructing the blood flow in chorionic vessels produced similar trends in resistance between modalities, with <5% difference in model predictions of the relationship between the level of obstruction and change in resistance. However, the two models showed differences in blood flow heterogeneity throughout the placenta.

Conclusion: Vascular networks generated from 2D photographs approximate the overall haemodynamics of high-resolution microCT-derived networks from the same placenta and responses to perturbation were comparable. This suggests that the photograph-based method offers a viable alternative for simulating placental haemodynamics.

P1.8.
DOCETAXEL TREATMENT OF HUMAN VESSELS IN AN IN VITRO CULTURE BASED ON PLACENTA TISSUE

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Objectives: The aim was to establish a vessel in vitro culture to investigate toxic effects of drugs or chemicals in human tissue. We used the chemotherapeutic drug docetaxel as an example of breast cancer treatment during pregnancy. Effects on fetal vessels of the placenta were examined, with particular interest in senescence, over a 14-day long-term culture.
Methods: After dissection of vascular explants from the fetal side of the placenta immediately after birth, various concentrations of docetaxel mimicking a bolus dose were added the following day. Subsequently, arteries and veins were cultured for 14 days. To assess cell activity, MTS cell viability assay was performed on days 1, 7, and 14. An interleukin-6 ELISA was conducted to monitor the stress response. To investigate morphological changes and differentiation, histological staining (HE, Movat pentachrome staining of Verhöff, and multiplex immunofluorescence) was performed. Furthermore, qPCR for the senescence markers p16 and p21 was carried out to measure senescence across the entire vascular explant. Multiplex immunofluorescence staining of the aforementioned senescence markers in the vascular explants was performed to differentiate the senescent cells.
Results: Analyses show vessel tissue viability over a 14-day period under control conditions. We were able to distinguish and track different cell types of the typical vascular layers, such as endothelial cells, connective tissue, or smooth muscle cells. Preliminary results of the toxicologic evaluations showed a decrease in cell activity, morphological changes, and an increase in weight with rising concentrations of docetaxel, which is also microscopically evident through cell hypertrophy. Furthermore, histological alterations and remodeling processes in the vascular explants have been demonstrated through long-term culture. Additional results are currently being analyzed.
Conclusion: This model enables long-term toxicological testing of drugs in fetal placental vessel tissue, indicating senescence and structural changes after docetaxel exposure.
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P1.9.
MATERNAL HYPERTENSION DISORDERS IN AN UNEQUAL WORLD: GLOBAL DISPARITIES, TEMPORAL TRENDS, AND FUTURE BURDEN: ANALYSIS OF GLOBAL BURDEN OF DISEASE (GBD) STUDY 2021

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Objectives: Maternal Hypertensive Disorders (MHD) cause significant maternal and perinatal morbidity, yet global disparities remain unclear. This study aims to conduct a comprehensive epidemiological assessment of MHD, examine global disparities, analyze temporal trends, and project future burden to inform targeted public health strategies.
Methods: Using 2021 Global Burden of Disease (GBD) database, we analyzed MHD burden trends (1990–2021) in age-standardized incidence rate (ASIR), age-standardized prevalence (ASPR), age-standardized mortality rate (ASMR), and disability-adjusted life years (DALYs) at global, regional, and national levels. Geographic disparities and the impact of Socio-Demographic Index (SDI), were examined by stratifying data into SDI quintiles. Joinpoint regression, decomposition, and health inequality analyses assessed trends and disparities, while Bayesian Age-Period-Cohort (BAPC) model predicted future incidence.
Results: Low-SDI regions had the highest increases in incidence (0.631, 95% UI: 0.602–0.663) and prevalence (0.618, 95% UI: 0.581–0.659), while high-middle SDI regions showed the greatest declines in ASMR and age-standardized DALYs. The burden was primarily driven by population and epidemiological shifts, with the highest impact in young adults (20–34 years old). Central Sub-Saharan Africa had the highest ASMR (4.173, 95% UI: 2.926–5.755) and DALYs (255.216, 95% UI: 182.058–348.264). East Asia recorded the lowest ASIR (108.743, 95% UI: 84.212–140.959) and ASPR (23.84, 95% UI: 14.514–35.824). Nigeria had the highest DALYs (36.291, 95% UI: 22.35–53.409), while India led in incidence, prevalence, and mortality. San Marino had the lowest global burden. Health inequities persist despite partial mitigation, with low-SDI regions furthest from the health frontier. BAPC predicts a global and Chinese ASIR decline despite rising MHD cases in China post-2030.
Conclusion: The global MHD burden has declined, but disparities persist. BAPC predicts a continued ASIR decline, suggesting effective maternal health management is reducing the burden. Strengthening prenatal care and hypertension control remains essential.

P1.10.
THE NEXT STEPS IN CLINICAL TRANSLATION: SULFORAPHANE IN THE MATERNAL AND FETAL CIRCULATION

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Objectives: Preeclampsia is characterised by hypertension with maternal end-organ dysfunction and/or fetal growth restriction. In recent years, in vitro studies have demonstrated that sulforaphane, a naturally occurring antioxidant found in broccoli sprouts, can mitigate oxidative stress and inflammation in placental tissue, and protect against endothelial dysfunction in human blood vessels. These findings suggest that sulforaphane, via a broccoli sprout extract, may offer protective effects in preeclampsia. This study aimed to compare circulating sulforaphane concentrations from three extracts in pregnant women and determine whether sulforaphane crosses the placenta to the fetus.
Methods: Healthy non-pregnant (n=18) and uncomplicated pregnant women (n=18, gestation between 28-36 weeks) were assigned to one of three broccoli sprout extracts (EnduraCell, AVMACOL®, or BROQ™; ~21mg sulforaphane). Blood was collected over 8 hours and sulforaphane levels were measured using liquid chromatography-mass spectrometry (LCMS). Blood pressure and heart rate were recorded using the Uscom BP+ device. Additionally, uncomplicated pregnant patients (n=8) scheduled for elective caesarean sections received a single dose of EnduraCell pre-operation, with maternal blood, urine, umbilical cord blood, and placenta collected at birth. A second dose was administered postnatally, followed by maternal blood and breast milk collection two hours later.
Results: The area under the curve of sulforaphane was analysed and compared across three extracts in the non-pregnant group (EnduraCell: 343.1 ± 90.13, AVMACOL®: 90.97 ± 30.78, BROQ™: 245.3 ± 17.67) and pregnant participants (EnduraCell: 179.2 ± 22.63, AVMACOL®: 42.05 ± 15.67, BROQ™: 105.0 ± 10.66). Sulforaphane was detected in maternal (70.10ng/ml ± 11.90) and umbilical cord blood (vein: 23.91ng/mL ± 3.11, artery: 18.81ng/mL ± 2.36), placental tissue (10.99ng/mg ± 2.11) and breast milk (1.33ng/mL ± 2.29).
Conclusion: Different extracts demonstrated distinct circulating sulforaphane profiles in pregnant and non-pregnant women. Further, sulforaphane is present in umbilical cord and breast milk, providing the world's

first evidence of sulforaphane maternal-fetal transfer and opening avenues for future research into fetal implications.

P1.11.
SYMPTOMATIC PEDUNCULATED LEIOMYOMAS IN PREGNANCY; CASE REPORT AND REVIEW OF THE LITERATURE

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Objectives: Symptomatic pedunculated leiomyomas in pregnancy; Case report and review of the literature
Methods: Evaluation 37 case reports
Results: Case report: A 36-year-old Caucasian primigravida was referred symptomatic at 16+0 weeks due to a 13,5 cm myoma causing pain, constipation, urine retention and dysesthesias. Our patient underwent myomectomy at 17+0 weeks. One pedunculated leiomyoma was successfully removed.
Conclusion: Myomectomy can be performed and are safe for pedunculated fibroids in pregnancy. Based on the size of the fibroids and expected adhesions, a laparotomy is a safe option and is not a contraindication for vaginal birth in the case of pedunculated fibroids. Myomas larger than 10cm should be removed by laparotomy.

P1.12.
ARE THERE IMMUNOLOGIC EXPLANATIONS FOR THE INCREASED RISK OF POSTPARTUM HEMORRHAGE IN OOCYTE DONATION PREGNANCIES?

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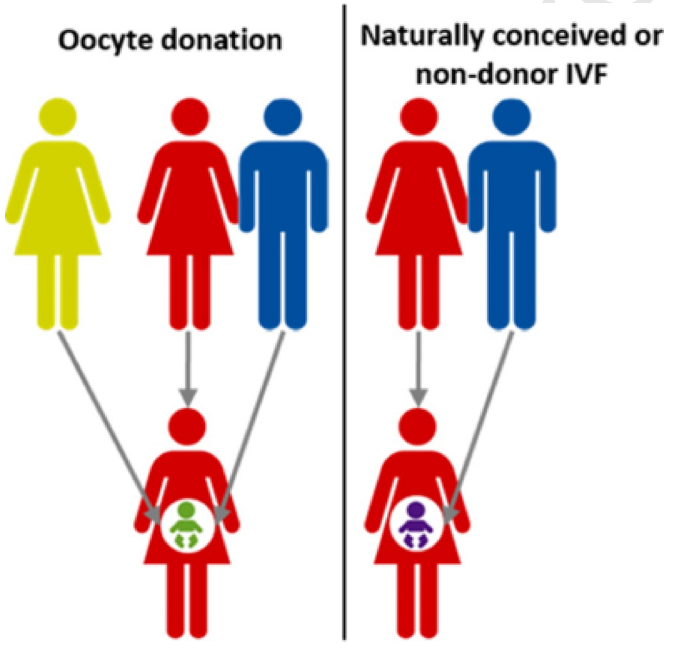
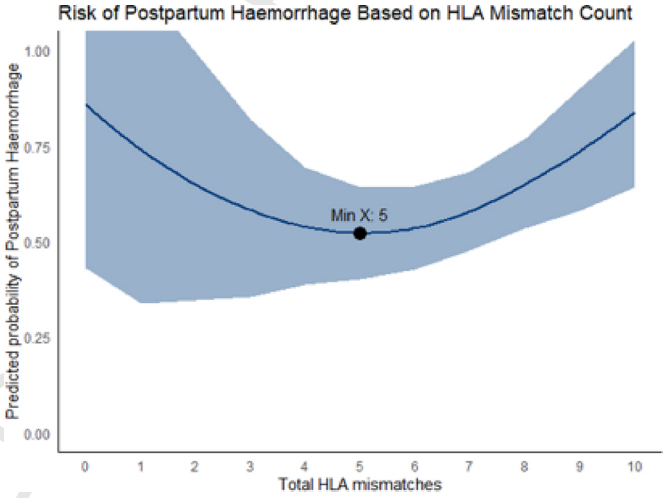
Objectives: Oocyte donation (OD) is an assisted reproductive technology that involves an oocyte donor and recipient.



As OD use increases, obstetric complications, including postpartum hemorrhage (PPH), are becoming more frequent. PPH can result from abnormal placentation, but the immunogenetic and immunohistochemical aspects of placentation in OD pregnancies are not fully understood.
Objective: A previous meta-analysis by our research group found OD to increase the risk of PPH compared to natural conception and autologous oocyte IVF, suggesting an immunological factor may contribute. This study aims to investigate the pathophysiology of PPH in OD pregnancies.
Methods: This nested case-control study, using data from the DONOR cohort, includes singleton OD pregnancies. Cases with PPH (>500cc) are compared to controls without PPH. Donor-recipient relationship was assessed in three categories: unrelated, related 1st and 2nd degree. Placental pathology is analyzed, and fetal-maternal HLA typing (HLA-A, -B, -C, -DRB1, -DQB1) is conducted using maternal and umbilical cord blood to quantify mismatches. PIRCHE scores are be calculated (this score focuses on predicting how well the recipient's immune system will recognize and respond to the donor based on HLA epitopes).



Results: Donation by relatives shows a significantly lower risk of PPH compared to unrelated donors. There was no significant difference between the individual loci mismatches, class-based mismatches, total mismatch count or PIRCHE scores. However, the predicted probabilities showed trends that extreme dissimilarities in total mismatch count are associated with increased risk, particularly for 9 and 10 mismatches reached significance. Severe PPH (>1000 mL) was associated with a higher incidence of villitis basalis.
Conclusion: While there is no proven increased risk for PPH related to HLA typing, preliminary analysis shows that both extreme dissimilarity and similarity increase the risk. Indicating that a more average number of mismatches might be beneficial. Further analysis is necessary to properly investigate this non-linear relation.

P1.13.
CLUSTERING ANALYSIS FOR THE ASSESSMENT OF CLINICAL OBSERVABILITY AMONG ETIOLOGICAL SUBTYPES OF PREECLAMPSIA

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Objectives: The purpose of this work is to assess the clinical utility of our previously discovered etiological subtypes, which are defined by a maladaptive placenta ("canonical"), a maladaptive maternal immune response ("immunologic") and a maternal cardiovascular maladaptation with no placental or fetal involvement ("maternal").
Methods: We repurposed patient information collected from the Screening for Pregnancy Endpoints (SCOPE) international cohort study (n = 278) and sought to apply a novel tool, Similarity Network Fusion (SNF) Metaclustering, to refine diagnoses according to these potential subtypes, exclusively through data at the clinical level.
Results: From this unsupervised learning approach, we found a highly stable 3-cluster solution of PE cases. To assess cluster model validity, 1000 resamplings of 80% of the data were generated and pairwise comparisons of the resampled solutions had a good mean pairwise adjusted Rand index (0.612), greater than 0.5. A comparison of observations co-clustering

between the candidate solution and the resampled solutions further shows stability, with observations mirroring the co-clustering 84.6% of the time. We observed support for our past observations of PE etiological subtypes, detecting a "canonical" cluster showing an alignment of low placental weight and a high uterine artery Resistance Index with more preterm births, more admissions to the neonatal unit, more small-for-gestational-age babies and the highest measurements of antepartum proteinuria; an "immunologic" cluster showing an alignment of shorter length of sexual relationship with the father and admissions to the neonatal unit; and a "maternal" cluster showing an alignment of a family history of ischemic heart disease in the cases with term deliveries, with higher Apgar Scores and fewer admissions to the neonatal unit.
Conclusion: Overall, our results reinforce growing evidence that prognosis heterogeneity arises from distinct mechanisms, advancing patient subtyping as a promising avenue for developing better targeted diagnostic screens and treatment plans.

P1.14.
PLACENTAL CRISES: DISRUPTIVE SELECTION AND MATERNAL UNDER-INVESTMENT AS THE FOUNDATIONS OF MAMMALIAN PLACENTAL EVOLUTION AND DYSFUNCTION

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Objectives: Historically, investigations into the evolution of the mammalian placenta have been grounded in 'the efficiency paradigm', the assumption that certain placental configurations permit easier nutrient exchange, but this paradigm has struggled to explain the diversity of mammalian placentation strategies. We aimed to re-evaluate our understanding of mammalian placental structures and life history strategy to arrive at a new explanatory paradigm for placental evolution in mammals.
Methods: Here, we use multidimensional plotting of recorded placental structures, quantitative metrics for mammalian maternal investment, and illustrative computational modelling of physiological processes, to propose the 'placental cost paradigm' of mammalian placental evolution.
Results: We find that mammal placental structures co-occur across scales and exist at two discrete clusters of possible morphological combinations. Species with Cluster 1 placentas (e.g hemochorial labyrinthine) invest significantly less in pregnancy than Cluster 2 placentas (e.g epitheliochorial villous) ($p < 0.001$). We propose that the ancestral placental design in mammals was a result of maternal under-investment associated with altricial r-selected offspring and that, when a group expands into a larger-bodied, longer-lived niche, this design induces a 'placental crisis' characterised by chronic gestational dysfunction, triggering an arms race through the interaction of disruptive selection and materno-fetal conflict. As a group, primates invest significantly less in pregnancy than any other group, and we propose this, along with inappropriate Cluster 1 placentation, as the foundation of primate-specific gestational orders, like pre-eclampsia.
Conclusion: We argue that the ancestral mammalian placenta is not a streamlined 'highly efficient' design, but rather a product of low maternal investment, with fitness costs that manifest as gestational demand increases. We conclude that the ancestral mammalian placental design was not an innovation that allowed placentation to dominate the clade, but rather an idiosyncrasy of mammal-specific biology, which may have hindered mammalian expansion into larger-bodied niches.

P1.15.
ISOLATION-FREE IDENTIFICATION AND PHENOTYPING OF FIRST-TRIMESTER EXTRAVILLOUS TROPHOBLASTS RECOVERED FROM CERVICAL FLUID

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Objectives: Placental malplacentation underlies pregnancy disorders such as pre-eclampsia (PE) and fetal growth restriction (FGR), yet current blood-based screening assays perform optimally only in mid-gestation. Direct interrogation of placental cells during the first trimester could transform risk stratification, but existing trophoblast retrieval and isolation from the cervix (TRIC) is technically demanding. We therefore developed an isolation-free, swab-based workflow coupled to mass cytometry to sensitively identify and characterize extravillous trophoblasts (EVTs) in first-trimester cervical fluid.
Methods: Endocervical specimens (9 – 14 weeks gestation) were collected with flocked swabs, shipped and stored under defined conditions, and processed without enrichment. Samples were bar-coded with palladium, spiked with metallotope-labeled reference cell lines, and stained with a Cytometry by Time of Flight (CyTOF) panel targeting HLAG, CK7, PAPPA, CD45, and HLA-ABC, among others. Signal drift was corrected by dual normalization to EQ™ beads and reference-cell intensities. EVT gates were defined as HLAG+ CD45– CK7+. Stability of cell recovery and antigen expression was assessed after shipping and –80 °C storage.
Results: HLAG+ CD45– CK7+ EVTs were reproducibly detected in every sample (n=22). PAPPA was highly expressed within this gate, confirming placental origin, whereas maternal cells lacked these proteins. Cell yield, viability, and biomarker intensity were unchanged after shipping or frozen storage. Preliminary analyses indicated significantly different biomarker expression profiles in EVTs from pregnancies later diagnosed with PE/FGR, relative to uncomplicated controls.
Conclusion: We present the first isolation-free protocol for quantitative phenotyping of cervical EVTs in early pregnancy. The platform is robust to routine clinical logistics and reveals altered protein expression profiles in pregnancies destined for malplacentation. Integration of this assay into first-trimester care could enable earlier, biology-based prediction of PE and FGR.

P1.16.
FSHR EXPRESSION IN GRAVID UTERINE SMOOTH MUSCLE CELLS AND ITS DOWNREGULATION IN UTERINE ATONY REVEALED BY SINGLE-NUCLEUS TRANSCRIPTOMICS

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Objectives: To investigate the cellular localization of follicle-stimulating hormone receptor (FSHR) in human myometrium at term, and to evaluate its differential expression in patients with uterine atony, a major contributor to postpartum hemorrhage.
Methods: We performed single-nucleus RNA sequencing (snRNAseq) on biopsies from the lower uterine segment of term cesarean deliveries (n=15). Cell populations were identified and annotated using canonical markers. For comparative analysis, we selected 4 samples from patients with uterine atony and 4 matched controls. Differential gene expression was analyzed in uterine smooth muscle cells (uSMC). FSHR expression was validated by RNA in situ hybridization (RNAscope), immunofluorescence, and Western blot in independent patient samples.
Results: snRNAseq identified 19 distinct cell types, including two smooth muscle populations: oxytocin receptor–positive uterine smooth muscle cells ($OXTR^+$ uSMCs) and neurogenic locus notch homolog protein 3–positive vascular smooth muscle cells ($NOTCH3^+$ vSMCs). FSHR transcripts were detected in uSMCs and were significantly downregulated in atony samples (log2FC −2.3, adjusted $p < 0.0001$). Validation assays confirmed FSHR presence at the RNA and protein level in uSMCs across all samples.
Conclusion: This study provides single-nucleus resolution of gene expression in the human gravid uterus and identifies transcriptomic alterations in uterine atony. FSHR is expressed in uterine smooth muscle cells and shows reduced transcript levels in atony, suggesting a potential

role in regulating myometrial tone. These findings raise the possibility that FSH–FSHR signaling could represent a novel therapeutic target for improving uterine contractility.

P1.17.
TRANSCRIPTOMIC ANALYSIS OF THE HUMAN UTERUS IN PRETERM LABOR VERSUS PRETERM NON-LABORING REVEALS IMMUNE AND REGULATORY SIGNATURES

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Objectives: Preterm labor (PTL) is a major cause of neonatal morbidity and mortality. While inflammation is implicated, the molecular mechanisms driving the shift from uterine quiescence to contractility remain unclear. This study aimed to identify transcriptomic differences in uterine tissue from women with PTL versus preterm non-laboring controls, focusing on immune pathways and candidate regulators of contractile activation.
Methods: Uterine smooth muscle samples were collected from the midline of the hysterotomy during preterm cesarean deliveries from women in spontaneous labor ($n = 4$) and non-laboring controls ($n = 8$). Samples were obtained with informed consent under IRB-approved protocols. Bulk RNA sequencing was performed, and differentially expressed genes (DEGs) were identified using an adjusted p-value<0.1 and $\log_2$ fold change $\geq$1. Functional enrichment was assessed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.
Results: A total of 46 differentially expressed genes (DEGs) were identified, including 33 upregulated and 13 downregulated in PTL. Upregulated genes reflected robust activation of immune pathways, including markers of neutrophil activation and infiltration (NLRP3, ICAM1, CXCR1), immune regulation and antigen presentation (CD74, LILRB4), and cytokine receptor signaling (TNFRSF10C). GO and KEGG pathway analysis highlighted biological processes such as regulation of T cell differentiation, lymphocyte activation, leukocyte adhesion, and hemopoiesis. Conversely, ADORA1, which encodes the adenosine A1 receptor, a G protein-coupled receptor that inhibits cAMP and reduces smooth muscle contractility, was significantly downregulated in PTL ($\log_2$FC = –2.8, adj. p = 0.06), suggesting a loss of tonic inhibitory signaling that may contribute to myometrial activation.
Conclusion: PTL is characterized by a dual transcriptomic profile associated with upregulation of immune and inflammatory mediators, and reduced expression of ADORA1, a key regulator of uterine quiescence. ADORA1 emerges as a mechanistically relevant gene that may contribute to human myometrial activation and represents a potential novel target in preterm labor.

P1.18.
PER- AND POLYFLUOROALKYL SUBSTANCES IN THE MOUSE PLACENTA: DETECTION USING 19-F NMR AND THEIR IMPACT ON PLACENTAL METABOLISM

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Objectives: Per- and polyfluoroalkyl substances (PFAS) are a class of persistent organic pollutants that are used in a variety of products including cookware, cosmetics and firefighting foams. Legacy PFAS (e.g., perfluorooctanoic acid, PFOA) have been banned following the link to numerous health issues. However, these "forever chemicals" remain in the environment, potentially causing adverse health effects at all stages of life including pregnancy. Our group recently detected fluorotelomer ethoxylates (FTEOs), a novel PFAS, in dust samples taken from healthcare facilities. The bioaccumulation potential and toxicity of these novel compounds are unknown. The objective of this abstract is to investigate bioaccumulation of legacy and novel PFAS in the placenta and determine their impact on placental metabolism.
Methods: Pregnant CD-1 mice were exposed to PFOA or FTEOs through their drinking water. To investigate placental metabolism, ^{1}H high-resolution solid-state magic angle spinning nuclear magnetic resonance (MAS NMR) was used. Bioaccumulation in placental tissue samples was determined using ^{19}F solid-state MAS NMR.
Results: Maternal exposure to PFOA and FTEOs had a significant impact on the placental metabolome, with the relative concentration of several essential nutrients for fetal development (e.g., lysine, glucose) altered in the PFAS-exposed groups. The effect was dependent on the type of pollutant. PFAS was found to bioaccumulate in the placenta and the detection frequency was higher for male placentas compared to females.
Conclusion: Exposure to both legacy and novel PFAS results in alterations in placental metabolism and biochemical pathways. These adverse prenatal exposures may predispose individuals to metabolic syndromes in adulthood. ^{19}F solid-state MAS NMR is a promising tool for detection of PFAS in the placenta. The observed increase in detection in male placentas is consistent with males being at increased risk of complications following exposure to PFAS. This study emphasizes that efforts should be made to minimize exposure to PFAS during pregnancy.

P1.19.
THE ROLE OF DEL-1 IN THE ENDOMETRIUM: IMPLICATIONS FOR ENDOMETRIAL CELLULAR SENESCENCE AND INFLAMMATION

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Objectives: Oxidative stress induces epigenetic modifications that may compromise decidual function during pregnancy, increasing the risk of miscarriage and pregnancy complications. Developmental Endothelial Locus-1 (DEL-1), a multifunctional protein primarily secreted by vascular endothelial cells, is known to promote inflammation resolution. While DEL-1 has been shown to protect lung tissues from LPS-induced inflammation and is implicated in aging-related cellular senescence, its potential role in endometrial function has not been explored. This study aimed to investigate DEL-1 expression in mouse endometrium and its characteristics in human endometrial stromal cells (ESCs).
Methods: We examined DEL-1 expression in mouse uterine tissue and cultured ESCs, including normal endometrial and endometriotic stromal cells. Additionally, human ESCs derived from endometriotic lesions were cultured in a 3D Matrigel system and transplanted into the peritoneal cavity of nude mice adjacent to the dissection site of ovariectomy excision. After three weeks, the tissues were analyzed by immunostaining. To assess DEL-1 production, ESCs were stimulated with several inflammatory factors or H_2O_2, followed by detection of DEL-1 protein using western blot and assessment of senescence-associated β-galactosidase (SA-β-Gal) activity.
Results: DEL-1 was strongly expressed in luminal and glandular epithelial cells and detected in stromal cells of the mouse uterus. Intense staining was also observed in human ectopic ESCs transplanted into the peritoneal cavity. DEL-1 secretion was markedly higher in ESCs from endometriotic lesions than in eutopic ESCs in the primary culture system. Thrombin, a pro-inflammatory factor increased DEL-1 secretion. Moreover, Oxidative stress (H_2O_2) increased intracellular DEL-1 content and the number of SA-β-Gal positive ESCs.
Conclusion: DEL-1 appears to be involved in oxidative stress-induced cellular senescence in ESCs. Its excessive secretion from endometriotic ESCs may be associated with chronic inflammation in endometriotic lesions, highlighting a potential role in endometrial pathology.

P1.20.
ENDOMETRIAL STROMAL FIBROBLAST SIGNALLING PRODUCES A DAMPENED GENE EXPRESSION RESPONSE IN DEVELOPING VILLOUS AND EXTRAVILLOUS TROPHOBLAST IN PRE-ECLAMPSIA

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Objectives: Decidualisation of the endometrium is essential for placental development, with previous evidence associating aberrant decidualisation with pre-eclampsia (PE). We sought to test whether decidualised endometrial stromal fibroblasts (dESF) could cause trophoblast maldevelopment in PE.
Methods: ESF isolated from non-pregnant donors (n=5 controls, no previous obstetric complication; n=5 PE, ≥2 pregnancies with PE onset <34 weeks), were decidualised for 8 days (1μM medroxyprogesterone acetate, 500μM 8-bromoadenosine cyclic monophosphate) before culture for 1 day in serum-free media containing medroxyprogesterone acetate. dESF-conditioned media was used as the basal media to grow 3D villous trophoblast organoids and 2D extravillous trophoblast (EVT) cells from trophoblast stem cells. Non-conditioned media organoids and EVT were generated alongside (n=3). Trophoblast RNA extractions were sequenced using Illumina NextSeq500 and differentially expressed genes (DEG) determined using DESeq2 (p_{adj}<0.05).
Results: Profound gene expression changes were seen in both organoids and EVT cells formed in control dESF-conditioned media compared to non-conditioned media (3689 and 3281 DEG, respectively). Gene set enrichment analysis revealed downregulation of cell projection processes in organoids and downregulation of inflammatory responses in EVT formed in control conditioned media (FDR <0.05). Many of the same genes were regulated by PE dESF-conditioned media when compared to non-conditioned media (2591 organoid DEG and 2263 EVT DEG overlapping with control dESF-conditioned media). Overlapping organoid DEG showed no functional enrichment, while overlapping EVT DEG exhibited downregulation of inflammatory response. Control-specific organoid DEG showed strong downregulation of cell projection processes (FDR<0.01), while control-specific EVT DEG consisted of up- and down-regulated hormone metabolism genes (FDR<0.05). PE-specific organoid and EVT DEG were not enriched for any biological process.
Conclusion: dESF signals regulate gene networks affecting villous trophoblast morphology and EVT inflammatory signalling and hormone metabolism. In PE, these signals are aberrant and produce a dampened effect on trophoblast gene expression, leading to maldevelopment and subsequent placental dysfunction.

P1.21.
IN-DEPTH CHARACTERIZATION OF THE IMMUNOPHENOTYPE OF DECIDUA BASALIS AND DECIDUA PARIETALIS AT TERM

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Objectives: Both the decidua basalis (DB) and decidua parietalis (DP) are essential components of the uterine lining during pregnancy. The DB represents the maternal side of the placenta and directly contacts fetal cells. The DP contours the remainder and is in contact with other fetal cells, including those from the chorion. Despite the importance of the decidua, much about immune balance at term pregnancy is still unclear. Here, we investigated several immune features in the decidua.
Methods: Thirteen term placentas from healthy pregnancies were included. Leukocytes from the DB and DP were isolated for suspension mass cytometry (SMC). We analyzed 47 markers orienting on various leukocyte subsets. Additionally, DB and DP were processed for immunohistochemistry (IHC). Gene expression analysis was performed by RNA sequencing (Illumina NovaSeq) and quantitative PCR (qPCR), on isolated DB and on DP attached to surrounding amnion and chorion from six placentas.
Results: SMC showed that of the general immune cell lineages, only B cell frequencies ($CD20^+/IgM^+/HLA\text{-}DR^{high}$) were significantly higher in DB compared to DP (fraction B cells of $CD45^+$ cells: median 0.77 vs. 0.26, P=0.0017), depicted in Figure 1A. IHC results, illustrated in Figure 1B, confirmed this trend in the DB versus DP ($CD20^+$ cells per mm^2: median 7.5 vs. 2.7, P=0.019), as did qPCR (CD19: 5.1-fold higher). As an internal control HLA-G expression was assessed and found mostly in DB (IHC: 3.1-fold higher, qPCR: 2.2-fold higher). RNA sequencing analysis showed >3,100 differentially-expressed protein-coding genes between DB and DP, many of which related to immune pathways, but a definitive picture concerning immune cell lineage involvement will emerge after deconvolution and pathway analyses.

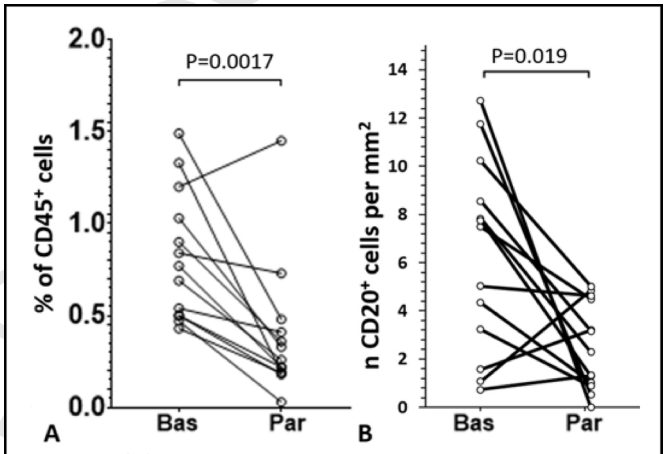

Figure 1. (A) SMC data showing the fraction of B cells in all $CD45^+$ cells of 13 paired DB and DP samples. (B) The amount of cells that stained positive for CD20, manually counted in 13 paired samples by microscopy.

Conclusion: DB and DP have distinct cellular and molecular features, and should be approached as two unique compartments. The different B cell counts may point to a hitherto unexplored involvement of this cell type in pregnancy-related regulatory mechanisms.

P1.22.
STIMULATORY EFFECTS OF SELECTIVE-PROGESTERONE RECEPTOR MODULATORS (SPRMS) ON HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION

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Objectives: Decidualization of human endometrial stromal cells (ESCs) is a critical process for the successful establishment of embryo implantation and subsequent placental development. During decidualization, ESCs produce IGF-binding protein 1 (IGFBP1) and prolactin (PRL) in response to progesterone (P4) and cAMP. A subset of ESCs exhibits senescence-like phenotypes during this differentiation process. Selective P4 receptor modulators (SPRM) are synthetic steroids with mixed agonistic and antagonistic activities targeting tissue-specific P4 receptors (PR). Ulipristal acetate (UPA) is used as an emergency contraceptive and for the treatment of uterine fibroids, while mifepristone (MFP) is administered as a medical abortifacient.
However, the effects of SPRMs on ESC decidualization remain incompletely understood. The present study aimed to elucidate the impact of SPRMs on ESC decidualization using an in vitro model system.

Methods: Human ESCs were treated with a cAMP derivative (db-cAMP) and P4 together with UPA or MFP for 2 days and the expression of decidual markers IGFBP1 and PRL, and their transcription factors FOXO1 and HAND2 were assessed by real-time RT-PCR. The effects of SPRMs on decidualization were investigated under the siRNA-mediated PR knockdown condition. Furthermore, senescence-associated β-galactosidase (SA-β-Gal) activity was measured to evaluate decidual cell senescence.
Results: Treatment with UPA or MFP upregulated the db-cAMP-induced IGFBP1, PRL, FOXO1 and HAND2 expression. Even in the presence of P4, UPA and MFP further promoted the db-cAMP-induced IGFBP1, PRL and FOXO1 expression. While PR knockdown reduced db-cAMP-induced HAND2 expression in cells treated with UPA or MFP, the stimulatory effects of SPRMs on IGFBP1, PRL, and FOXO1 expression remained evident. Furthermore, the SPRMs enhanced the SA-b-Gal activity in the presence of decidual stimuli.
Conclusion: These findings indicate that UPA and MFP facilitate ESCs decidualization and senescence through both PR-dependent and -independent mechanisms under db-cAMP/P4 stimulation. These results provide novel insights into the mechanisms of action of SPRMs on endometrial function.

P1.23.
IFNγ MEDIATED DECIDUAL STROMAL CELL DYSFUNCTION IN VILLITIS OF UNKNOWN ETIOLOGY PATHOGENESIS

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Objectives: Villitis of unknown etiology (VUE) is a CD8 T cell driven inflammatory pathology diagnosed in 10-15% of all placentas post-delivery. Previous whole transcriptome analysis shows that interferon gamma (IFNγ) signaling is significantly enriched in VUE placentas, especially decidual stromal cells (DSC). This study aimed to elucidate how IFNγ influences DSC function and phenotype.
Methods: IFNγ staining was performed on VUE and control placental tissues. Human endometrial stromal cells were decidualized in vitro for 7 days (estradiol, medroxyprogesterone acetate, 8-bromo cyclic AMP), and confirmed by prolactin (PRL) and insulin growth factor binding protein 1 (IGFBP1) secretion via ELISA. DSCs were chronically exposed (days 7-18) to either IFNγ (5ng/mL) or conditioned media from activated CD8 T cells. IFNγ neutralizing antibody (1ug/mL) was added on day 14-18. PRL, IGFBP1, and CXCL10 secretion were measured by ELISA.
Results: Staining revealed an increase in IFNγ-positive cells in VUE decidua as compared to controls. In vitro, chronic IFNγ treatment of DSCs led to increased secretion of T cell chemoattractant CXCL10 and decreased secretion of IGFBP1, while PRL levels remained stable. Conditioned media from activated CD8 T cells also significantly upregulated CXCL10 secretion. Addition of IFNγ neutralizing antibodies reversed these functional differences.
Conclusion: Chronic IFNγ exposure induces CXCL10 secretion by DSCs, potentially promoting enhanced T cell recruitment to the maternal fetal interface. Decreased IGFBP1 production suggests that IFNγ may impair DSC function, contributing to a loss of maternal fetal tolerance. Overall, these results implicate IFNγ as a key mediator in VUE pathogenesis and suggest it may be a promising therapeutic target.

P1.24.
ADVANCED AGE PREGNANCY ELICITS IMPAIRED DECIDUAL ANGIOGENIC VASCULAR REMODELLING

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Objectives: Advanced age pregnancies (AAP) (when a pregnant person is over 35 years old) represent a growing population worldwide and are associated with higher risk for pregnancy complications. It has been shown that delayed decidualization in AAP mice drives increased reproductive risk; however, the effects on the decidual vasculature are unknown. This is critical since vascular attrition is a hallmark of aging. In the murine decidua, it is known that endothelial-cell-driven vascular remodelling occurs with an initial angiogenic burst followed by proliferation-independent vascular enlargement resulting in larger caliber vessels by mid-gestation. We hypothesize that impaired decidual vascular remodelling will be more prevalent in AAP.
Methods: Young (11–20-week-old) and AAP (9–16-month-old) C57BL/6J dams were bred with young studs. Dams were sacrificed and dissected at E10.5. Uterine horns were fixed, serially cryosectioned, stained with anti-Erg and anti-CD31 (endothelial cell markers), and Hoechst. Plasma was collected and analyzed using multiplex angiogenic factor assay. Results were analyzed using unpaired student's t-tests and Chi square tests.
Results: AAP dams had a 56.6% reduction in litter size ($p<0.0001$) and visibly smaller and darker implantation sites compared to young. A trending 5.4% decrease in the proportion of endothelial cells ($p=0.06$) and a significant shift in vascular diameters towards smaller calibre vessels ($p<0.0001$) was observed in AAP decidua. Plasma analyses showed that AAP dams had a 27.3% ($p= 0.01$) reduction in circulating VEGF-C with no change in VEGF-A or VEGF-D.
Conclusion: Aged dams have a fewer decidual endothelial cells and smaller calibre vessels compared to young dams, suggesting impaired angiogenic remodelling. The decrease in circulating angiogenic factor VEGF-C may play a key role in altered decidual vascular remodelling in AAP. Impaired decidual angiogenesis and vascular remodelling may contribute to increased reproductive risk in AAP.

P1.25.
MATERNAL DIABETES INDUCES ALTERATIONS IN PATHWAYS RELATED TO DECIDUALIZATION FROM THE FETAL STAGE: EFFECTS OF A MATERNAL DIET ENRICHED WITH EXTRA VIRGIN OLIVE OIL

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Objectives: Diets enriched in extra virgin olive oil (EVOO) can activate PPAR pathways and induce antioxidant effects. Previous studies have shown programming of alterations in prolactin and PPAR pathways, relevant to decidualization, in the decidua of early pregnant offspring of diabetic rats. Aim: To evaluate prolactin and PPAR pathways in the fetal uterus of diabetic dams and to address putative beneficial effects of an EVOO-enriched maternal diet
Methods: A mild pregestational diabetic rat model was induced in F0 females by neonatal administration of streptozotocin (90 mg/kg sc). Control and diabetic females bred to healthy males, were fed a standard diet supplemented or not with 6% EVOO from day 1 to day 21 of gestation. The uteri of 21-day fetuses were collected (CICUAL Res. 2373/2017). Levels of prolactin and 4-hydroxynonenal (4-HNE, a prooxidant marker) were evaluated by immunohistochemistry, mRNA of prolactin receptor and PPARs by RT-qPCR and microRNAs (miRs) regulating PPARs by stem-loop RT-qPCR. n=6-8 dams per group.
Results: The uterus of the fetuses of diabetic rats showed reduced prolactin levels and reduced prolactin mRNA levels (37% and 0.57-fold, respectively, $p<0.05$), alterations not observed when the dams received the EVOO-enriched diet. No changes in the mRNA expression of PPARα and miR-21 (a negative PPARα regulator), but a reduction of PPARγ expression, observed in parallel with an increase in miR-19b (a negative PPARγ regulator) levels, were found in the fetal uteri of diabetic dams (0.34- and 2.73-fold respectively $p<0.05$), alterations not prevented by the maternal EVOO-enriched diet. The fetal uteri of diabetic rats showed increased 4-HNE levels (51%, $p<0.05$), an alteration prevented by the EVOO-enriched maternal diet ($p<0.05$ vs. diabetic group).
Conclusion: The uteri of fetuses from diabetic dams showed alterations in pathways relevant to the function of this reproductive organ, which were partially prevented by a maternal diet enriched in EVOO.

P1.26.
SINGLE AND COMBINED EXPOSURE TO BISPHENOL A AND BENZOPHENONE 3 SHOWS NO MAJOR EFFECTS ON TROPHOBLASTS AND MAST CELLS

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Objectives: During placentation, trophoblast cells, immune cells, and hormones interact closely, though the mechanisms of this interplay remain unclear. The impact of environmental factors such as endocrine-disrupting chemicals (EDCs), known for their hormone-like activity, on this interaction is also not well understood. This study investigates the effects of two common EDCs—bisphenol A (BPA) and benzophenone-3 (BP3) —on trophoblasts, mast cells, and their potential interactions.
Methods: Human mast cell line HMC-1 and extravillous trophoblast cell line HTR-8/SVneo were used as in vitro models. Cells were exposed to BPA, BP3, or their mixtures at concentrations ranging from 0.001 μM to 10 μM. Viability and proliferation were assessed after 24, 48, and 72 hours using a fixable viability dye and Ki67 staining, followed by flow cytometry analyses. Trophoblast migration was evaluated over 24 hours using a wound healing assay, following direct treatment with BPA or BP3.
Results: In HTR-8/SVneo cells, proliferation increased after exposure to BPA at 0.1 μM and BP3 at 1 μM (48 h). No effect on viability was observed, and mixtures showed no impact on either parameter. HMC-1 cells showed no significant change except increased viability for a mixture of 0.001 μM BPA and 10 μM BP3 (48 h). Trophoblast migration was reduced following BPA exposure at 0.01 μM (6 and 12 h), 10 μM (12 h), and BP3 at 10 μM (6 h). However, EDC-induced changes lacked a consistent concentration- or time-dependent pattern.
Conclusion: Single and combined exposures to BPA and BP3 caused limited, variable effects on trophoblast and mast cell functions. Further research is needed to assess their relevance within this cellular interaction model.

P1.27.
THIRD-TRIMESTER PLACENTAL EXPLANTS' IMMUNE RESPONSE AFTER POLYSTYRENE MICROPLASTICS EXPOSURE

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Objectives: Plastic pollution is an escalating global concern, with microplastics increasingly identified in human tissues, including the placenta. Nevertheless, the biological impact of MPs on placental health remains poorly understood. Here, we investigated the effects of polystyrene microplastics (PS-MPs) on the immune response of human term placental explants.
Methods: Placental villi explants from 15 healthy third-trimester placentas were cultured with 1 and 100 μg/mL of PS-MPs for up to 72 h. Supernatants were collected and analyzed for IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-17A, CXCL-10, CCL-2, TNF-α, IL-6, IFN-γ, and free active TGF-β levels using cytometric bead array kits.
Results: Exposure to 1 μg/mL of PS-MPs did not alter cytokine levels. In contrast, 100 μg/mL exposure significantly increased IL-2 ($p < 0.001$), CXCL-10 ($p < 0.001$), and IL-4 ($p < 0.001$), along with moderate elevations in IL-1β ($p < 0.05$) and IL-17A ($p < 0.05$), while reducing IL-12p70 ($p < 0.05$) and CCL-2 ($p < 0.01$). Other cytokines remained unaffected.
Conclusion: These findings demonstrate that low-level PS-MP exposure, similar to current estimated placental and maternal blood concentrations, may not trigger immune responses in third-trimester placental tissue. However, higher concentrations provoke a persistent and complex immune profile, characterized by Th2 and Th17 activation, T cell recruitment, and mild but sustained IL-1β secretion. The reduction in CCL-2 and IL-12p70 further suggests impairment of monocyte/macrophage-mediated functions. Given the placenta's pivotal role in fetal development, our data highlight critical concerns regarding the silent exposure to MPs at current presumable environmental levels and the potential immunological risks posed by higher contamination burdens during pregnancy.

P1.28.
DEVELOPMENT OF A PLACENTAL INFLAMMATION INDEX AS A BIOLOGICAL PROXY OF GESTATIONAL STRESS AND INTRAUTERINE ADVERSITY

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Objectives: Placental pathologies could serve as proxies of gestational stress, reflecting maternal and fetal clinical alterations that influence intrauterine development and birth outcomes. Our proposal was the development of a placental inflammation index, examine its association with a gestational stress construct, and evaluate their relationship with neonatal outcomes.
Methods: A group of 481 births from the São Paulo Western Region Birth Cohort (ROC) were included in the study. This study was approved by the ethics committee. Inflammatory placental lesions were classified by a certified surgical pathologist according to the Amsterdam Placental Workshop Group Consensus Statement. Latent constructs of placental inflammation and gestational stress were assessed using confirmatory factor analysis (CFA). The associations between constructs and neonatal outcomes were tested through structural equation modeling (SEM), using the lavaan package in R.
Results: The gestational stress construct comprised latent domains representing metabolic stress, psychosocial stress, and alcohol/tobacco exposure ($\chi2$(gl): 12.368(11); $\chi2$– p-valor:0.337; CFI:0.97; TLI:0.95; SRMR:0.03; RMSEA:0.02 (0.000-0.056)). The placental inflammation index included acute and chronic lesions: maternal, fetal, and mixed chorioamnionitis, chorionic vasculitis, funisitis, and chronic villitis ($\chi2$(gl): 6.302(6); $\chi2$ – p-valor:0.390; CFI:0.99; TLI:0.99; SRMR:0.03; RMSEA:0.01 (0.000-0.061)). In the final SEM model, metabolic stress was negatively associated with placental inflammation, which was, in turn, negatively associated with the birth weight/placental weight (BW/PW) ratio, a proxy for placental efficiency ($\chi2$(gl): 93.346(81); $\chi2$– p-valor:0.164; CFI:0.95; TLI:0.94; SRMR:0.05; RMSEA:0.02 (0.000-0.038)).
Conclusion: Our results suggest that placental inflammation plays an important role as a proxy for gestational stress, with potential implications for birth outcomes and fetal growth efficiency.

P1.29.
PLACENTA VASCULAR ALTERATIONS AND INFLAMMATORY LESIONS ASSOCIATED WITH PLACENTAL EXPRESSION OF PROTEINS ARE RELATED TO MATERNAL-FETAL NUTRIENTS SIGNALING AND EXPOSURE TO GLUCOCORTICOIDS

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Objectives: Placental pathologies are associated with maternal clinical alterations during pregnancy and adverse perinatal outcomes. O-linked N-acetylglucosamine transferase (OGT), an enzyme involved in nutrient sensing and cellular stress responses, and 11β-Hydroxysteroid dehydrogenase - which functions as a protective barrier that limits fetal exposure to maternal glucocorticoids, has been suggested as key biomarkers of gestational stress. Although these pathways, particularly O-GlcNAcylation regulated by OGT, have been extensively studied in animal models and are known to play a critical role in placental development, studies in humans remain scarce. The expression of these proteins has not yet been investigated in relation to histopathological placental alterations in pregnant women under social vulnerability, a condition associated with worse pregnancy outcomes.
Methods: Samples were selected from two randomized clinical trials (RCTs) (NCT02807818 and NCT04362098) with high-risk pregnant adolescents enrolled in a psychosocial pre-parenting intervention. Placenta vascular alterations and inflammatory lesions were classified by a pathologist according to Atlas of Placental Pathology. Immunohistochemistry (IHC) staining was performed to determine OGT and 11βHSD2 protein expression using primary antibodies anti-OGT (ab96718; Abcam) and anti-HSD11B2 (ab203132; Abcam). IHC slides were scanned on an Aperio ScanScopeXT, and image analysis was conducted using the Positive Pixel Count V9 algorithm. Protein expression were calculated using the IHC Index formula.
Results: OGT and 11βHSD2 expression was observed in syncytiotrophoblasts, the transporting epithelium which mediates maternal-fetal exchange of nutrients, and whose differentiation and maturation are influenced by the glucocorticoid system. Placenta vascular alterations (chorangiosis, arteriolar medial hyperplasia/mural hypertrophy) were associated with lower OGT expression, while inflammatory lesions (chorioamnionitis, funisitis and deciduitis) were associated with higher 11βHSD2 expression.
Conclusion: These results indicate that placental pathological alterations are associated with the expression of proteins involved in maternal-fetal nutrient signaling and fetal glucocorticoid exposure, which may represent key mechanisms through which stress program adverse outcomes in human offspring.

P1.30.
DETERMINING PLACENTAL UPTAKE OF POLLUTANT NANOPARTICLES USING IN VITRO AND EX VIVO MODELS.

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Objectives: Prenatal exposure to air pollutants such as combustion-derived black carbon (BC) is associated with adverse outcomes including low birth weight and preterm birth. BC nanoparticles (<100nm) cross the alveolar epithelial barrier and enter the blood where they accrue a proteinaceous coating known as a corona. BC has been identified in placental tissue but currently the mechanisms underlying its uptake and intracellular trafficking are not fully understood; this study aims to elucidate the mechanisms responsible.
Methods: Trophoblast stem cell-derived syncytiotrophoblasts were treated with 0.1-100μg/ml fluorescent carbon nanoparticles (10nm) to model BC. Nanoparticles were pre-incubated in maternal serum to form a protein corona. Uptake of particles was quantified and compared by Mann-Whitney U and Friedman tests (n=3). Samples were stained with endocytic antibodies to track the trafficking of particles through intracellular vesicles, colocalisation between nanoparticles and vesicles was determined through overlap coefficient analysis (r equates to the correlation between signals). To ascertain uptake mechanisms, syncytiotrophoblasts were treated with Dynasore to inhibit dynamin-dependent endocytosis. Ex vivo placental villous explants collected from healthy term pregnancies were treated with 1-10μg/ml carbon nanoparticles to determine the translocation of particles across the placental barrier.
Results: Carbon nanoparticles were visualised in syncytiotrophoblasts with uptake occurring in a time-dependent manner (p=0.0083), trending towards dose-dependency (p=0.0952). Following uptake, particles progressively co-localised with early endosomes (r=0.921), late endosomes (r=0.889) and lysosomes (r=0.912). Particles accumulated in enlarged lysosomes following 24hours of culture. Initial results suggest uptake was not affected by the presence of a protein corona nor Dynasore inhibition. Uptake of carbon nanoparticles in placental explants was also observed in the syncytium.
Conclusion: Uptake of pollutant nanoparticles by placental syncytiotrophoblasts occurs through dynamin-independent endocytosis and increases with particle dose. Particles are trafficked through endocytic vesicles to lysosomes where they accumulate and may cause lysosomal dysfunction through steric effects or co-emitted toxin action.

P1.31.
SIMULATING CHRONIC INFECTION TO STUDY MATERNAL CELLULAR MIGRATION USING THE EX VIVO HUMAN PLACENTA PERFUSION SYSTEM – A PH.D. PROJECT PLAN

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Objectives: Maternal microchimerism (MMc) is a natural process where a small number of maternal cells, such as leukocytes, are transferred to the fetus. These cells play a role in the newborn's development. Maternal health conditions, like HIV infection, may affect this transfer due to immune system alterations. Studies have shown lower levels of MMc in CD8 + T cells of HIV-exposed but uninfected infants. However, it remains unclear if this same tendence is linked to impaired placental transmigration of other leukocytes (like T cells and NK) or their extracellular vesicles (EVs). This project aims to assess the impairment of cellular and extracellular vesicle transmigration in the context of chronic infection, utilizing the ex vivo human placental perfusion model.
Methods: Double-side *ex vivo* human placenta perfusion will be performed as implemented by Zabel et. al, 2022. Healthy term placentas will be exposed to interferon-γ (IFN-γ) to mimic the microenvironment of a chronic viral infection. Peripheral blood mononuclear cells (PBMCs) will be isolated from healthy adult volunteers and stained with PKH67. Labeled cells will be perfused through the maternal circuit during 6 h. The transmigration of immune cell and extracellular vesicle (EVs) across the placental barrier will be analyzed by Western Blot and flow cytometry by staining for CD3+ T cells, CD56+ NK cells and CD9+ CD63+ALIX+CD40+ PLAP+HLAG+ EV.
Results: We expect that the chronic inflammatory environment induced by interferon-γ will result in an decreased transmigration of maternal immune cells, particularly T cells, NK cells and EVs, across the placental barrier.
Conclusion: This protocol is intended to serve as a valuable tool that may contribute to a deeper understanding of how chronic inflammatory may enhance cellular trafficking and vesicle release, potentially altering the natural balance of maternal microchimerism.

P1.32.
DEVELOPMENTAL PROGRAMMING OF OFFSPRING IMMUNE SYSTEM: ROLE OF CORTICOSTEROIDS

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Objectives: Environmental factors play a crucial role in shaping organisms, beginning with early development. While maternal exposure to environmental stressors during pregnancy has been extensively studied, paternal contributions to developmental programming are less well understood. Recent research in mice has identified non-germ cell factors, such as glucocorticoids (GCs) in seminal fluid, as key mediators of paternal programming on offspring health. Disruptions in paternal circadian rhythms before conception have been shown to affect metabolic health

and feeding behavior in male offspring, emphasizing the significance of GCs in these processes. Here, we investigated the role of glucocorticoids in developmental programming of immune landscape.

Methods: We compared multiple mouse models of stress in the context of corticosteroid-mediated programming- (a) paternal restricted feeding-induced circadian disruption, (b) glucocorticoid receptor heterozygous (GR-het) genetic model, and (c) maternal dexamethasone treatment. We employ bulk as well as single cell/nuclei multiomic approaches (transcriptomics and chromatin assays) to understand the programming of placenta and fetal tissues, along with spectral flow cytometry for immune phenotyping.

Results: Using bulk RNA-seq deconvolution in E18.5 fetal brain in models (a) and (b), we showed that various cell types like astrocytes, neuronal precursors and NK cells are differentially affected. These cell types were also interestingly shown to be glucocorticoid-signalling responsive using single-cell ATAC-seq of fetal brain. Single cell multiomics of E15.5 Placenta shows its immune compartment to be affected upon paternal circadian disruption, which is further validated for corticosteroid-mediated programming by looking at preliminary results of placenta immune compartment phenotyping using spectral flow cytometry in GR-het model (b). Glucocorticoid programming was also explored further with flow cytometry of placental immune compartment upon maternal dexamethasone treatment, showing differences in neutrophils and macrophages.

Conclusion: Altogether , our published and preliminary data suggest glucocorticoids as critical endocrine signal at conception with long-lasting impact throughout fetal development and for offspring health outcomes"

P1.33.
INFLUENCE OF MATERNAL SMOKING ON PREMATURE SENESCENCE AND ANTIOXIDATIVE CAPACITY IN THE PLACENTA

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Objectives: Smoking during pregnancy generates oxidative stress due to increased production of reactive oxygen species (ROS) and insufficient neutralization by antioxidant defense mechanisms like superoxide dismutase (SOD). There is a causal relationship between oxidative stress and senescence, the cellular phenotype of aging. The placenta naturally ages during pregnancy with an increase in senescence marker expression and a decrease in its functionality. Pregnancy complications are associated with oxidative stress, premature senescence and a premature loss of placental function. The aim of this study was to investigate whether maternal smoking during pregnancy induces premature cellular senescence in the placenta.

Methods: We performed multiplex immunofluorescence (mIF) staining on formalin-fixed paraffin-embedded (FFPE) placental samples from smokers and non-smokers across all three pregnancy trimesters (n=3 each). We examined senescence markers (p16, p21) and antioxidant capacity (SOD2) expression in stromal (vimentin+), endothelial (CD31+), and trophoblast (cytokeratin-7+) cells.

Results: First-trimester placentas from smokers showed significantly lower proportions of trophoblast cells with markedly reduced p21 expression compared to non-smokers. Simultaneously, first-trimester placentas from smokers showed significantly higher p21 expression in fetal endothelial cells. An upregulation of SOD2 expression was observed in smokers' placentas from term cesarean sections compared to non-smokers.

Conclusion: Our findings suggest disturbed proliferation of trophoblast cells in the first trimester placentas from smokers. Decreased p21 expression in trophoblasts in this context rather indicates disrupted cell fusion. In contrast the elevated p21 expression in fetal endothelial cells from smokers' first-trimester placentas indicates that smoking-induced oxidative stress might promote premature senescence of placental vasculature, as endothelial cells do not undergo fusion. The increase in antioxidant defense (SOD2) in term placentas of smokers, suggests a compensatory mechanism in response to prolonged oxidative stress during pregnancy. Collectively, our results demonstrate that maternal smoking may affect early placental development through cell type-specific alterations in senescence pathways and increases oxidative stress response in term placenta.

P1.34.
ANGIOGENIC MARKERS IN PLACENTAL TISSUE OF AUTOIMMUNE DISEASE PATIENTS

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Objectives: This study aimed to evaluate the placental expression of key angiogenic markers in women with AID and compare it to healthy pregnancies and cases of fetal growth restriction (FGR).

Methods: This prospective study utilized retrospectively collected data from placentas of pregnant women diagnosed with SLE, APS, or NC-OAPS, managed between 2010 and 2019 at the high-risk pregnancy units of Hospital Clínic Barcelona and Jena University Hospital. A total of 48 placentas were analyzed: 13 from AID patients with APO, 9 from AID patients without APO, 13 from FGR cases without AID, and 13 from healthy controls. Four angiogenic factors were evaluated by RT-qPCR: soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), vascular endothelial growth factor (VEGF), and soluble endoglin (sEng). Four samples were excluded due to insufficient RNA quality.

Results: Of the four markers studied, PlGF expression was significantly upregulated in placental tissue from AID patients without APO compared to healthy controls (Figure 1B). No statistically significant differences were observed in the expression of sFlt-1, VEGF, or sEng (Figures 1A, 1C, and 1D, respectively).

Conclusion: Increased placental expression of PlGF appears to be a distinctive feature of AID pregnancies without APO. This finding may reflect a compensatory placental mechanism that helps prevent adverse outcomes by enhancing angiogenesis. Moreover, it could potentially explain the previously reported reduction in circulating PlGF levels in AID patients. Further studies involving larger cohorts are warranted to validate these findings and better understand the role of placental angiogenic balance in autoimmune pregnancies.

P1.35.
GESTATIONAL INHALATION OF PARTICULATE MATTER INFLUENCES PLACENTAL GLUCOSE TRANSPORTER EXPRESSION AND LOCALIZATION

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Objectives: The inhalation of particulate matter (PM) during pregnancy is associated with reduced utero-placental perfusion and fetal growth restriction (FGR). Reduced perfusion can have negative consequences on nutrient delivery, including glucose, the primary substrate for energy in the fetus and placenta. The purpose of this study was to investigate placental glucose transport mechanisms after PM exposure to determine its role in FGR development.

Methods: Pregnant Sprague-Dawley rats were exposed to titanium dioxide nanoparticles (nano-TiO_2) from gestational day (GD) 4 to 19 via

whole-body inhalation to mimic exposure to ultrafine PM (<100 nm in diameter). Dams were fasted for 15 hours before sacrifice on GD 20, after which maternal and fetal blood glucose were measured, and maternal insulin was assessed using a commercially-available bioassay. Male and female placentas were collected at GD 20 and prepared for qRT-PCR, immunohistochemistry, and immunofluorescence confocal microscopy to quantify gene and protein expression and localize glucose transporters (GLUTs), respectively.

Results: Maternal and fetal blood glucose and maternal insulin were unaffected by nano-TiO_2 exposure. GLUT3 RNA was significantly reduced in exposed placentas compared to naïve. Reductions in GLUT1 protein were accompanied by an increase in GLUT4. Further analyses revealed that female placentas had a more pronounced increase in GLUT4 protein expression compared to male littermates. Confocal microscopy identified increased membrane localization of GLUT1 associated with placentas exposed to nano-TiO_2 while GLUT4 localization was unaffected.

Conclusion: Collectively, glucose transport in the placenta is affected by gestational exposure to ultrafine PM but it is unclear if these changes perpetuate the development of FGR. Furthermore, these changes may be sex-related, supporting that sex is an important factor of nutrient access. Future studies will assess glucose transport in real time using placental perfusion.

P1.36.
INVESTIGATING PLACENTAL BARRIER INTEGRITY UNDER EXPOSURE TO A REAL-LIFE PFAS MIXTURE

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Objectives: Per- and polyfluoroalkyl substances (PFAS) are persistent pollutants that cross the placenta and impair fetal growth, though whether effects result from fetal exposure or placental disruption remains unclear. This study explores the impact of PFAS on placental barrier formation and integrity using a real-life PFAS mixture, rather than single substances.

Methods: An in vitro placental barrier model was established on a collagen-coated Transwell (3 μm pore size) by coculturing the BeWo b30 trophoblast cell line (apical) with primary HUVEC endothelial cells (basolateral). The composition of a real-life PFAS mixture was chosen based on a screening of 56 PFAS measured in 1st trimester human placentas, resulting in a mixture of PFNA, PFOS, PFBA, PFOA, PFHxS, and PFDA in a ratio of 80:7:5:4:2:2. The in vitro placental barrier was exposed to this PFAS mixture at 100 μM or to the solvent control (0.1% DMSO). The treatment was added daily to the apical (maternal) side during media refreshment. Barrier formation was monitored daily via transepithelial electrical resistance (TEER) and Na-fluorescein translocation measurements. Placental integrity was assessed with FITC-Dextran (40 kDa) translocation over 1–21 hrs.

Results: Compared to the acellular control, TEER values increased and Na-fluorescein values decreased over time in all culture conditions, validating the in vitro placental barrier formation. BeWo cells in mono- and coculture showed the highest TEER values, while those of HUVEC in monoculture were similar to the acellular control. FITC-Dextran translocation decreased with culture time, with the coculture having the lowest permeability. PFAS mixture (100 μM) treatment did not alter any placental barrier endpoints in the co- or monocultures when compared to the DMSO control (p-value > 0.05).

Conclusion: Our findings suggest that a mixture of six PFAS at 100 μM in the ratio found in human placenta does not measurably affect formation or integrity of an in vitro placental barrier model.

P1.37.
SECONDHAND SMOKE VS. E-CIGARETTE AEROSOLS: UNVEILING MATERNAL LUNG INFLAMMATION, APOPTOSIS AND PLACENTAL RISK IN PREGNANCY

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Objectives: Environmental toxicants like secondhand smoke (SHS) and e-cigarette aerosols (eCigs) threaten maternal-fetal health, yet their differential impacts on maternal lung inflammation and apoptosis remain under-explored. This study dissects these effects in pregnant C57BL/6 mice exposed to SHS or eCigs for 4 or 6 days during late gestation.

Methods: Pregnant C57BL/6 mice were exposed to SHS or eCigs for four or six days at two gestational time points (E12.5–E18.5 and E14.5–E18.5). Lung inflammation was assessed via bronchoalveolar lavage fluid (BALF) and tissue cytokine profiling, while apoptotic responses were analyzed through intrinsic and extrinsic pathway markers.

Results: SHS exposure triggered a robust inflammatory response, characterized by increased pro-inflammatory cytokines (IL-1β, TNF-α, IL-6), chemokine activation (MCP-1, MIP-1α), and oxidative stress markers, including receptor for advanced glycation end-products (RAGE). This response intensified over time, transitioning into a chronic inflammatory state with persistent extracellular matrix remodeling. In contrast, eCig exposure induced a transient, less pronounced inflammatory response with moderate IL-6 and MCP-1 upregulation, coupled with increased anti-inflammatory IL-10 expression.

Apoptotic analyses revealed SHS-induced sustained activation of pro-apoptotic markers (Bax, cytochrome c, BID) alongside suppression of anti-apoptotic proteins (Bcl-2, XIAP), leading to impaired tissue homeostasis. Conversely, eCig vapor exposure elicited a transient apoptotic response with preserved anti-apoptotic signaling, suggesting a greater capacity for tissue repair. Both exposures activated extrinsic apoptosis via Fas/FasL, but SHS exposure exhibited more pronounced effects, correlating with increased oxidative stress and inflammatory cytokine release.

Conclusion: These findings underscore the significant yet distinct inflammatory and apoptotic trajectories induced by SHS and eCig exposure. SHS exposure results in persistent lung inflammation, prolonged oxidative stress, and chronic apoptotic activation, while eCig vapor leads to a more transient response. These data highlight the critical need for maternal-fetal exposure prevention strategies, reinforcing the importance of limiting SHS exposure and reevaluating the perceived safety of eCigs during pregnancy.

P1.38.
MICROPLASTIC EXPOSURE ALTERS METABOLIC AND HORMONAL PROFILES IN HUMAN PLACENTAL EXPLANTS

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Objectives: To investigate the biochemical impact of 5 μm polystyrene microplastics (PS-MP) on term placental chorionic villi explants, focusing on their metabolic and hormonal changes.

Methods: Human placental term explants were exposed to 100 μg/mL of 5 μm polystyrene microplastics (PS-MP) up to 72 h. The supernatants were collected and metabolomic profiling was obtained by nuclear magnetic resonance, while estrogen, progesterone and beta chorionic gonadotrophin (β-hCG) levels were measured using ELISA.

Results: The exposure to PS-MPs revealed substantial metabolite differences, separating samples in two different clusters OPLS-DA with a high

degree of separation between groups and good predictive performance through cross-validation (Q2 0.747, R2Y 0.855, and $p < 0.01$). The VIP score highlighted multiple significant alterations, with formate, valine, tyrosine, isoleucine, isobutyrate, hypoxanthine, and alanine being the most important variables. The relative concentrations, showed lower levels of alanine ($p = 0.03$), formate ($p < 0.001$), glutaric acid ($p = 0.02$), and maltotriose ($p = 0.03$), with increase in valine ($p = 0.03$) and tyrosine ($p < 0.001$) in the exposed group. Hormonal analysis showed no significant changes.

Conclusion: These findings suggest potential impairments in folate, and amino acid metabolisms, and alterations in the TCA cycle. Given the placenta's vital role in pregnancy, these disruptions may have implications for fetal development. This study underscores the urgent need for further investigation into the effects of microplastics exposure on placental health and function.

P1.39.
MATERNAL HYPOXIA AT MID-GESTATION LOWERS UTERINE OXYGEN DELIVERY AND UPTAKE AND PLACENTAL BUT NOT FETAL WEIGHT

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Objectives: Fetal hypoxia is strongly associated with fetal growth restriction (FGR). However, the mechanisms whereby hypoxia initiates FGR are unclear. The objective of this study was to test the effects of maternal hypoxia from 60% of pregnancy (~103 days in 148-day gestation in sheep) and sustained for 12 days on uteroplacental hemodynamics and placental and fetal biometry.

Methods: Pregnant sheep were submitted to surgery for vascular sampling catheters (maternal femoral artery; uterine vein; fetal carotid artery), perivascular flow probe (uterine artery [UtA]; umbilical artery), and maternal tracheal tube placement. ~5 days post-surgery, sheep were exposed to intratracheal infusion of compressed air (CON; n=4) or nitrogen (targeting maternal paO2 of ~55mmHg; HOX; n=3). Blood samples and hemodynamic assessment were taken at baseline and at necropsy. Placental and fetal weights were obtained, and vasoreactivity in 4th generation UtA was assessed by wire myography. Preliminary analysis of this ongoing study was performed with Mann-Whitney t-test or Mixed-model analysis.

Results: Maternal and fetal paO2 decreased by ~40% (p=0.0002) and ~26% (p=0.160), respectively, in CON versus HOX. Despite similar UtA blood flow, umbilical artery blood flow increased only in CON across the study (p=0.057). Uterine oxygen delivery (p=0.056) and uterine oxygen uptake (p=0.029), supplying the placenta and fetus, were lower in HOX versus CON. Hypoxia increased maternal (p=0.0238) but not fetal heart rate. UtA from HOX had increased vasoconstrictor response to serotonin. Total uteroplacental (p=0.057) and placentome weight (p=0.10) were lower in HOX, yet placentome number and fetal biometry were similar.

Conclusion: Mid-gestation hypoxia lowers uterine oxygen delivery and uptake and reduces placental weight, without decreasing fetal growth. This suggests that placental effects may precede constrained fetal growth or that adaptive mechanisms protect fetal growth. Ongoing studies with additional animals will further interrogate relationships between vascular responses, oxygen and nutrient flux, and fetal growth.

P1.40.
MICROPLASTIC ACCUMULATION IN PLACENTAS AND UMBILICAL CORDS FROM PREGNANCIES IN BRAZIL

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Objectives: This study aimed to investigate the presence of microplastics (MPs) in the placentas and umbilical cords of Brazilian pregnant women from the Alagoas State, providing the first evidence of MP accumulation in pregnancies from a Latin American population

Methods: A total of 20 pregnant women were randomly selected for inclusion. Following delivery, each placenta was placed in a glass container and transported to the laboratory. Socio-demographical and clinical data were collected, and samples were weighted in a free-plastic protocol. All samples were washed with glass-filtered solutions, firstly with Milli-Q water and later with a 10% potassium hydroxide solution (1:9, w/v) for one week at room temperature. The digested samples were glass-filtered and stored in metal containers for subsequent analysis with optical microscopy and Raman spectroscopy. Data analysis was performed using the KnowItAll software (Wiley) and the SLOPP/SLOPPe MP libraries.

Results: Microplastic particles were detected in all analyzed placental ($n = 20$) and umbilical cord ($n = 20$) samples. In placental tissue, the major identified polymers were polyethylene (PE), polyurethane, and polyamide (PA). In umbilical cords, PE, PA, and polyethylene vinyl acetate (PEVA) were the most frequently detected. Additionally, several chemical additives were found associated with MPs, but they majorly varied amid the samples.

Conclusion: Our findings provide the first evidence of MPs contamination in a Latin-American pregnant population, with all their samples being contaminated with MPs, also adding to information on umbilical cord MP presence, which strongly indicates that fetuses are being exposed to plastic polymers and associated chemicals during their development. These results underscore the urgent need for better regulations on plastic pollution and environmental contamination, and further research into the long-term health effects of MP exposure during pregnancy

P1.41.
ENDOMETRIAL STROMAL CELL DERIVED SIGNALING FACTORS AS POTENTIAL MEDIATORS OF TROPHOBLAST INVASION

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Objectives: Embryo implantation and placentation require proper endometrial signaling for successful pregnancy establishment. Disruptions in maternal-embryo crosstalk can result in implantation failure and placental abnormalities. Key signaling factors include extracellular vesicles (EV) and cytokines. In this study, menstrual blood mesenchymal stromal cells (MenSCs) were used as a model to study maternal-embryo signaling by characterizing MenSC derived EVs and cytokines and evaluating the ability of these cells to promote trophoblast invasion using an in vitro 3D model.

Methods: MenSCs were isolated from the menstrual blood of five healthy women. A 3D invasion model was performed using spheroids formed from the extravillous trophoblast derived HTR8/SVneo cell line and MenSCs embedded in Matrigel. Large (lEV) and small EV (sEV) fractions were isolated from the supernatant of decidualized and non-decidualized MenSCs and characterized by Western blot, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). Cytokine expression was analyzed using a human cytokine array kit.

Results: In the 3D model, MenSCs triggered the invasion of trophoblast spheroids compared to the negative control. MenSCs produced EVs with concentrations of lEVs ranging between 1.2×10^{10} - 1.8×10^{10} particles/mL and sEVs ranging between 1.8×10^{10} - 5.1×10^{10} particles/mL with an average particle size of 162.5 nm for lEVs and 108 nm for sEVs. EVs expressed characteristic sEV markers CD63, CD9, and ALIX and lEV markers GAPDH and CD47. Total RNA content was higher in EVs from decidualized cells compared to those from non-decidualized cells. MenSCs expressed multiple cytokines, such as IL-6, PAI-1, IL-8, SDF, GM-CSF, ICAM1, CCL2, and CXCL1.
Conclusion: MenSCs were used as a non-invasive alternative to endometrial biopsies to model implantation by promoting invasion of trophoblast spheroids. These cells produce EVs and a variety of cytokines that may play important roles in embryo implantation and regulation of trophoblast invasion.

P1.42.
THE INVOLVEMENT OF MIR-150-5P-CONTAINING EXOSOMES IN THE PATHOGENESIS OF PREECLAMPSIA.

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Objectives: Exosomes are extracellular vesicles secreted by cells, and contain nucleic acids such as microRNA (miRNA), proteins, and lipids. They participate in intercellular communication by being taken up by recipient cells. We hypothesize that intercellular communication via exosomes contributes to the pathogenesis of preeclampsia (PE), and performed in vitro experiments.
Methods: 1. Exosomes were purified from the plasma in patients with early-onset preeclampsia (EoPE) and normal control by differential ultracentrifugation. MiRNAs were extracted from exosomes and analyzed comprehensively using RNA sequencing (RNA-seq). MiRNAs with False Discovery Rate <0.05 and more than two-fold difference in expression between the two groups were selected. 2. The expression level of miRNAs which showed the differences following the RNA-seq of placental tissues in patients with EoPE (n=5) and normal control (n=12) was evaluated using real-time PCR. 3. The concerned miRNA was transfected into a human umbilical vein endothelial cell line (HUVEC) using lipofection. CCK8 and tube formation assays were conducted. 4. Exosomes purified from choriocarcinoma cell line JAR, which the miRNA had been transfected were used to examine their effects on HUVEC. 5. The expression level of MYB (a target gene of the miRNA) was evaluated in HUVEC transfected with the miRNA.
Results: 1. Only miR-150-5p significantly upregulated in patients with EoPE following RNA-seq. 2. The expression of miR150-5p of placentas in patients with EoPE was significantly higher than those with normal control. 3. MiR-150-5p significantly inhibited the proliferation and angiogenesis of HUVEC. 4. The proliferation and angiogenesis of HUVEC co-cultured with exosomes derived from JAR/miR-150 were suppressed. 5. MYB was reduced in HUVEC transfected with miR-150-5p.
Conclusion: Exosomes released from villous trophoblast in patients with EoPE abundantly contain miR-150-5p. The exosomes flow into maternal circulation and may impair the proliferation and angiogenesis in vascular endothelial cells, contributing to PE pathogenesis.

P1.43.
HYPOXIA ALTERS THE PROTEOME OF SMALL HUMAN PLACENTAL EXTRACELLULAR VESICLES AND THEIR EFFECT ON ENDOTHELIAL DYSFUNCTION.

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Objectives: Extracellular vesicles (EVs) have important roles in placentation and vascular homeostasis in pregnancy. Hypoxia is proposed to alter secretion and biochemical composition of placental EVs. Here, we investigated the effect of placental hypoxia on the proteome of placental EVs and their effect on endothelial function (model for preeclampsia).
Methods: Human placental tissue was collected at term caesarean section (n=5). Placental explants were cultured (48 hours) under hypoxic (1% O_2) or physiological (control) conditions (8% O_2). EVs were isolated from media by differential centrifugation. EV size and concentration was determined by NanoSight Tracking Analysis. Protein extracted from EVs was assessed by Liquid Chromatography-Mass Spectrometry (matched vesicle concentration). Human umbilical vein endothelial cells (HUVECs, n=6) were cultured with control or hypoxic placental EVs (6 hours), to investigate effects on endothelial function. Anti-angiogenic and endothelial dysfunction markers were analysed (qPCR, ELISA), in addition to functional endothelial tube formation and leukocyte adhesion assays. Wire myography assessed human omental artery response, following incubation with control or hypoxic placental EVs (n=5), to vasoconstrictor, endothelin-1, and vasodilator, bradykinin.
Results: Mass spectrometry identified 6,421 proteins in placental EVs; 43 proteins were uniquely expressed in hypoxic placental EVs. The most abundant were associated with inflammation, angiogenesis, cellular repair, and survival. HUVECs cultured with placental EVs from control or hypoxic conditions demonstrated upregulation in mRNA expression of endothelial dysfunction markers (VCAM, ICAM), pro-inflammatory (CCL2, CCL7, CX3CL1), oxidative stress (NOS3) and anti-angiogenic factors (sFLT1; increased secretion); in contrast downregulated CXCL8. Interestingly, hypoxic EVs reduced endothelial tube formation, all placental EVs increased leukocyte adhesion, however neither treatment altered omental artery vasoreactivity.
Conclusion: EVs from placenta cultured under hypoxia demonstrated an altered proteome. However regardless of oxygen tension, placental EVs drive endothelial dysfunction and increase anti-angiogenic sFlt-1 production. These data provide new knowledge regarding EV actions in human models aimed at understanding the pathology driving preeclampsia.

P1.44.
SURFACE PROTEOME OF PLASMA EXTRACELLULAR VESICLES AS CLINICAL BIOMARKERS OF PREECLAMPSIA

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Objectives: Placental EVs (pEVs), which are shed from placental villi into the maternal circulation, reflect placental function in normal and pathological pregnancies. The main objectives of this study is to characterize the surface proteomic signatures of total circulating EVs and placental EVs in maternal plasma and assess their potential utility in predicting preeclampsia (PE) and preterm delivery (PTD) compared to term delivery (TD) controls.
Methods: Plasma samples were collected from women with normal pregnancies, PE, and PTD. Surface proteins on total EVs and pEVs were profiled using a multiplex EV-array printed with a wide panel of capturing antibodies. Total EVs were detected and identified using biotinylated antibodies against common EV markers (CD9, CD63, and CD81), while placental EVs were detected via biotinylated anti-PP13, followed by fluorescent streptavidin labeling. Signal intensities were measured by fluorometry.
Results: Heatmap analysis revealed distinct surface protein expression patterns across total EVs and pEVs in PE, PTD, and control groups. Univariate analysis showed significant differential expression of CD63, TNFRII, C1q, CD81, and PP13 in PE compared to controls. Combined marker analysis produced an area under the ROC curve (AUC) of 0.89 (95% CI: 0.72–1.00). In PTD, a unique proteomic profile including C1q, CD62E, LFA-1, and Alix was observed. For differentiating PE from PTD, a combination of C1q, CD81, CD63, and PP13 achieved an AUC of 0.91, with 80% sensitivity and 90% specificity.
Conclusion: The surface proteomic profiles of total and placental EVs effectively distinguish between normal and pathological pregnancies. This EV-based microarray platform offers a promising and accessible tool

for the early and differential prediction of major pregnancy complications, including PE and PTD.

P1.45.
SURROGATE CIRCULATING EXTRACELLULAR VESICLE BIOMARKERS FOR RISK ASSESSMENT OF PREECLAMPSIA

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Abstract

Objective: The Fetal Medicine Foundation (FMF) algorithm is a validated first-trimester screening method for the early identification of women at high risk of developing preeclampsia (PE), combining maternal factors with biophysical and biochemical markers assessed at 11–13 weeks' gestation. While the uterine artery pulsatility index (UtA-PI) is a key component for predicting early-onset PE, it relies on Doppler ultrasound, which can be challenging to implement in low-resource settings. This study aimed to identify extracellular vesicle (EV) protein biomarkers that can reliably distinguish between high- and low-risk pregnancies for PE, offering a minimally invasive and scalable alternative to Doppler-based assessments.

Methods: Plasma samples were collected at two time points—11–13 weeks and 36 weeks' gestation—from pregnant women (n = 120). Women were stratified into low-risk (n = 102) and high-risk (n = 18) groups based on FMF algorithm results and high-risk women were prescribed 150 mg of aspirin from approximately 13 to 36 weeks' gestation. EVs were isolated using an immunoaffinity magnetic bead-based capture system and quantitative proteomics was performed. Longitudinal changes in EV protein profiles were analysed using linear mixed-effects modelling.

Results: In early gestation, 886 EV-associated proteins were identified, with 6 significantly upregulated and 2 downregulated in high-risk pregnancies. In late gestation, 765 proteins were detected, with 14 upregulated and 4 downregulated. ROC analysis in early gestation demonstrated excellent discriminatory performance of the EV biomarker panel (micro-average AUC = 0.93), with an estimated sensitivity of 90%, specificity of 85%, positive predictive value of 83%, and negative predictive value of 91%. Additionally, longitudinal modelling revealed dynamic and distinct trends in EV protein expression between risk groups across gestation.

Conclusion: This study identifies circulating EV protein biomarkers with strong diagnostic performance for early and longitudinal risk assessment of PE, providing a non-invasive and scalable alternative to Doppler-based screening.

P1.46.
DNA BREAK MAPPING IN PLACENTAL CELLS UNCOVERS VULNERABILITY OF REPETITIVE SEQUENCES IN PREGNANCY COMPLICATION

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Objectives: During placental development, trophoblast cells increase their ploidy through many rounds of replications, either by endocycle or by syncytium formation. During this process, they accumulate DNA damage, likely due to replication error from the dampening of the ATR (ATM and RAD3-related) DNA damage response pathway. As gestation progresses, there is a gradual, physiological increase in DNA damage. The objective of this study is to map DNA damage and associated loci in the placental cells.

Methods: We performed γH2A.X chromatin immunoprecipitation (ChIP) in mouse parietal trophoblast giant cells (P-TGCs) at the 9.5*dpc* stage of pregnancy, as well as in human term placental cells. Using in-vitro human trophoblast stem cell culture model and blood samples from different stages of human pregnancies, we have developed a q-PCR based protocol to detect DNA breaks in cell-free DNA (cfDNA) during gestation.

Results: We were able to identify 51 reproducible DNA damage sites in P-TGCs. Analysis of their genomic positions revealed that these sites, are mostly located within low or medium expression levels, with a mean gene size of approximately 84kb. Since repetitive DNA sequences are more prone to DNA damage, we also analyzed several repetitive elements. Our finding suggest that placental cells show more breaks in specific micro-satellite repeats. We hypothesize that these repeats accumulate damage over the course of pregnancy and may be especially susceptible to breaks under pathophysiological conditions such as fetal growth restriction (FGR). Across different stages of pregnancies, we showed that flanking sequences corresponding to these repeats can be detected as cfDNA and the amount of these repeats in cfDNA increases in the case of FGR.

Conclusion: In conclusion we have mapped DNA damage sites in placental cells and we propose that detection of these sites in maternal cfDNA could serve as potential marker for early detection of pregnancy complication.

P1.47.
PLACENTAL EXTRACELLULAR VESICLES EXPRESSION IN RESPONSE TO HYPERGLYCEMIA USING THE EX-VIVO PLACENTAL PERFUSION MODEL

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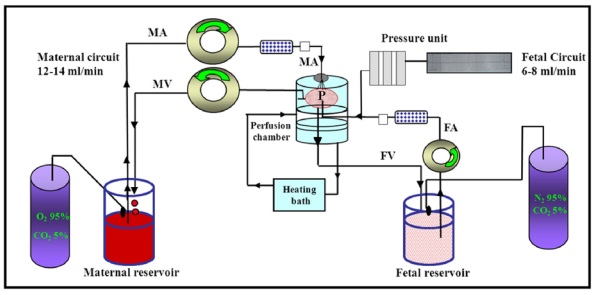
Objectives: Gestational diabetes mellitus (GDM), affecting 5–10% of pregnancies, is associated with adverse maternal and neonatal outcomes. Its pathophysiology is closely linked to inflammation and oxidative stress. This study investigated how hyperglycemia influences placental inflammatory responses by examining the secretion and protein cargo of placental extracellular vesicles (EVs) using an ex-vivo dual-perfused human placental cotyledon model. We focused on inflammasome-associated Caspase-1 and oxidative-inflammatory regulators including thioredoxin-interacting protein (TXNIP), Thioredoxin (TRX), suppressor of cytokine signaling 3 (SOCS3), and signal transducer and activator of transcription 3 (STAT3).

Methods: Placentas from term cesarean deliveries were perfused for 180 minutes under normoglycemic (100 mg/dL) or hyperglycemic (400 mg/dL) conditions using M-199 culture medium. Placental viability was assessed by monitoring maternal and fetal flow rates, fetal artery inflow pressure, reservoir pH, and hCG production. EVs were isolated from maternal perfusates using differential ultracentrifugation. Western blot analyses evaluated the expression of trophoblast marker PLAP, exosomal markers CD9 and CD63, and target proteins Caspase-1, TXNIP, TRX, SOCS3, and STAT3.

Figure1- Schematic presentation of the ex-vivo placental perfusion model.



Kovo M, Golan A. In Vitro Models Using the Human Placenta to Study Fetal Exposure to Drugs. *Clinical medicine Reproductive health*. 2008

Results: EVs were successfully isolated from 11 placentas (normoglycemic n=5; hyperglycemic n=6). PLAP expression confirmed their trophoblastic origin (Figure 2) while the lack of CD9 and CD63 expression indicated minimal contamination from other EV subtypes, supporting the specificity of the EV isolation. Under hyperglycemic conditions, Caspase-1and SOCS3 expression were significantly elevated compared to normoglycemic controls (fold change [FC]= 1.48 and 1.2; $p<0.05$), suggesting enhanced inflammasome activation. Conversely, TXNIP and STAT3 levels were reduced in hyperglycemic EVs (FC= 0.55 and 0.63; p=0.08 and p=0.025, respectively), indicating altered regulation of oxidative stress (Figure 3).

Figure 2. Extraction of placental exosomes (EXs) and extracellular vesicles (EVs) from maternal and fetal perfusates with comparative analysis of exosome/EV markers

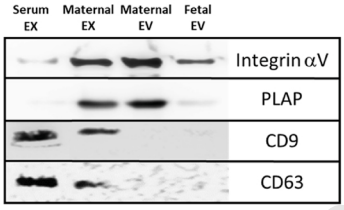
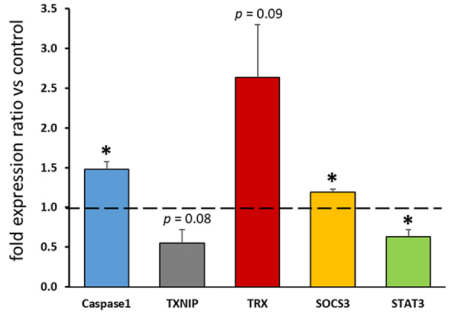


Figure 3 – Relative expression of placental EVs proteins (Caspase-1, TXNIP, TRX, SOC-3 STAT3) in maternal hyperglycemic perfusate compared to the normoglycemic perfusates



Conclusion: Hyperglycemia modulates the protein composition of placental EVs, reflecting changes in inflammatory and stress-related pathways. These alterations may contribute to GDM-associated placental dysfunction and could serve as potential biomarkers for early detection and monitoring of GDM-related complications.

P1.48.
PLACENTAL SMALL EXTRACELLULAR VESICLES SHOW EMT/ENDOMT SIGNATURES IN EARLY-ONSET PREECLAMPSIA

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Objectives: Extracellular vesicles (EVs) mediate intercellular communication by transporting bioactive molecules, playing key roles in both normal and pathophysiological pregnancies. In preeclampsia (PE), placental dysfunction may contribute to altered fetal development, potentially mediated by placental-derived small EVs (sEVs). This study aims to elucidate their role by characterizing the sEVs proteome and identifying disease-specific signatures in early-onset PE (EO-PE).
Methods: Endothelial cells (fpECs) were isolated from chorionic vessels from term (T, n=4), preterm (PT, n=5), and EO-PE (n=4) placentas. sEVs were isolated from fpEC-conditioned media via differential ultracentrifugation and characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and Western blotting. Global proteomic profiling of EVs was performed using nanoLC-MS/MS, and data were analyzed by unsupervised gene set enrichment analysis.
Results: sEVs were isolated from fpEC-conditioned media, showing a characteristic size range (30–200 nm) and morphology. Marker analysis confirmed the presence of sEVs-associated proteins (Syntenin-1, Alix, CD81) and the endothelial marker CD31. Proteomic profiling identified 1,329 proteins, with 877 shared across all groups. Notably, sEVs from the EO-PE cohort showed a distinct enrichment of proteins associated with epithelial-to-mesenchymal transition (EMT), with a normalized enrichment score of 2.31 (EO-PE vs. PT, p=<0.001) and 2.69 (EO-PE vs. T, p=<0.001).
Conclusion: This study provides the first proteomic evidence of EMT-like signatures in fpEC-derived sEVs from EO-PE placentas. Given the endothelial origin of sEVs and the parallels between EMT and endothelial-to-mesenchymal transition (EndoMT), our findings suggest EO-PE sEVs may modulate EMT/EndoMT-related pathways in recipient cells. These vesicles may play a mechanistic role in fetal programming and vascular dysfunction linked to EO-PE.

P1.49.
METABOLIC GLYCAN LABELLING FOR THE STUDY OF PLACENTAL EXTRACELLULAR VESICLE TRANSFER

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Objectives: Extracellular vesicles (EVs) are mediators of cellular communication. Fetal EVs are believed to be present in maternal circulation, suggesting EV crosstalk across the placental barrier. However, the mechanisms underlying EV transfer are poorly understood and bidirectionality has not been established. We aim to study bidirectional EV transfer across a placenta-on-a-chip with metabolic glycan labelling, a cutting-edge technique currently used to label cell-surface glycans. Our objectives include optimising the metabolic glycan labelling of EVs, generating cell lines capable of implementing this novel labelling strategy, and quantifying and imaging EV transfer across a placenta-on-a-chip.
Methods: K562 cells were fed chemically modified glycans. EVs were isolated and labelled with click reactions tagging alkyne-bound fluorophores to azido glycans. EV fluorescence was assessed with nanoparticle flow cytometry. To enhance labelling specificity, K562 and trophoblast cell lines capable of incorporating modified glycans at higher rates were generated by stable transfection of an artificial biosynthetic pathway or

bump-and-hole glycosylation enzymes. A placenta-on-a-chip model using BeWos and HUVECs was used to quantify and visualise bidirectional EV transfer, with nanoparticle flow cytometry and confocal microscopy. The use of metabolic glycan labelling on the placenta-on-a-chip will be tested.

Results: Up to 61.8% of wild-type K562 EVs were labelled by metabolic glycan labelling with Ac_4ManNAz. The incorporation of an artificial biosynthetic pathway doubled the proportion of EVs labelled with Ac_4GalNAz, from 8.92% to 16.8%, although replicates remain to be carried out with the newly optimised conditions. EV transfer across placenta-on-a-chips was quantified and visualised.

Conclusion: We outline here the successful optimisation of a novel labelling strategy to study EVs in a traceable manner. This work will be used to explore EV transfer across the placental barrier, with future experiments focusing on implementing a metabolic glycan labelling approach to quantify and visualise EV transfer across a placenta-on-a-chip.

P1.50.
PRIMING OF MATERNAL PLATELETS DURING PREGNANCY – THE ROLE OF PREGNANCY SPECIFIC BETA-1-GLYCOPROTEIN 11 IN PLATELET ACTIVATION

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Objectives: During pregnancy platelets underlie certain dynamic changes, including increased activity and a decrease of the platelet count towards the end of pregnancy. Previous studies revealed that a tightly regulated cross talk between platelets and the placenta seems to be of high importance.

Pregnancy-specific glycoproteins (PSG) are the most abundant trophoblast-derived proteins in the maternal blood during human pregnancy and several studies indicate that PSGs play a critical role in the regulation of the immune response and platelet activation. Here, we tested the hypothesis whether PSG11 is selectively taken up by maternal platelets and whether platelet priming is a crucial process during pregnancy.

Methods: Platelets were isolated from blood samples of either healthy pregnant women in the first and third trimester, preeclamptic patients or healthy non-pregnant women and afterwards subjected to proteomic analysis as well as to RNA Sequencing in order to elucidate dynamic changes of the platelet proteome and transcriptome over the course of gestation and in preeclampsia. Furthermore, platelets from non-pregnant women were incubated with plasma from healthy pregnant women or with recombinant PSG11 and subsequently analysed on protein level, via electron microscopy or via aggregometry.

Results: Our proteomics data showed an abundance of PSG11 in platelets from pregnant women and an increase over the course of pregnancy. Furthermore, we could detect PSG11 in platelets from healthy non-pregnant women after incubation with plasma from pregnant women. Notably, pre-incubation of isolated platelets from non-pregnant with PSG11 hampered the collagen Type I induced platelet aggregation nearly by 50% compared to controls, whereas the Thrombin Receptor Activator Peptide (TRAP) 6 induced platelet aggregation was slightly increased.

Conclusion: Our data suggests that platelets increasingly sequester PSG11 over the course of gestation, which tempts us to speculate that platelet priming by placenta-derived factors is a common process to adapt maternal platelets to the haemostatic challenges in pregnancy.

P1.51.
PLACENTA ACCRETA SPECTRUM BIOMARKER DISCOVERY USING DIA-MS PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLE ENRICHED FRACTIONS

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Objectives: Placenta accreta spectrum (PAS) refers to a continuum of disorders characterized by abnormal invasion of placental villi into or through the myometrium, thereby impeding natural placental separation during delivery. This condition is associated with significant adverse outcomes, including postpartum hemorrhage, hysterectomy, and even maternal mortality. To date, no specific circulating biomarkers for PAS have been applied in clinics, and the understanding of its pathogenesis remains limited.

Methods: In this study, maternal serum samples were collected from 10 pregnant women diagnosed with PAS who delivered at Peking University First Hospital between 2020 and 2023, as well as from 10 matched control pregnant women without PAS complications. Proteomic analysis was performed on the extracellular vesicle (EV)-enriched fraction of serum, which was isolated using ultracentrifugation and analyzed via 4D-data-independent acquisition mass spectrometry (4D-DIA-MS).

Results: A total of 3,112 proteins were identified in the EV-enriched fraction, among which 1,248 proteins demonstrated statistically significant differences between the pulmonary arterial sclerosis (PAS) and control groups ($P < 0.05$). These findings unveiled both known and novel pathways associated with PAS pathophysiology. Notably, elevated levels of collagen type III alpha 1 chain (COL3A1) and syndecan-1 (SDC1) were identified as potential biomarkers for PAS. Additionally, insights into specific disease-related pathways, such as extracellular matrix (ECM)-receptor interaction and neutrophil activation, were obtained.

Conclusion: In conclusion, our study has identified promising proteomic biomarkers and significantly advanced our understanding of the pathogenesis underlying PAS. Further validation studies in larger PAS cohorts are warranted to assess the clinical utility and reliability of these biomarkers for diagnostic purposes and targeted therapeutic interventions in PAS.

P1.52.
PLACENTA-DERIVED SVEP1 MAY PRIME MATERNAL PLATELETS DURING PREGNANCY

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Objectives: During hemochorial placentation, rapid and effective hemostasis is essential for successful implantation and to ensure the survival of both mother and fetus. Throughout pregnancy, maternal platelet count gradually decreases, potentially due to hemodilution, increased sequestration, and consumption within the placental circulation. In this study, we aimed to further investigate the tightly regulated cross-talk between the placenta and maternal platelets across different stages of gestation.

Methods: Platelets were isolated from whole blood samples collected from healthy pregnant women in the first and third trimesters, women diagnosed with preeclampsia, and healthy non-pregnant controls. Platelet fractions were purified by magnetic-beads based depletion of red blood cells and leukocytes and subjected to proteomic and transcriptomic analysis. In addition, platelets as well as placental tissue samples were stained for SVEP1 (Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1) via immunohistochemistry. The potential role of SVEP1 in promoting platelet activation was further assessed through aggregometry measurements.

Results: Proteomic analysis revealed that SVEP1 is upregulated in platelets from pregnant women compared to non-pregnant controls. SVEP1 appears to be selectively taken up by platelets. In placental tissue, SVEP1 expression was higher in both preeclamptic cases and gestational age-matched controls relative to first trimester samples.
Conclusion: Our findings highlight SVEP1 as a pregnancy-associated protein that is enriched in maternal platelets and placental tissue. The presence of SVEP1 in maternal platelets suggests a potential role in modulating platelet activation and maternal-fetal vascular interactions. These results provide new insights into the dynamic cross-talk between platelets and trophoblasts, and suggest a potential role of SVEP1 in maternal vascular and immune adaptation during pregnancy and diseases such as preeclampsia.

P1.53.
ASTROCYTE AND MICROGLIA ACTIVATION MAY EXACERBATE SEV-MEDIATED BLOOD-BRAIN BARRIER DISRUPTION IN PREECLAMPSIA

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Objectives: Preeclampsia (PE) is associated with systemic endothelial dysfunction and an increased risk of cerebrovascular complications, including blood-brain barrier (BBB) disruption. While brain endothelial cells (BECs) are the primary structural component of the BBB, astrocytes and microglia are essential for maintaining its integrity and modulating neuroinflammation. Plasma-derived PE-derived small extracellular vesicles (PE-sEVs) are implicated in BBB breakdown, and previous evidence has shown their ability to enter the brain parenchyma. We hypothesize that astrocytes and microglia are subsequent targets of PE-sEVs, contributing to BBB disruption through the activation of inflammatory mechanisms.
Methods: We used human-based in vitro BBB models to assess the effects of circulating PE-sEVs on astrocytes and microglia, and their downstream impact on BECs. Glial activation was evaluated through GFAP and Iba1 expression, assessment of cell morphology, IL-6 release, and wound closure assays. Microglial phagocytosis of sEVs was also examined. Changes in BECs were monitored by tight junction protein expression (occludin and claudin-5) and analysis of barrier permeability.
Results: PE-sEVs increased GFAP expression, IL-6 secretion, and enhanced migration. Microglia phagocytosed PE-sEVs and upregulated Iba1 expression. The presence of glial cells reduced BBB disruption upon acute PE-sEV exposure, likely due to the mounting of a reactive response.
Conclusion: PE-sEVs activate astrocytes and microglia, with protective effects in acute stimulation; however, in the long term, the occurrence of glial inflammatory responses may contribute to BBB dysfunction. The astrocyte-microglia axis emerges as a potential amplifier of BBB breakdown via sEVs in PE and a potential target to mitigate its cerebrovascular complications.

P1.54.
GENETIC CO-OCCURRENCE NETWORKS IDENTIFY POLYMORPHISMS IN THE VASCULAR ENDOTHELIAL GROWTH FACTOR SIGNALLING PATHWAY HIGHLY ASSOCIATED WITH PREECLAMPSIA.

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Objectives: Preeclampsia is a heterogeneous disease likely caused by the interactions of multiple genetic variants. Genome-wide association studies capture only single-variant relationships to a disease. We developed a novel statistical framework to assess standard SNP array data to identify the cooccurrence of gene polymorphic variants with a disease. These were then applied to maternal genetics data for preeclampsia.
Methods: A statistical framework that used a cooccurrence test and chi-square enrichment test to identify significantly associated SNPs to a disease was designed. The method was tested first with synthetic data and then applied to EGAD00000000022 and PE dataset EGAD00010000854. Enriched SNPs were mapped into a network graph and tested for enrichment to gene ontologies using GREAT. Enriched ontologies were grouped, clustered, and annotated using Cytoscape and the Enrichment-Map plugin.
Results: Analysis of synthetic data showed a strong ability to identify co-occurring SNPs from the background, with bonafide co-occurring SNPs showing higher degrees and connectivity compared to the background. Application of the cooccurrence methods on real-world data of 3172 individuals (1320 control, 1852 PE) showed graph structures of co-occurring SNPs in strong agreement with synthetic data experiments. Cooccurrence methods identified several 100 cooccurring SNPs to preeclampsia. Divisive clustering produces 6 clusters of patients, including a highly resistant cluster of control samples and two PE-enriched clusters. Ontological analysis identified ontologies representing immune, metabolic and endocrine pathways. Highly enriched were SNPs associated with genes in the VEGF signalling pathway. A group of PE cases representing one of the two PE-enriched clusters were significantly enriched in SNPs in VEGF signalling genes.
Conclusion: Cooccurrence shows increased sensitivity in discovering associated SNPs to multigenic diseases like preeclampsia. Enriched SNPs may form the basis of early or pre-pregnancy risk diagnosis for preeclampsia. Genetic subgroups with potentially pure or environmentally triggered preeclampsia were identified.

P1.55.
JQ1-MEDIATED BROMODOMAIN INHIBITION REVEALS TREML2 AS A MASTER REGULATOR OF TROPHOBLAST FUSION AND IDENTIFIES ITS DECREASED EXPRESSION IN PREECLAMPTIC PLACENTAS.

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Objectives: This study aims to identify novel epigenetic regulators that play crucial roles in trophoblast syncytialization through systematic high-throughput screening of an epigenetic compound library. We will validate the most promising candidates through functional assays in relevant trophoblast cell models and confirm their expression in human placental tissues. Our primary objective is to discover previously uncharacterized molecular targets that control the process of cytotrophoblast fusion and differentiation into syncytiotrophoblast.
Methods: BeWo, a choriocarcinoma cell line, was utilised for in vitro trophoblast syncytialization studies and to screen an epigenetic compound library comprising 101 small-molecule inhibitors. To assess trophoblast differentiation, β-HCG ELISA was performed alongside qRT-PCR analysis of key differentiation markers GCM-1 and Syncytin-1. Ultrastructural alterations during trophoblast cell fusion were studied by electron microscopy. Chromosomal dynamics were investigated through parallel analysis of ATAC-Seq and RNA-Seq datasets. Additionally, immunohistochemistry and genetic knockdown experiments were conducted to further validate the findings.
Results: High-throughput screening of an epigenetic compound library identified JQ1, a bromodomain inhibitor, as a critical regulator of trophoblast syncytialization through inhibition of β-HCG production. Treatment with JQ1 resulted in the disappearance of cell-cell adhesion markers, including desmoplakin, E-cadherin, and ZO-1, while ultrastructural imaging revealed reduced syncytium formation. Integrated ATAC-Seq and RNA-Seq data analysis identified Triggering Receptor Expressed on Myeloid Cells-like 2 (TREML2) as a key driver during syncytialization. siRNA-mediated silencing of TREML2 significantly impaired the syncytialization process, as evident by β-hCG production. Immunohistochemical localisation demonstrated reduced TREML2 expression in preeclamptic placental tissue samples compared to normotensive controls (n=12).
Conclusion: This study identifies JQ1, a bromodomain inhibitor, as a potent regulator of trophoblast syncytialization. Our findings establish TREML2 as a novel master regulator of syncytiotrophoblast formation, with significant implications for understanding placental development and pathophysiology.

P1.56.
LOSS OF MATERNALLY IMPRINTED SFMBT2 MICRORNA CLUSTER RESULTS IN PLACENTAL JUNCTIONAL ZONE ABNORMALITIES AND FGR IN MICE

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Objectives: The Sfmbt2 gene is maternally imprinted and robustly expressed in mouse placentas. Previous studies on the inbred C57Bl6 strain have shown that deleting the paternal allele's 10th intron, containing over 70 microRNA precursors, resulted in fetal death, accompanied by abnormal placental morphology, including the shrinking of the placental junctional zone. Here, our objective was to investigate the microRNA cluster deletion in the outbred CD1 strain and assess pup viability and placentation.
Methods: We generated Sfmbt2 microRNA deletion using a CRISPR-Cas9 approach, and founder heterozygous males of CD1 background were mated to wild-type females. We dissected pregnant females at embryonic days 11.5, 15.5, and 17.5 (E11.5, E15.5, E17.5), measured placental and pup weights, and performed histological analyses on collected placentas. Additionally, using CRISPR-Cas9 targeting, we deleted the microRNA cluster in trophoblast stem cells and generated mutant placental organoids to assess the deletion's effect on differentiation.
Results: Surprisingly, Sfmbt2 miRNA deletion on the outbred background is fully viable. However, we observed a dramatic weight decrease of up to 25% in Sfmbt2 microRNA+/- pups and fetuses with decreased placental weights as early as day E11.5. Histological analyses revealed a decreased placental size, reduction in the glycogen compartment, and shrinkage of the junctional zone. TS cells lacking Sfmbt2 microRNA differentiated significantly slower.
Conclusion: In contrast to published data, paternal deletion of Sfmbt2 microRNA in outbred mice results in growth restriction but is not lethal. This hints at specific genetic variations between strains that compensate for the absence of the microRNA cluster. Moreover, we are currently analyzing glycogen cell markers in our Sfmbt2 microRNA+/- organoid model to elucidate the role of Sfmbt2 microRNAs in trophoblast developmental trajectories. Further investigation into inter-strain differences may shed light on the role of Sfbt2 microRNA in placentation and fetal growth.

P1.57.
GENETIC FACTORS CONTRIBUTING TO PLACENTAL INSUFFICIENCY: WHOLE-GENOME SEQUENCING OF TRIOS WITH RECURRENT PREGNANCY LOSS AND LIVE BIRTHS

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Objectives: Placental insufficiency (PI), broadly defined as poor placental function, is a major contributor to adverse pregnancy outcomes, including recurrent pregnancy loss (RPL), preeclampsia, and fetal growth restriction. Despite diagnostic advancements, PI is often undiagnosed until complications arise. Genetic biomarkers of PI will be effective tools for guiding diagnostic and risk stratification approaches in pregnancy, however, genetic factors that contribute to PI remain poorly understood. Whole-genome sequencing (WGS) offers a comprehensive approach to uncover genetic variations that contribute to PI in unexplained cases.
Methods: We performed WGS on 14 trios, i.e., 14 parents, 31 placentae affected by suspected PI (cases) and 32 live births unaffected by PI (controls) from the same parents. Suspected PI was defined as any of the following: unexplained RPL (embryonic losses, fetal deaths, and/or stillbirths), severe preeclampsia, spontaneous preterm birth, or small-for-gestational age. We used the Illumina platform for WGS and state-of-the-art protocols to identify chromosomal abnormalities and variants (e.g., structural variants [SVs] as deletions or duplications). We compared variants between suspected PI cases to controls.
Results: Among 14 trios, 10 passed genotype quality control, which included 22 suspected PI cases and 32 controls. Chromosomal coverage analysis identified a previously undetected whole-chromosome gain-trisomy 11-in one PI case (embryonic loss at 8 weeks' gestation) in a trio with three embryonic losses and fetal deaths. This rare aneuploidy, known as partial trisomy 11q, is associated with intrauterine growth restriction and craniofacial abnormalities in previous studies. Our finding highlights the utility of WGS in uncovering cryptic chromosomal abnormalities not detected by karyotype or microarray testing.
Conclusion: This study leveraged WGS in trios with suspected PI and unaffected controls to discover genetic contributions to suspected PI. The findings will enhance our understanding of the genetic contributors to PI in RPL, providing new avenues for risk stratification and genetic counseling to improve pregnancy outcomes.

P1.58.
OPTIMIZING CUT&TAG TO STUDY HISTONE MODIFICATIONS IN THE HUMAN PLACENTA

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Objectives: The placenta has a unique DNA methylation (DNAme) profile that may interact with diverse histone post-translational modifications (PTMs) to mediate the distinctive sex and cell-influenced epigenetic regulation during pregnancy. However, the profiles of these histone modifications in different placental cell types and by sex has not been studied. Cleavage Under Targets and Tagmentation (CUT&Tag) can be used to investigate histone marks in challenging tissues such as the multinucleated syncytiotrophoblast, as it is adapted to low input DNA obtained from isolated nuclei.
Methods: We developed a strategy to use CUT&Tag to assess H3K27me3, H3K4me3, H3K9me3, H4K20me1 and H420me3 in placenta, assumed to be associated with sex and cell-influenced features. Nuclei isolation was optimized to obtain single nuclei from enzymatically separated syncytiotrophoblast (STB) and trophoblast-depleted mesenchyme (DM) and checked by microscopy to ensure intact nucleus and clumping. 150K nuclei along with the SNAP-CUTANA K-metStats were used together to optimize CUT&Tag and to normalize the sequencing data. Flash-frozen tissues showed preservation of the histone marks but signal-to-noise ratio was high. We aim to perform CUT&Tag in fresh placentas from at least 3 XX and XY placentas, for all histone PTMs for both STB and DM including 2 replicates and a negative control.
Results: We isolated the heterogeneous nuclei of complex placental tissues and used the products for histone PTM CUT&Tag. The DM and STB nuclei generated DNA libraries showing the regular sizes of nucleosomal ladders. Our pilot sequencing data on the histone PTMs such as H3K27me3 and H3K4me3 (well-characterized in other tissues) showed the expected traces in the proximal/distal regions of TSS and promoters confirming the success of CUT&Tag.
Conclusion: The optimized protocols will be applied to a larger number of placentas to determine how histone modifications differ by sex and cell type and their relationship to DNA methylation.

P1.59.
IN-VITRO AND FUNCTIONAL CHARACTERIZATION OF PLAC9 IN EARLY PREGNANCY ESTABLISHMENT

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Objectives: To characterize in-silico the PLAC gene and protein

To determine by PLAC9 gene loss/gain of function assays, the functional impact on migration, invasion and differentiation processes in trophoblast cells under basal conditions and under altered conditions in PE.

Methods: We use bioinformatics platforms, such as Gene Mania, STRING and SWISSPROT to identify protein and gene characteristics. MEME was used to detect motifs and the ALGGEN PROMO platform to identify transcription factors that bind to these motifs.

For the functional assay we use two first-trimester EVT cell lines: HTR-8/SVneo and Swan71. Lentiviral constructs of and overexpression and PLAC9 silencing vectors were designed and packaged by VectorBuilder.

The vectors were transformed into competent E.coli bacteria, and the extracted DNA was used to transfect the cell lines. The transfections were assessed by real-time PCR.

Cell invasion was determined by a transwell invasion assay.

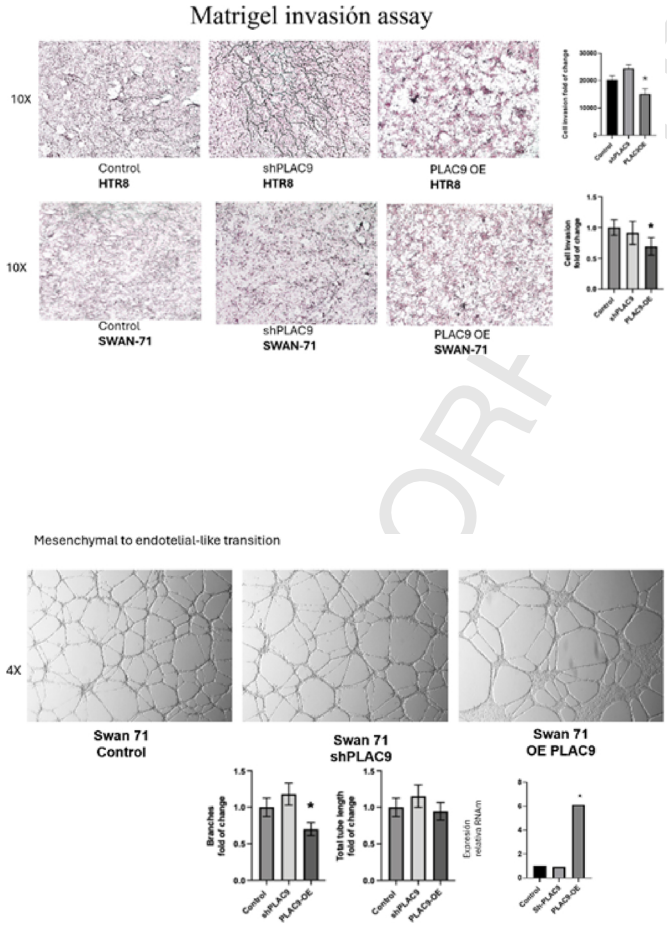
Cellular differentiation was documented by evaluating the role of PLAC9 silencing and overexpression in the process toward the endothelial cell phenotype. This was represented by the cells ability to form tubular structures in a polymerized Matrigel matrix.

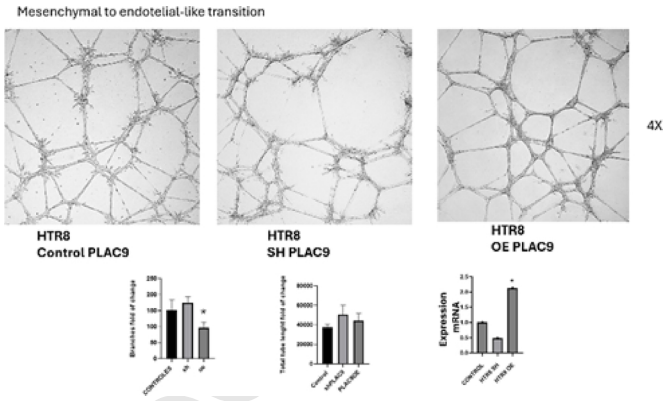
Results: We found that PLAC9 is co-expressed with genes that are involved in biological processes important for normal pregnancy development, such as the formation and function of the placenta.

Interactions between the PLAC9 and proteins such as, TWIST2, PODN and FGF10 suggesting a possible multifaceted role in various cellular processes such as angiogenesis and development of placenta

We identified two unique PLAC9 motifs that bind transcription factors related to pregnancy, preeclampsia, and apoptosis.

Overexpression of PLAC9 showed a decrease in cell migration and a significant decrease in the number of branches.





Conclusion: PLAC9 has interactions with genes and proteins important for normal pregnancy

The TFs that bind to the gene are related to PE, preeclampsia and apoptosis

Overexpression of PLAC9 decreases angiogenesis and cell migration

P1.60.
EVALUATION OF NUCLEOTIDE VARIATIONS IN THE ADORA2A GENE AND THEIR ASSOCIATION WITH PREECLAMPSIA

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Objectives: To identify the placental nucleotide variations in the ADORA2A gene and their association with preeclampsia.

Methods: The local Ethics Committee approved this case study (women with PE, n=50) and control study (healthy pregnant women, n=50) (registration number 596/022). PE was classified as early or late. Placental DNA was used to evaluate five SNVs in ADORA2A: rs2298383, rs4822489, rs2236624, rs8192446, and rs17650937 by real-time PCR using TaqMan probes. The association between SNVs and PE was analyzed using multivariate regression analysis, and normally distributed variables are expressed as mean ± SD values.

Results: We found significant differences in the genotype and allele frequencies of the SNV rs8192446 studied in PE compared to controls. An association between this same SNV rs8192446 and PE was observed (OR=27.46, 3.42-220.48, p=0.0001), rs17650937 was also found associated with PE, but in strong Hardy Weinberg Disequilibrium. The variants rs2298383, rs4822489, and rs2236624 were not associated with preeclampsia. Haplotype analysis revealed a strong linkage disequilibrium between SNVs rs17650937 (T) and rs8192446 (C) (D' = 0.0068). This haplotype TC was significantly associated with an increased risk of developing preeclampsia [OR = 20.67; 95% CI: 2.49–171.67; p = 0.0061].

Conclusion: An association was observed between PE and placental SNV rs8192446 (OR=27.46; 95% CI: 3.42-220.48; p<0.0001). Haplotype analysis revealed a strong linkage disequilibrium between rs17650937 and rs8192446 (D'=0.0068), indicating that the haplotype TC is associated with preeclampsia (OR = 20.67 (95% CI: 2.49-171.67; p = 0.0061).

P1.61.
EPIGENETIC EFFECTS OF *STOX1* TRANSCRIPTION FACTOR IN TROPHOBLAST CELLS

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Objectives: We study the epigenetic effects of the transcription factor *Storkhead Box 1 (STOX1),* particularly on the methylation pattern of a CpG island in the promoter region of *FAD dependent oxidoreductase domain containing 2 (FOXRED2).* Furthermore, we assess the role of FOXRED2 in

key pathophysiological aspects of placental dysfunction like ER stress response and oxidative stress management.

Methods: We profiled the global methylation landscape with the Illumina Methylation EPIC array in JEG-3 cells overexpressing STOX1A. In parallel, gene expression was determined using the ClariomD microarray. STRE1, a STOX1 DNA-binding site found up-stream of the CpG island, was mutated in STOX1A overexpressing JEG-3 cells through CRISPR-Cas9 technology. The methylation levels in mutant cells were determined with the EZ DNA Methylation Kit. Oxidative stress management and ER stress response were assessed by live cell-imaging.

Results: We identified 17 494 methylation sites that were significantly differentially methylated. The most differentially methylated region is a CpG island present in the promoter region of *FOXRED2*, a gene involved in endoplasmic reticulum function. The overexpression of STOX1A suppresses the gene expression of *FOXRED2* through hypermethylation of a CpG island found in the promoter region of *FOXRED2*. After mutating STRE1, the complete disruption of the binding sequence caused a demethylation of the CpG island. Hence, the methylation pattern of the promoter region in mutated cells seems to revert to normal conditions of wild-type JEG-3 cells. Low levels of FOXRED2 are associated with higher levels of ER stress and poor oxidative stress management.

Conclusion: We conclude that the expression of *FOXRED2* in JEG-3 cells is largely driven by DNA methylation. STOX1A binds to STRE1, a DNA binding site of STOX1 present upstream of the CpG island, and enables the observed epigenetic modifications and pathophysiological alterations. Thus, changes in FOXRED2 expression lead to ER and oxidative stress, key aspects of placental dysfunction.

P1.62.
PRECONCEPTION-PREGNANCY MICRONUTRIENT INTERVENTION ALTERS DNA METHYLATION IN OFFSPRING UC-MSCS: IMPLICATIONS FOR THE BRAIN-ADIPOSITY AXIS

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Objectives: Maternal micronutrient status is important for healthy feto-placental development, and our NiPPeR randomised trial recently reported that improving maternal micronutrient status during preconception/pregnancy can reduce the risk of rapid infant weight gain and child obesity. We hypothesised that preconception/pregnancy micronutrient intervention alters DNA methylation in offspring, particularly in genes involved in adiposity development, thereby reducing obesity risk at age 2 years.

Methods: Umbilical cords were collected at birth as part of the NiPPeR trial, in which participants were randomised to receive either a standard micronutrient control supplement or an intervention supplement additionally containing myo-inositol, probiotics, and other vitamins, taken from preconception through to delivery. Umbilical cord mesenchymal stem cells (UC-MSCs) were isolated from 18 offspring samples (8 intervention/10 control), cultured, and DNA extracted. A Duet-ModC library was prepared for each sample using a novel technology capable of simultaneously detecting all four canonical bases and modified cytosines. Differentially methylated regions (DMRs) were identified between UC-MSCs from intervention-born and control-born offspring.

Results: Analysis of DNA methylation identified 3,171 significant DMRs, encompassing genes enriched in key pathways related to synaptic protein-protein interactions, cell-cell adhesion, and synapse organisation. Among the top 100 DMRs with greatest fold change, six genes (*KCNQ5, GPC6, HYDIN2, UTRN, LRP1B, PAPOLA*) associated with the hypothalamus were more hypomethylated in the UC-MSCs of intervention-born offspring.RNA-seq is in progress to validate the gene expression changes related to the identified DMRs.

Conclusion: Preconception/pregnancy nutritional supplementation alters offspring DNA methylation profiles, especially involving genes important in central nervous system and synaptic processes. Those in the intervention group had a greater proportion of hypomethylated DMRs, suggesting activation of related gene pathways. These may impact offspring adiposity risk postnatally through the gut-brain-adiposity axis, which plays a crucial role in regulating appetite, energy balance and metabolism.

P1.63.
SPATIOTEMPORAL EXPRESSION OF $P57^{KIP2}$ DURING MOUSE PLACENTAL DEVELOPMENT

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Objectives: The development and growth of the placenta is precisely regulated at each stage of pregnancy. However, the mechanism is still not fully understood. Previous gene editing mice studies suggested that the cyclin-dependent kinase inhibitor $p57^{Kip2}$ plays a crucial role in placental development and growth. However, it is unclear how $p57^{kip2}$ regulates placental development. In this study, we analyzed the histological expression of $p57^{kip2}$ during the course of pregnancy in mice.

Methods: Placentas were obtained from ICR mice at days 9, 12, 15 and 18 post-coitum (p.c.). Paraffin-embedded tissue sections were used for immunohistochemistry. The $p57^{Kip2}$ expression was histologically assessed.

Results: The expression of $p57^{kip2}$ changed during pregnancy. Trophoblast giant cells (TGCs) at day 9.0 p.c. showed the highest intensity, not only in the nucleus but also in the cytoplasm. At day 9.0 p.c., $p57^{kip2}$ expression was observed only in TGCs, but later it was also observed in the spongiotrophoblast cells (SpT) and labyrinth (Lb). In SpT and Lb, positive staining cells were increased, and all cells were positive staining at day 15 and 12 p.c. respectively. At day 18.0 p.c., TGCs showed negative staining and SpT showed both negative and positive staining cytoplasm, which was the highest positive intensity in SpT.

Conclusion: These results suggest that $p57^{kip2}$ may play different roles at different stages and may be involved in functions in the cytoplasm other than regulating the cell cycle.

P1.64.
INVESTIGATING THE ROLE OF OXYGEN IN PLACENTAL DEVELOPMENT USING APICAL-OUT TROPHOBLAST ORGANOIDS

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Objectives: The human placenta is essential for maternal and fetal health, significantly influencing pregnancy outcomes. Despite its critical role, the mechanisms of human placental development remain poorly understood. This study aims to unveil how oxygen levels impact placental development using apical-out trophoblast organoids (TOs) from first-trimester placental tissues. The newly developed apical-out model with physiological polarity allows us to study the impact of oxygen on the in vivo relevant syncytiotrophoblast layer.

Methods: To investigate the impact of oxygen on trophoblast differentiation, we cultured in parallel apical-out TOs at 2% or 21% oxygen. Immunofluorescence was used to observe phenotypic changes, while transcriptomic analysis was conducted to identify metabolic shifts and gene expression changes.

Results: Our findings reveal significant phenotypic and transcriptomic changes in TOs cultured under low oxygen (2% O_2) compared to standard conditions (21% O_2). TOs at 2% oxygen exhibited reduced size and altered

cell type proportions. Transcriptomic analysis indicated a metabolic shift towards glycolysis and increased expression of cytotrophoblast cell column gene markers in the 2% oxygen conditions rather than differentiation of the cytotrophoblast towards the syncytiotropblast.
Conclusion: These findings enhance our understanding of oxygen's role in placental development and trophoblast differentiation. The research highlights the potential of primary apical-out TOs as a model for studying placental biology, providing insights into the mechanisms of human placental development and the impact of oxygen levels on trophoblast differentiation.

P1.65.
INTERFERON-TAU (IFNτ) AND ESTRADIOL (E2) LEAD TO AN INCREASED EXPRESSION OF SPECIFIC EPITHELIAL CELL MARKERS IN BOVINE CARUNCULAR EPITHELIAL CELLS (BCEC) IN VITRO

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Objectives: The majority of embryonic death in dairy cattle occurs prior to implantation and leads to economic losses for the farmers. During the peri-implantation period the ovarian steroid hormones estradiol (E_2) and progesterone (P_4) as well as the ruminant-specific pregnancy recognition signal interferon-tau (IFN_τ) influence the uterine gene expression and prepare the endometrium for successful implantation. Since epithelial cells are usually non-adhesive, it is assumed that the endometrial epithelial cell programme is changed (epithelial-mesenchymal transition) to allow attachment to the trophoblast. Therefore, we hypothesise that E_2, P_4 and/or IFN_τ lead to a modified expression of epithelial cell markers in preparation for embryo implantation.
Methods: In experiment 1, bovine caruncular epithelial cells (BCEC) were stimulated with 100 and 1.000 ng/ml IFN_τ for 24 h. In experiment 2, BCEC were stimulated with E_2 (20 pg/ml), P_4 (20 ng/ml) or P_4 (20 ng/ml) in combination with IFN_τ (100 ng/ml) for 48 h. BCEC grown in serum-reduced medium or vehicle medium (PBS, ethanol) served as controls. The mRNA-expression of ezrin (*EZR*), cytokeratin 18 (*CK18*), E-cadherin (*CDH1*), occludin (*OCLN*) and zonula occludens-1 (*ZO-1*) was examined via quantitative real time-PCR. Additionally, the protein expression of EZR, CK18 and CDH1 was investigated via Western blotting.
Results: In experiment 1, both IFN_τ-concentrations significantly upregulated the mRNA-expression of *EZR* ($p<0,001$), *CDH1* ($p<0,05$), *OCLN* ($p<0,05$, $p<0,001$) and *ZO-1* ($p<0,05$, $p<0,001$) in a dose-dependent manner. In experiment 2, only E_2 led to a significant upregulation of *EZR*-($p<0,001$) and *CK18*-mRNA ($p<0,05$). However, in neither experiment significant differences were observed regarding the protein expression.
Conclusion: In conclusion, application of IFN_τ and E_2 leads to an increased expression of specific epithelial cell markers and thus might support preparation of the endometrium for embryo implantation. Further studies on the protein localisation of these epithelial cell markers in BCEC are planned.

P1.66.
THE ROLE OF THE PLACENTA IN THE RISK FOR SCHIZOPHRENIA

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Objectives: Schizophrenia is a mental disorder with a complex aetiology. Along with a strong genetic component, the early life complications (e.g., complications during the pregnancy) have been linked to an increased schizophrenia risk. Several ultra-rare risk genes with a high schizophrenia risk have been identified to date. One of these genes, CUL1, has been demonstrated to have a role in the implantation and placentation of the embryo, pointing to a potential impact of these genes on an early and very critical stage of the embryo development.
We hypothesise that the ultra-rare schizophrenia risk gene variants affect the placental function, and in conjunction with the environmental risk factors, this could lead to an erroneous foetal neurodevelopment and a later risk for the development of schizophrenia. We selected a set of ultra-rare schizophrenia risk genes expressed in the placenta (TRIO, 3q29del, XPO7, GRIA3) with an unknown effect on the implantation and placentation, along with the CUL1. The first study objective is to develop 3D *in vitro* placental models from the embryonic stem cells (ESCs) to investigate the functionality of the placenta. The second objective of the study is applying these placental models to assess the impact of the ultra-rare schizophrenia risk gene variants.
Methods: To obtain the 3D placental organoids, we are reversing the primed embryonic stem cells (ESCs) to a naive-like state and deriving the trophoblast stem cells (TSCs), which will further be differentiated to extravillous trophoblasts (EVTs) and syncytiotrophoblasts (SCTs). Functional testing of these cell types will be performed in 2D and 3D models. Further, the CRISPR/Cas9 edited ESCs will be utilised.
Results: We have validated the naive-like ESC and TSC protocols, and the work on the characterisation and further differentiation is ongoing.
Conclusion: Conclusions to date indicate the robustness of the applied protocols for the ESC conversion and the derivation of TSCs.

P1.67.
CRAC CHANNELS IN THE HUMAN PLACENTA: EXPRESSION PATTERNS AND FUNCTIONAL IMPLICATIONS

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Objectives: Aberrations in calcium (Ca^{2+}) signaling and impaired Ca^{2+} homeostasis have been associated with severe placental disorders, including gestational diabetes (GDM), intrauterine growth restriction (IUGR) and preeclampsia (PE). Our previous results showed increased mRNA and protein expressions of Ca^{2+}-release activated Ca^{2+} (CRAC) channels, composed of Orai1-3 subunits and the ER Ca^{2+} sensor proteins stromal interaction molecules (STIM1 and 2) upon syncytium formation. In placental tissue affected by GDM, IUGR and PE, Orai3 expression was significantly higher compared to healthy controls. However, the underlying mechanisms governing intracellular Ca^{2+} signaling in the placenta, particularly the functional role of Ca^{2+}-regulatory proteins, remain largely unknown.
Methods: In this study, we aimed to explore the potential role of CRAC channels, and their activators, STIM1 and 2, during trophoblast differentiation using healthy primary human trophoblasts and placental cell lines (HTR8-SVneo and BeWo). To investigate the contributions of Orai isoforms to SOCE and its downstream signaling, as well as to Ca^{2+}-dependent cellular functions in the human placenta, we performed Fura-2AM-based Ca^{2+} imaging with pharmacological CRAC channel inhibitors, xCELLigence and CRISPR-Cas9 technology.
Results: We observed that upon spontaneous syncytialization of primary trophoblast cells and forskolin-induced syncytium formation of BeWo cells Fura-2AM-based detection of SOCE was elevated. Application of CRAC channel inhibitors effectively blocked SOCE in both HTR8-SVneo and BeWo cells. Interestingly, despite the pharmacological inhibition of CRAC channels in the extravillous trophoblast (EVT) cell line HTR8-SVneo, key cellular functions such as proliferation and migration remained unaffected. In contrast, the deletion of Orai1 led to an increase in both proliferation and migration in HTR8-SVneo cells compared to the control group.
Conclusion: In conclusion, our data provide evidence for SOCE and suggest that CRAC channels play a potential role in the regulation of EVT proliferation and migration. Future research will focus on understanding the functional role of CRAC channels in syncytium formation.

P1.68.
INVESTIGATING THE MOTILITY AND FUNCTION OF ENDOMETRIAL CILIA.

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Objectives: This study aims to visualise and characterise the beat pattern and structure of motile cilia from endometrial biopsies and develop an endometrial organoid model to study the endometrial epithelial apical surface for cilia motility.
Methods: Endometrial samples are collected from women with various fertility conditions and a control group of those who have had children without intervention. Tissue samples are stored, and endometrial organoids are cultured. High-speed video microscopy is used to analyse cilia mobility within 1 hour of sample collection. Epithelial cells are cultured into organoids and suspended in Matrigel; following this, they are removed from Matrigel and cultured in suspension to promote apical membrane differentiation on the outer surface of the organoids.
Results: In conflict with previous studies, endometrial motile cilia have been visualised beating in coordination within and between cells. Evidence that they may play a role in generating a current within the womb lining or glandular structures. Furthermore, apical membrane-out endometrial organoids have been cultured for the first time using novel techniques in this field, providing a new model for fertility interventions and treatments.
Conclusion: Cilia may have a coordinated beat pattern in the endometrium, suggesting roles in mucus clearance or embryo movement. This result, alongside the novel apical-out endometrial organoid model, can provide the foundations for investigating and understanding the role of motile cilia in the womb. Future research using these techniques could improve understanding of how the endometrium prepares for pregnancy and lead to new therapeutic strategies for reproductive disorders.

P1.69.
TRPV2-MEDIATED CALCIUM SIGNALING CHARACTERIZES SYNCYTIOTROPHOBLAST DIFFERENTIATION.

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Objectives: Crucial for placental development is the establishment of the syncytiotrophoblast (STB), a multinucleated cell formed by trophoblast fusion, with important roles in embryo implantation and hormone secretion. Previous research of our group has demonstrated that TRPV2, a calcium-permeable ion channel, is indispensable for normal placental development in mice, through its contribution in syncytium formation. Moreover, loss of TRPV2 results in fetal growth restriction (FGR). It remains unclear whether TRPV2 plays a conserved role in human STB differentiation and FGR. Here, we aim to unravel how calcium signals are regulated during human STB differentiation and whether TRPV2-induced calcium influx precedes or results from STB fusion/differentiation.
Methods: To do so, the molecular expression of TRPV2 was assessed during STB differentiation of hTSC by qRT-PCR and FISH, whereas calcium microfluorimetry including TRPV2 agonists and antagonists evaluated channel functionality. The exact timing of TRPV2-induced calcium influx will be determined by CRISPR/Cas9-mediated gene-tagging of TRPV2 with the genetically encoded calcium indicator GCaMP6f, combined with endogenous tagging of genes informative for STB fusion/differentiation.
Results: Calcium microfluorimetry revealed that intracellular calcium levels significantly increase during human STB differentiation. Interestingly, both qRT-PCR and RNAscope showed that the calcium-permeable ion channel TRPV2 is highly upregulated during STB differentiation. This increase in molecular TRPV2 expression relates to channel functionality, as shown by functional calcium microfluorimetry. Furthermore, high intracellular calcium levels correlate with TRPV2 expression and TRPV2 seems to be co-expressed with SDC1, a marker of differentiated STB. Live-imaging of hTSC in which TRPV2 was endogenously tagged showed GCaMP6f signals during STB differentiation, indicating a role for TRPV2-mediated calcium influx in early human STB differentiation.
Conclusion: To conclude, our results strengthen the hypothesis that calcium influx through TRPV2 is important for human STB differentiation but TRPV2's specific role and the consequences of its absence remain to be elucidated.

P1.70.
NATURAL KILLER-INVASIVE TROPHOBLAST CELL DYNAMICS IN THE ESTABLISHMENT OF THE RAT HEMOCHORIAL PLACENTA

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Objectives: During placentation, natural killer (**NK**) and invasive trophoblast (**IT**) cells have roles in transforming the uterine environment, including spiral artery remodeling. NK cells enter and expand in number within the post-implantation uterus. After mid-gestation, NK cells disappear from the uterine-placental interface (**UPI**) as IT cells migrate into the uterus. IT cells take two routes of entry into the uterus: endovascular and interstitial. Thus, IT cells and NK cells cooperate but act in temporally distinct phases of gestation. In this work, we explore roles for IT and NK cells in transformation of the UPI and their influence on maternal adaptations during pregnancy
Methods: Gravimetric, histological, biochemical, and molecular phenotypes were investigated in wild type, NK cell deficient (*Il15* null), interstitial IT cell deficient (*Plac1* null); total IT cell deficient (*Tfap2c* conditional null), and NK and IT cell doubly deficient rat models.
Results: Litter size and viability among breeding combinations at gestation day (gd) 7.5 were similar. Maternal NK cell deficiency was associated with abnormal intrauterine trophoblast cell invasion at gd 18.5, whereas IT cell deficiency models were associated with changes in placentation and retention of NK cells in the UPI. Double-deficient pregnancies (absence of maternal NK cells and interstitial IT cells or all IT cells) exhibited abnormalities in placentation, impaired fetal growth, and increases in fetal death. Postnatal offspring survival was negatively affected in double mutant pregnancies. Evidence for disruptions in maternal adaptations associated with NK and IT cell deficiencies were also observed.
Conclusion: A "single hit" adversely affecting NK cells, interstitial IT trophoblast cells, or total IT cells altered placental site development; however, a "double hit" affecting both NK and IT cells led to more severe impacts on placental and fetal development and survival. These findings underscore the remarkable cellular plasticity at the UPI and the cooperative roles of maternal and extraembryonic cells

P1.71.
INVOLVEMENT OF NR2F6 IN HEMOCHORIAL PLACENTATION

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Objectives: The placenta is crucial for fetal growth throughout pregnancy. During hemochorial placentation, the extravillous trophoblast (**EVT**) cells from the placenta enter the uterus. This enables the remodeling of uterine arteries that deliver nutrients from the maternal bloodstream to the fetus. Disruptions in this process are associated with pregnancy diseases, such as early pregnancy loss, pre-eclampsia, or preterm birth. Because of the poor understanding of EVT cell development and function regulation, it is crucial to identify the mechanisms underlying the regulation of this process. Nuclear receptor subfamily 2 group F member 6 (**NR2F6**) is a transcription factor predicted to contribute to the regulation of EVT cell development and function.

Methods: *Nr2f6/NR2F6* expression in human and rat placentation sites was assessed by *in situ hybridization* and RT-qPCR. We investigated the role of NR2F6 in hemochorial placentation using the human trophoblast stem (**TS**) cells and a loss-of-function approach using short hairpin RNA (**shRNAs**). We also tested an *in vivo* role for NR2F6 in placentation using rats, which possess a deep intrauterine trophoblast cell invasion, like humans. CRISPR-Cas9 genome editing was used to generate *Nr2f6* mutant rats.
Results: *NR2F6* was expressed in basal cytotrophoblast and EVT cell progenitors of first-trimester EVT cell columns. In human TS cells, *NR2F6* expression increased during the transition from the stem state to EVT cells. Disruption of *NR2F6* resulted in impaired cell differentiation into EVT cells, which is associated with a downregulation of EVT cell-associated transcripts and an upregulation of stem state-associated transcripts. In rats, *Nr2f6* is expressed in all trophoblast cell compartments of the placentation site, including invasive trophoblast cells in the uterine-placental interface. Efforts are underway to investigate potential placentation site phenotypes in *Nr2f6* mutant rat models.
Conclusion: NR2F6 is a candidate conserved regulator of the EVT cell lineage.
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P1.72.
AGE-RELATED DECIDUALIZATION DEFECTS IN PLACENTAL-DERIVED ENDOMETRIAL STROMAL CELLS

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Objectives: Advanced maternal age ($\geq$ 35 years) is associated with adverse pregnancy outcomes, e.g. preeclampsia. Women who developed preeclampsia reveal a molecular signature of impaired endometrial maturation suggesting a decidualization defect already before pregnancy. Therefore, we aim to broaden the knowledge about endometrial aging and decidualization defects which affects women`s reproductive health.
Methods: Human endometrial stromal cells (ESC) were isolated from the placental basal plate of healthy pregnant women of optimal maternal age (OMA; 18-30 years) (n=8) and advanced maternal age (AMA) (n=8). The aging profile of AMA ESC was analyzed by immunocytochemistry (p21, γH2AX^{S139}) and telomere length assay. To investigate age-related effects of decidualization, OMA and AMA ESC were decidualized for 6 and 9 days with a hormone cocktail (10 nM estradiol, 1 µM progesterone, 0.5 mM cAMP) and the mRNA expression levels of key decidualization (IGFBP1, PRL) and angiogenic markers (VEGF, PIGF, sFLT1,ENG) were quantified by qRT-PCR.
Results: ESC from women of AMA exhibited an altered cellular profile, characterized by increased expression of p21 and γH2AX^{S139} compared to those from women with OMA. On day 6 of decidualization, AMA ESC showed reduced mRNA expression level of IGFB1 and PRL relative to OMA ESC. In addition, the angiogenic markers VEGF, PIGF and ENG were decreased in AMA ESC at day 6, whereas the mRNA expression levels were comparable to those from OMA ESC by day 9.
Conclusion: Our results give first insights that placenta-derived ESC of women with AMA exhibit an impaired aging and decidualization profile, which may affect stromal cell functions and proteins involved in the decidualization process. Further studies are needed to investigate the properties of the human endometrium at different developmental stages, e.g. in the luteal phase, that may change with increasing women's age.

P1.73.
LAMB3 IN EARLY PLACENTAL DEVELOPMENT

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Objectives: Endoglandular trophoblast invasion, the infiltration of uterine glands by extravillous trophoblasts (EVTs), is essential for early placental development and embryo nourishment, yet key factors remain poorly understood. Preliminary analysis of publicly available scRNAseq and spatial transcriptomics data identified Laminin-subunit-beta 3 (LAMB3) as a gene of interest. LAMB3 is a promising candidate involved in the development and maintenance of the maternal-fetal interface. Given its putative role in epithelial remodeling, dysregulation of LAMB3 may contribute to impaired placentation and reproductive failure, including conditions such as early pregnancy loss (RM) or implantation failure (RIF). This study aims to (1) characterize the expression and spatial distribution of decidual LAMB3 during early placental development, and (2) evaluate the association between LAMB3 expression and trophoblast invasion.
Methods: Formalin-fixed, paraffin-embedded first-trimester decidual tissue sections (GA 6–12 weeks) from 32 healthy pregnancies were subjected to double immunofluorescence staining using antibodies against LAMB3 and HLA-G. HLA-G served as a marker to delineate EVT-invaded areas from non-invaded regions. A quantitative image analysis pipeline was developed using *CellProfiler* to measure LAMB3 fluorescence intensities. This approach enabled a spatial comparison of LAMB3 expression across EVT-invaded and non-invaded regions.
Results: Initial analyses demonstrated region-specific expression patterns of LAMB3 in the early decidua. Only glandular and decidual surface epithelium expressed LAMB3. A consistently increased fluorescence signal of LAMB3 in glandular epithelium was observed in areas invaded by EVTs compared to non-invaded regions. These findings suggest a potential spatial association between LAMB3 expression and endoglandular trophoblast invasion.
Conclusion: This study contributes to identifying key molecular factors involved in endoglandular trophoblast invasion, a critical yet understudied process in early human pregnancy. Future work, including functional experiments, will explore whether compromised glandular LAMB3 expression is associated with reproductive failure and compare endometrial and decidual LAMB3 expression between healthy pregnant and non-pregnant patients and those with reproductive complications.

P1.74.
ENDOPLASMIC RETICULUM STRESS, SENESCENCE AND MECHANOCOMPETENCE: RESHAPING OUR UNDERSTANDING ON ENDOMETRIAL RECEPTIVITY

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Objectives: Decidualization of human endometrial stromal cells (HESC) is a differentiation process that generates two distinct subpopulations, both essential for embryo implantation: mature decidual cells (mDec) and senescent decidual cells (snDec). Since endoplasmic reticulum (ER) stress is linked to the decidual phenotype, we aim to investigate its role in senescence and implantation, focusing on how decidualization alters cellular mechanocompetence and how ER-stress affects embryo implantation.
Methods: We performed an *in silico* analysis and identified expression patterns of mDec and snDec markers. These findings were validated in endometrial samples from fertile and recurrent implantation failures (RIF) patients and using decidualized HESC cell line pre-treated or not with Thapsigargin (Tg), an ER-stress inducer. RT-qPCR was assessed alongside flow cytometry. Cell nanomechanical properties were measured using atomic force microscopy and force spectroscopy. To evaluate implantation, we used CFSE-labeled blastocyst-like spheroids (BLS) and analyzed

their attachment and expansion index on decidualized HESC monolayers pre-treated or not with Tg.

Results: Decidualization significantly upregulated mDec markers like FOXO1 and snDec markers, such as LUM and β-galactosidase activity, suggesting that physiological senescence accompanies decidual differentiation. However, Tg-induced ER-stress reduced the expression of both mDec and snDec markers, as FOXO1, FTL, LUM, DIO2, and decreased X-gal staining. These indicates that ER-stress impacts the maturation of HESC, but also reduces senescent cells, leading to an imbalance between both subpopulations, as detected on RIF biopsies.

Regarding cells' nanomechanical properties, decidualization significantly decreased cellular stiffness. Tg pre-treatment reversed this, increasing cellular stiffness reaching levels similar to undifferentiated HESC and reducing BLS both attachment and expansion index. These results marked a reduction in the cells' permissiveness to trophoblast invasion.

Conclusion: ER-stress disrupts decidualization by impairing both decidualization and senescence, altering cell nanomechanical properties associated with endometrial receptivity. These findings highlight cellular mechanocompetence as a critical, previously unrecognized determinant of implantation success.

P1.75.
AN EX VIVO MODEL FOR STUDYING REGULATED CELL DEATH IN SYNCYTIOTROPHOBLAST USING HUMAN PLACENTAL EXPLANTS

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Objectives: To gain an enhanced understanding of the fundamental biology of programmed cell death in syncytiotrophoblast (STB), and to then probe these processes in the context of an infection, malaria, that is known to impact STB function and integrity, we have pursued investigation of STB cell death pathways utilizing placental explant cultures.

Methods: Placentas were obtained from healthy donors *via* cesarean delivery. Villous explants measuring 3 to 4 mm were cultured in DMEM/F12 medium supplemented with insulin, hydrocortisone, and retinyl acetate for up to 12 days at 5% CO_2. STB integrity was tracked by measurement of secreted chorionic gonadotropin (hCG) and released lactate dehydrogenase (LDH) by ELISA. Culture conditions known to induce necroptosis and ferroptosis plus Toll-like receptor (TLR) agonists were applied to induce these pathways in explants at day 8. Lipid peroxidation was estimated *via* malondialdehyde (MDA) detection using the thiobarbituric acid method.

Results: Placental explants were optimal for assays at day 8, as assessed by high hCG and low LDH levels. Necroptosis was successfully induced, as evidenced by LDH release and MDA formation, upon application of TNFα, Zvad-FMK, and cycloheximide, which was reduced by inclusion of the inhibitor, necrostain. TLR agonists (lipopolysaccharide (LPS), TLR4; polyI:C, TLR3; PAM3csk4, TLR2) did not augment cell death. RSL3 outperformed erastin as an inducer of ferroptosis-associated LDH release, and ferrostatin-mediated inhibition of LDH release and MDA formation confirmed activation of this cell death pathway. LPS, TLR4 and PAM3csk4 combined with RSL3 did not augment LDH release. Finally, LDH release and MDA formation in response to both necroptosis and ferroptosis conditions did not vary as a function of placental sex.

Conclusion: This study effectively establishes a reliable *ex vivo* placental explant culture model that offers optimal STB function and sustainability after 8 days in culture and is amenable for studies of programmed cell death in STB.

P1.76.
NEUTROPHIL-DRIVEN OXIDATIVE STRESS IN SYNCYTIOTROPHOBLAST

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Objectives: Oxidative damage in syncytiotrophoblast (STB) is commonly observed in pathological pregnancies, such as preeclampsia, and also in infectious diseases like malaria. Important drivers of, and outcomes related to, this pathology are incompletely understood. The purpose of this study is to assess the roles of respiratory burst in innate immune cells and exposure to malaria toxins in driving oxidative stress in STB.

Methods: This study employed forskolin-syncytialized choriocarcinoma BeWo cells, and primary human trophoblasts (PHTs) and villous explants isolated from cesarean-delivered term placentas to investigate the effects of neutrophil-derived ROS on STB. Human peripheral blood neutrophils were isolated from healthy donors using immunomagnetic negative selection, and then pre-loaded with 2',7'-dichlorodihydrofluorescein diacetate probe (DCFDA), a ROS-sensitive fluorescent dye. Exposure to phorbol myristate acetate (PMA) was used to elicit respiratory burst, with confirmation by flow cytometry. BeWo cells, PHTs and explants were then co-cultured with unlabeled resting or PMA-activated neutrophils. Activation of DCFDA in STB was assessed by measuring fluorescence intensity longitudinally in a microplate reader.

Results: BeWo cells, PHTs and explants exhibited time-dependent increases in intracellular ROS levels when co-cultured with PMA-activated neutrophils, with marginal increases upon exposure to resting neutrophils during the four hour time course.

Conclusion: These findings suggest that activated neutrophils can induce significant oxidative stress in STB *in vitro*. Ongoing experiments are exploring the synergistic effects of neutrophil-derived ROS and the heme-containing malaria toxin, hemozoin, on STB oxidative stress, characterizing the specific structural and functional consequences of this stress on the STB, and identifying antioxidant mechanisms that are mounted by trophoblasts to counteract this damaging process.

P1.77.
A NOVEL ROLE OF PREGNANCY-SPECIFIC GLYCOPROTEIN 1 AS AN INDUCER OF DISULFIDE BOND FORMATION IN HUMAN PLACENTAL TROPHOBLASTS

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Objectives: The placental trophoblasts, predominantly in species with hemochorial placentation including primates and rodents, secrete a large amount of pregnancy-specific glycoprotein 1 (PSG1) in pregnancy. Although PSG1 has been suggested to be implicated in tackling challenges associated with direct exposure of fetal trophoblasts to maternal immune cells in hemochorial placentation, the role of PSG1 in trophoblasts *per se* remains elusive.

Methods: In this study, we investigated this issue by using the human placental villi of the first and third trimester.

Results: We demonstrated that PSG1 expressed primarily in syncytiotrophoblasts rather than in cytotrophoblasts of the placental villi in both the first and third trimesters of human pregnancy. By using cultured primary human placental trophoblasts, we demonstrated that expression of PSG1 was significantly increased during syncytialization of cytotrophoblasts. Functional study showed that knock-down of PSG1 expression was accompanied by down-regulation of the protein disulfide isomerase (PDI) as well as the total disulfide bonds in syncytiotrophoblasts. Since the secretion of human chorionic gonadotropin (hCG) relies on the formation of disulfide bond formation, we investigated whether PSG1 affected hCG secretion in syncytiotrophoblasts. Knockdown of PSG1 had no effect on the mRNA level of the b subunit of hCG (b-hCG), but increased intracellular b-hCG protein abundance along with decreased extracellular b-hCG protein abundance in syntiotrophoblasts, suggesting that PSG1 may maintain hCG secretion in human placental trophoblasts by inducing disulfide bond formation in the subunit of hCG. Consistently, treatment of syncytiotrophoblasts with dithiothreitol, a reducing reagent breaking disulfide bonds, also increased intracellular b-hCG protein abundance while decreased extracellular b-hCG protein abundance in syncytiotrophoblasts.

Conclusion: We have demonstrated a novel role of PSG1 to maintain disulfide bond formation in secretory proteins such as hCG, in human placental trophoblasts.

P1.78.
THE PRO-INFLAMMATORY CYTOKINES IFN-α AND TNF-α INHIBIT EXTRAVILLOUS TROPHOBLAST INVASION

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Objectives: The maternal immune system plays an important role throughout pregnancy, and needs to adapt to the developmental status of the embryo and fetus. Consequently, dysregulation of the maternal immune system, as seen in immune-mediated inflammatory diseases (IMIDs), increases the risk for adverse pregnancy outcomes. Since these diseases particularly affect women of reproductive age, understanding their impact on pregnancy is of high clinical relevance. IMIDs are characterized by elevated activity of pro-inflammatory cytokines, such as interferon alpha (IFN-α) in systemic lupus erythematosus and tumor necrosis factor alpha (TNF-α) in various other IMIDs. Increased levels of these cytokines during pregnancy have been linked to preeclampsia and other placentation disorders, but their direct effect on early placenta development remains unclear. Here, we aim to investigate the effect of IFN-α and TNF-α on key placentation processes.
Methods: We use human trophoblast stem cells, trophoblast organoids and first-trimester placental and decidual tissue to study the effect of IFN-α and TNF-α on extravillous trophoblast (EVT) differentiation and invasion, which we assess by RT-qPCR, (3D) microscopy, and RNA sequencing.
Results: While IFN-α and TNF-α do not affect EVT differentiation, they significantly reduce EVT invasion into a 3D Matrigel matrix. RNA sequencing on EVTs derived from trophoblast organoids revealed several invasion-related genes modulated by these cytokines. Ongoing rescue experiments aim to validate their roles in mediating the observed reduction in EVT invasion. Additionally, we are investigating whether IFN-α/TNF-α treatment also disrupts trophoblast invasion into ex vivo decidual tissue.
Conclusion: Thus far, our findings indicate that the pro-inflammatory cytokines IFN-α and TNF-α impair trophoblast invasion, a process essential for spiral artery remodeling and a healthy pregnancy progression. This work improves our understanding of early placenta development, and uncovers mechanisms that may contribute to the increased risk for adverse pregnancy outcomes in patients with elevated IFN-α/TNF-α activity.

P1.79.
MOLECULAR MECHANISM OF TROPHOBLASTIC EPITHELIAL-MESENCHYMAL TRANSITION: A PCR-GUIDED INVESTIGATION OF GENE EXPRESSION IN HYPERGLYCEMIC PREGNANCIES

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Objectives: In pregnant women with type 1 diabetes mellitus (T1DM), perinatal mortality remains high, despite intensified care. The molecular mechanisms underlying placental insufficiency in T1DM remain largely unclear but manifest in impaired placental function, as measured by reduced umbilical cord blood oxygenation and increased perinatal mortality in these pregnancies. In other organ systems, such as the liver and kidney, diabetes has been associated with fibrosis and subsequent functional decline, triggered by glucose-induced epithelial-mesenchymal transition (EMT). While our preliminary studies demonstrated a significant increase in mesenchymal tissue in diabetic placentas, this study further investigates the transcriptional expression of mesenchymal and epithelial markers.
Methods: To examine the presence of EMT markers in T1DM placentas, their expression was analyzed via qRT-PCR in chorionic villi samples from 13 placentas of pregnancies with T1DM and corresponding healthy controls. Additionally, the transcription of EMT markers was studied in the trophoblastic cell line AC-1M32 after 72-hour and 7-day glucose treatment. Markers included TGFβ and Snail (profibrotic signaling initiators), collagen type 1A1 and vimentin (mesenchymal cells), N-cadherin and E-cadherin (epithelial cells) and β-catenin (Wnt signaling pathway).
Results: First results suggest that the findings from our preliminary study will be confirmed at the gene expression level, with increased expression of mesenchymal markers detectable in placentas from T1D pregnancies.
Conclusion: Our results will further validate the potential initiation of EMT as a cause of placental dysfunction in T1DM pregnancies. These insights enhance the understanding of placental failure mechanisms and may lead to improved treatment strategies for pregnant women with T1DM, potentially enabling better prediction of perinatal mortality risks.

P1.80.
HOW DOES TUBERCULOSIS INFECTION INFLUENCE PLACENTAL MORPHOLOGY AND FUNCTION?

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Objectives: Physiological immune modifications in pregnancy impact control of tuberculosis infection(TBI), which in turn triggers low-grade systemic inflammation. In a Swedish register study, based on women tested for TBI in antenatal care, TBI was found to be associated with severe pregnancy complications, such as preeclampsia and stillbirth. In this ongoing cohort study, we aim to investigate how placental morphology and function is affected by TB infection, deciphering underlying molecular mechanisms. We aim to compare placental histopathology and biomarkers of placental dysfunction in women with and without TBI to correlate these findings with the immune profiles in peripheral blood from women with TBI.
Methods: Pregnant women (n=700) are enrolled at Gandhi memorial hospital, Addis Ababa, Ethiopia, with follow-up of mothers and infants at 6 weeks postpartum. Maternal TBI status is determined by QuantiFERON blood test (obtained at inclusion during delivery). Detailed demographic and clinical obstetric data is registered. Placenta tissue is sampled for histopathological studies (including immune cell phenotyping and detection of Mtb by *in-situ* hybridization and immunohistochemistry), and for molecular studies, RNA and protein expression profiling (using RNA sequencing and mass spectrometry). The Mtb-specific and non-specific immune responses are characterized in peripheral blood, to be correlated with morphological placental findings. The project is based on a research constellation including experts in infection medicine, obstetrics, pathology, and immunology in Sweden and Ethiopia.
Results: Enrolment of participants is ongoing since October 2024, with anticipated completion of collection of data and samples in spring 2026. Among 120 women hitherto included, approximately 25% have TB infection.
Conclusion: The association between TBI and placental dysfunction has hitherto not been explored. Knowledge on this interaction may yield new strategies for management of TB in women of reproductive age, which could contribute to reduction of pregnancy disorders, especially in resource-limited settings in which both TBI and pregnancy complications are common.

P1.81.
A DATA-DRIVEN INVESTIGATION OF MATERNAL DIABETES HIGHLIGHTS CONSISTENT CHANGES IN IMMUNE AND VASCULAR PATHWAYS IN GDM PLACENTA

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Objectives: Diabetes in pregnancy is associated with short- and long-term health complications for both mother and offspring. These adverse

outcomes are likely linked to alterations in placental development and function; however, the underlying molecular mechanisms remain unclear. Exploiting advances in high-throughput technologies, we employed a data-driven approach to gain insights into disease pathways, which we subsequently validated through wet-lab analysis.

Methods: A systematic review was conducted to identify studies analysing placental molecular changes in pregnancies complicated by maternal diabetes using high-throughput experiments. Gene ontology and functional enrichment analysis (GO&FEA) was performed in g:Profiler. Experimental validation of predicted functions of differentially abundant proteins (DAP) or genes (DEG) was performed using RT-PCR and immunohistochemistry (IHC) in human placental tissue obtained from uncomplicated and gestational diabetes mellitus (GDM) pregnancies (n=12-19).

Results: 56 studies were identified, with the majority focusing on GDM (n=52). Of these, 42 reported differences in protein (n=12) and RNA (n=30) profiles. Despite variability across studies, eight proteins and 189 RNA species exhibited consistent expression and directional changes in GDM in at least two studies. GO&FEA revealed that DAPs and DEGs were enriched in immune and vascular pathways. Hofbauer cells (HBCs) are the only immune cell in placental villous tissue and have postulated roles in vascularisation. IHC demonstrated that HBCs were located in close proximity to endothelial cells in both non-GDM and GDM placenta. Consistent with the *in-silico* data, there was a reduction in both endothelial (*PECAM1*) and HBC-enriched markers (*FOLR2 and VSIG4*).

Conclusion: By using an integrated approach, we established that key molecular signatures associated with immune and vascular regulatory pathways are consistently altered in studies examining GDM placentae. Whilst further work is required, our data suggest that targeting molecular pathways linked to HBCs and vascularisation may serve as potential targets for therapeutic intervention to reduce complications associated with GDM.

P1.82.
PLACENTAS AFFECTED BY PREECLAMPSIA SHOW REDUCED CD4+ T-CELL NUMBERS IN DECIDUA BASALIS AND INCREASED EVTS IN THE CHORIONIC MEMBRANE

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Objectives: Interactions between immune cells and trophoblasts in the human placenta are crucial for maternal-fetal immune responses, contributing to development of preeclampsia. The aim of this study is to explore differences between these cell types at various placental sites between healthy controls and women with preeclampsia. We also investigate clinical features including maternal age, BMI, gestational age, fetal sex and differential blood count values.

Methods: We collected blood and placental tissue from women with preeclampsia (N = 20) and healthy controls (N = 20). Peripheral blood mononuclear cells (PBMCs) were collected from peripheral venous blood prior to delivery and differential blood count was performed on whole blood. We isolated immune cells from PBMCs, decidua parietalis and decidua basalis. Extravillous trophoblasts (EVTs) were isolated from decidua basalis and chorionic membrane. Isolated cells were sorted by fluorescence-activated cell sorting into populations of EVTs, monocytes/macrophages, natural killer (NK) cells, and $CD4^+$ T- and $CD8^+$ T-cells.

Results: Women with preeclampsia exhibited lower median gestational age compared to controls ($p = 0.004$). No differences in maternal age, BMI, fetal sex or differential blood count parameters were found between groups. The relative proportion of $CD4^+$ T-cells within the total $CD45^+CD14^-$ cell population was reduced in decidua basalis of placentas affected by preeclampsia compared to controls ($p = 0.046$), but not in decidua parietalis or PBMCs. EVTs were increased within the $CD45^-$ population in the chorionic membrane of placentas affected by preeclampsia ($p = 0.008$), but not in decidua basalis. No differences were found in frequencies of NK cells, $CD8^+$ T-cells or macrophages across placental sites or in PBMCs.

Conclusion: Our findings suggest a reduction in $CD4^+$ T-cells in decidua basalis and increased EVT numbers in chorionic membrane of placentas affected by preeclampsia, indicating altered immune cell and EVT recruitment and function at various placental sites in women with preeclampsia.

P1.83.
PLACENTA-RELATED PREGNANCY COMPLICATIONS AS ADVERSE EVENTS REPORTED FOR PSYCHIATRIC MEDICATIONS: AN ANALYSIS OF FDA ADVERSE EVENT REPORTING SYSTEM

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Objectives: Treated and untreated mental illness in pregnancy have both been linked with adverse pregnancy outcomes in epidemiological studies. This study investigates potential links by analysing data from the FDA Adverse Events Reporting System (FAERS).

Methods: FAERS data (2004-2024) was extracted using the DiAna R package for reports of pregnancy complications, small-for-dates-baby, foetal growth restriction, pre-eclampsia, stillbirth, gestational hypertension and haemorrhage in pregnancy, linked with antidepressant or antipsychotic medications identified as primary suspects. AE frequencies and associated outcomes were examined.

Results: A total of 2,974 cases, with 3,105 associated reports of AEs of interest and 4,480 outcomes, were included in the analysis. Figure 1 shows the frequency of AEs for selected drugs.

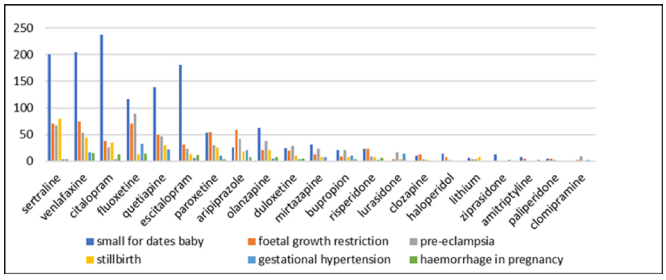

Figure 1. Frequency of AEs by selected medications. Included medications: ≥ 10 reported AEs of interest; n= 3,058. Total AE reports: small-for-dates-baby, 1,385 (44.6%); foetal growth restriction, 581 (18.7%); pre-eclampsia, 541 (17.4%); stillbirth, 331 (10.7%); gestational hypertension, 170 (5.5%); and haemorrhage in pregnancy, 97 (3.1%).

The use of venlafaxine was most frequently documented in reports of foetal growth restriction (12.9%) and haemorrhage (15.5%), fluoxetine in cases of gestational hypertension (18.8%) and pre-eclampsia (16.5%), and sertraline in stillbirth cases (24.2%). Of the 4,480 reported outcomes, fluoxetine was linked with the highest number of congenital anomaly reports (14.9%; 110/736) and hospitalisation reports (13.3%; 93/699). Disproportionality analyses for quantifying signal detection will be conducted to generate association hypotheses.

Conclusion: Despite some limitations, FAERS data provides access to reported outcomes in cases of placental disorders in the context of antidepressants or antipsychotics use during pregnancy. While not establishing causality, disproportionality analyses may highlight unexpected or rare AEs not evident in clinical trials, potentially aiding post-marketing surveillance and regulatory oversight. They can also inform aspects of future analysis of other observational data sources such as prospective cohort studies.

P1.84.
HISTOPATHOLOGICAL DIFFERENCES IN PLACENTAS EXPOSED TO DOLUTEGRAVIR- AND EFAVIRENZ-BASED ANTIRETROVIRAL TREATMENT REGIMENS AND HIV PROPHYLAXIS

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Objectives: Dolutegravir (DTG)-containing antiretroviral therapy (ART) is first-line treatment for HIV in pregnancy. However, there is mixed evidence of potential adverse effects of ART on the placenta. To investigate antiretroviral effects, we compared gross and histological placental findings by exposure to DTG-based ART, efavirenz (EFV)-based ART, and tenofovir-based pre-exposure prophylaxis against HIV infection (PrEP).
Methods: We prospectively enrolled 506 people with singleton pregnancies in Mbarara, Uganda and collected placentas at delivery (28-46 weeks' gestation). Of 414 (82%) placentas examined to date, 168 (41%) were EFV-ART-exposed, 146 (35%) DTG-ART-exposed, and 100 (24%) PrEP-exposed. We characterized placental findings using Amsterdam criteria and compared groups using Kruskal-Wallis, ANOVA, and t-tests.
Results: Mean maternal age was lower for PrEP than ART groups (26 vs 29 years, $P<0.001$). EFV-ART-exposed placentas were thinner (1.6cm) than DTG-ART- and PrEP-exposed placentas (both 1.8cm, $P<0.01$), though trimmed weight (mean 438-452g) was similar. DTG-ART-exposed placentas had a lower cord coiling index (0.10) than EFV-ART- and PrEP-exposed placentas (0.12 for both, $P<0.01$). Laminar necrosis was more common in EFV-ART-exposed placentas (11%) than DTG-ART- (1%) and PrEP-exposed (4%, $P<0.001$) placentas. Placental infarcts were more common in EFV-ART- (5%) and DTG-ART- (6%) than PrEP-exposed (1%, $P=0.06$) placentas. Maternal vascular malperfusion prevalence was similar between groups (29-38%), but fetal vascular malperfusion (FVM) was higher in PrEP- (19%) than EFV-ART- (5%) and DTG-ART-exposed (9%, $P=0.002$) placentas, including avascular villi (48% for PrEP- vs 11% for EFV-ART- and DTG-ART-exposed). Chronic villitis (of unknown etiology, 17%) was more prevalent in PrEP- than EFV-ART- and DTG-ART-exposed placentas (3-6%, $P<0.001$). Plasma cell deciduitis was less frequent in DTG-ART- (9%) than PrEP- (13%) and EFV-ART-exposed (22%) placentas.
Conclusion: Placentas exposed to PrEP and EFV-containing ART had more FVM and deciduitis than DTG-ART-exposed placentas. Future studies should explore links between antiretrovirals and vascular placental pathologies, underlying mechanisms, and associated pregnancy and child outcomes.

P1.85.
HISTOLOGIC PLACENTAL MICROVASCULAR SIGNATURE OF PLACENTAL DYSFUNCTION: EARLY-ONSET PREECLAMPSIA, EARLY-ONSET FGR, AND THEIR OVERLAP

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Objectives: Early-onset fetal growth restriction (EOFGR) and pre-eclampsia (EOPE) are pathologic associations of clinical early uteroplacental insufficiency (E-UPI). Studies within our lab suggest chorionic villous microvascular endothelial dysfunction plays a central role in EO-FGR and EO-PE. We used morphometric analysis to quantify whether total chorionic villus count (TCVC), chorionic villous diameter (CVD), total syncytial knot count (TSKC), total microvascular density (TMVD), and luminal count (LC) per villus differ among placentas from EOFGR, EOPE, EOFGR/EOPE, and healthy controls (H).
Methods: We investigated placental microvascular density in EOPE, EOFGR, EOPE/EOFGR, and H cohorts utilizing immunofluorescence-labeled CD31 and nuclear DNA markers to enable quantitative morphometric analysis by a blinded reviewer who determined: TCVC, maximal and minimal CVD, LC per villus, TMVD, and TSKC. Statistical analysis was performed with ANOVA to test for overall group differences with post hoc pairwise comparisons via Tukey's HSD.

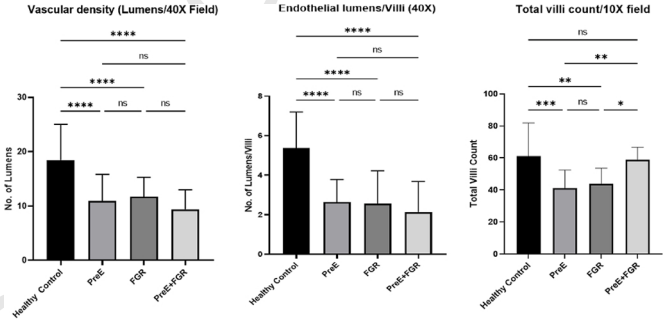

Figure 1. Quantification of morphometric and immunofluorescent parameters in placentas from gestations with EOFGR (early onset fetal growth restriction), EOPE (early onset preeclampsia), EOPE/EOFGR (early onset preeclampsia with concurrent early onset fetal growth restriction) vs. healthy controls

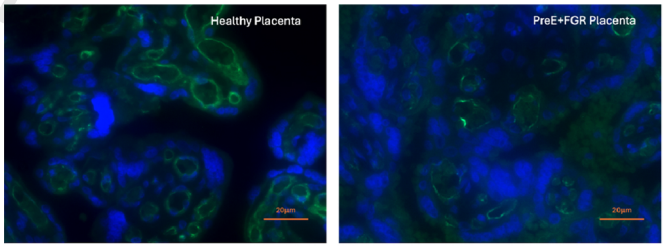

Figure 2. Immunofluorescence for CD31 at 40x in healthy (left) and combined EOPE/EOFGR (early onset preeclampsia with concurrent early onset fetal growth restriction)

Results: EOPE (N=4), EOFGR (N=3), EOPE/EOFGR (N=2) vs. H (N=4). Mean (M) delivery gestational age (GA): 32.2 ± 2.45 (EOPE); 37.6 ± 0.4 (EOFGR); 31.1 ± 5.6 (EOPE/EOFGR); 39.5 ± 0.3 (H). GA at early-onset diagnosis: 31.6 ± 2.2 (EOPE); 30.65 ± 3.3 (EOFGR). TMVD differed significantly from H (M=16.05) vs. diseased groups: M=11.73 (EOFGR); M=10.92 (EOPE); EOPE/EOFGR (M=9.38), (p=<0.001). TMVD inter-group differences were insignificant. H vs. diseased group LC differed significantly: M=4.90 (H); M=2.57(EOFGR); M=2.63 (EOPE); M=2.14 (EOPE/EOFGR); p=< 0.0001.) H (M=59.53) and EOPE/FGR (M=58.91) differed from EOFGR (M=43.73) and EOPE (M=41.29) CVCs, p=<0.0001. Maximal CVD was smaller in diseased groups vs. H ($p < 0.0001$); minimal CVD showed no differences. Pathologic group TSKCs were nearly twice those of H, (p=<0.0001).
Conclusion: Our preliminary findings reveal that EOFGR, EOPE, and EOPE/EOFGR are associated with significant chorionic villous and microvascular maldevelopment. Further studies are needed to understand whether EOPE, EOFGR, and EOPE/EOFGR represent an overlapping spectrum of etiopathology.

P1.86.
ANALYSIS OF THE POLARIZATION PROFILE OF DECIDUAL MACROPHAGES IN PLACENTA OF APS PATIENTS

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Objectives: Obstetric antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by pregnancy morbidity (PM) and the persistent presence of antiphospholipid antibodies (aPL). Recently, we demonstrated that extracellular vesicles released from aPL-treated endothelial cells influence maternal myeloid cells by altering monocyte differentiation. The aim of this follow-up study is to investigate the consequences on the polarization profile and percentage of tissue-resident macrophages.
Methods: Sections of formalin-fixed and paraffin-embedded placenta tissues from healthy volunteers (HP), patients complicated with PM (eclampsia, preeclampsia and/or fetal growth restriction) or APS were stained with hematoxylin and eosin to determine morphological abnormalities of the decidua. The presence of total macrophages (CD68), the M1 (CD86) and M2 (CD206) subpopulation as well as their activation status (vimentin) was determined by immunofluorescence staining. Marker-positive cells were detected by confocal microscopy and evaluated using QuPath and Excel.
Results: Our preliminary results show an increased amount of $CD68^+$ and $CD206^+$ cells in both PM- and APS-derived decidual sections compared to HP. Furthermore, $CD68^+vim^+$ and $CD206^+vim^+$ as well as $CD206^+CD68^+vim^+$ cells were increased in PM and APS compared to HP. $CD86^+$ cells were slightly increased in APS but reduced in PM compared HP. $CD86^+CD68^+$, $CD86^+vim^+$ and $CD86^+CD68^+vim^+$ were reduced in PM and APS compared to HP. $CD206^+$ cells were detected more frequently than $CD86^+$ cells in all groups.
Conclusion: Compared to HP, an increased amount of activated macrophages was detected in PM and APS decidua. The higher percentage of activated M2 compared to M1 macrophages in PM and APS, however, indicates a mainly immunosuppressive polarization state of these decidual macrophages. Unique $CD68^+CD206^+vim^+$ macrophages may be differentiated as a consequence of a previous inflammatory state in these regions to secrete immunomodulatory chemo- and cytokines. The sample size of this preliminary study will be increased to reinforce the obtained results.

P1.87.
TROPHOBLAST EXTRACELLULAR VESICLES ARE A MECHANISTIC LINK BETWEEN PREECLAMPSIA AND EARLY CARDIOVASCULAR MORTALITY IN WOMEN

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Objectives: Preeclampsia is a pregnancy-specific hypertensive disorder that doubles risk of premature cardiovascular disease and mortality. The precise mechanisms underlying this heightened cardiovascular susceptibility remain unclear. Trophoblast extracellular vesicles (TEVs), facilitate fetal and maternal communication and are released into maternal circulation throughout gestation. This study investigated whether pre-eclamptic TEVs contribute to sustained cardiovascular dysfunction postpartum.
Methods: Trophoblast EVs were isolated from women with early-onset preeclampsia (EOPE, n=8) and late-onset preeclampsia (LOPE, n=9), and FBS-derived vesicles were used as a vehicle control. These vesicles were administered to normotensive Wistar rats at five time points over ten days (DPC8.5 – DPC18.5). Systolic blood pressure was monitored non-invasively at baseline and monthly for 12 months postpartum. Echocardiographic assessments were performed quarterly. Vascular reactivity was evaluated by wire myography, measuring responses to phenylephrine, endothelin-1, U46619, acetylcholine, and sodium nitroprusside.
Results: Both EOPE and LOPE EV-treated rats exhibited a progressive, significant elevation in systolic blood pressure starting at three months postpartum, peaking at six months in the LOPE group (~30 mmHg increase, $p<0.01$) and at nine months in the EOPE group (~30 mmHg increase, $p<0.05$). Myograph analysis revealed distinct vascular responses in EOPE and LOPE groups. EOPE EV-treated rats demonstrated heightened vasoconstriction to phenylephrine ($p<0.0001$), endothelin-1($p=0.005$) and U46619 ($p<0.0001$), with no significant changes in relaxation responses. Notably, LOPE EV-treated rats exhibited significantly increased vasoconstriction in response to phenylephrine ($p=0.0079$) and impaired relaxation to acetylcholine ($p<0.0001$) and sodium nitroprusside ($p<0.0001$), indicating both endothelial and smooth muscle dysfunction.
Conclusion: Trophoblast EVs from preeclamptic pregnancies induce persistent hypertension and vascular dysfunction in postpartum Wistar rats, suggesting a causative role in the increased cardiovascular risk observed in women with a history of preeclampsia. These findings underscore the necessity for prolonged cardiovascular surveillance and targeted interventions in this high-risk population to mitigate long-term morbidity.

P1.88.
THE ROLE OF POLY (ADP-RIBOSYL) ATION IN THE ESTABLISHMENT OF PLACENTAL DYSFUNCTION OBSERVED IN INFLAMMATORY SUBCLASS OF PREECLAMPSIA

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Objectives: Preeclampsia (PE) is a leading cause of maternal and neonatal morbidity and mortality and is diagnosed after 20 weeks of gestation, when new-onset hypertension is accompanied by clinical evidence of maternal end-organ injury. Several subclasses of preeclampsia have been identified where solely the inflammation-mediated preeclampsia (I-PE) exhibits pronounced increase in PARylation together with a marked depletion of NAD^+. The present study investigates the mechanistic basis of this poorly studied phenotype by systematically profiling placental NAD^+-consuming enzymes and assessing whether selective inhibition of PARylating enzymes can restore and improve trophoblast function and overall placenta health.
Methods: HTR-8/SVneo trophoblasts cells were cultured and exposed with TNF-α, as an inflammatory insult that we have previously shown to mimic I-PE. HTR-8s were treated with two different co-treatments to inhibit PARylation activity from the PARP family: 1) PARP1/2 inhibitor Olaparib, 2) PARP5a/5b inhibitor XAV939. PARylation activity, migration function and viability of trophoblasts were analyzed in the different co-treatments of inhibitors in addition to mitochondrial health through seahorse assay.
Results: HTR-8/SVneo trophoblasts exposed to TNF-α induce-inflammation had increased PARylation levels, impaired mitchondrial health and showed reduction of migration function and cell viability. When treated with PARP1/2 inhibitor, PARylation levels were reduced just after 1h of TNF-α exposures and rescued the effects of TNF-α by increasing the migrating function and viability of trophoblasts. These changes were not observed with PARP5a/5b inhibitor.
Conclusion: In HTR-8/SVneo trophoblasts, inhibition of only PARP1/2 with Olaparib rescued PARylation levels and restored trophoblast function implicating that PARP1/2 is mainly responsible for the increase in PARylation rather than PARP5a/5b during this TNF-α insult. Targeting PARP1/2 is therefore a rational therapeutic strategy to re-establish NAD^+ levels and improve placental performance in inflammatory-mediated preeclampsia.

P1.89.
ALTERED PLACENTAL EXPRESSION OF ANNEXIN V AND COMPLEMENT PROTEINS IN PREGNANCY COMPLICATIONS OF PATIENTS WITH AUTOIMMUNE DISEASES

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Objectives: During pregnancy, antibody-mediated autoimmune diseases (AID), such as systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS), are associated with an increased risk of adverse pregnancy outcomes (APO), including fetal growth restriction, preeclampsia, preterm birth, and perinatal death. Placental development relies on tightly regulated processes, and the complement system plays a central role in maintaining immune balance at the maternal–fetal interface. Excessive complement activation has been implicated in the pathogenesis of APO in AID pregnancies. Similarly, annexin V, a key regulator of placental thrombogenicity, has been associated with pregnancy complications, although its role in autoimmunity remains unclear. In this study, we evaluated the expression of annexin V and complement proteins in placental tissue from patients with SLE and APS, with and without APO.
Methods: Placental samples from patients with SLE and APS were classified into two groups based on the presence (n = 13) or absence (n = 9) of APO. Additional control groups included cases of isolated FGR (n = 13) and healthy term pregnancies (n = 13). Tissue sections were stained with antibodies against human annexin V, C3b, C4d, and C5b-9, and expression was quantified as mean fluorescence intensity (MFI).
Results: Significantly lower expression of annexin V was observed in both AID groups (with and without APO) compared to healthy controls. Conversely, significantly higher levels of C3b, C4d, and C5b-9 were detected in all study groups compared to healthy controls. Complement depositions were localized in the villous stroma, cytotrophoblasts, syncytiotrophoblasts, and perivascular areas surrounding fetal vessels.
Conclusion: Placental tissue from patients with SLE and APS exhibited excessive complement activation and reduced annexin V expression, suggesting their involvement in the pathophysiology of APO in autoimmune pregnancies.

P1.90.
PLACENTAL NRP1 AND SERUM SNRP1 LEVELS CORRELATE WITH MATERNAL GLUCOSE CONTROL IN PREGESTATIONAL TYPE 1 AND TYPE 2 DIABETES

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Objectives: Neuropilin-1 (NRP1) is a Vascular Endothelial Growth Factor (VEGF) receptor that regulates placental microvascular and trophoblast development. Alternative transcript splicing and/or cleavage of NRP1 produces a soluble variant (sNRP1) that antagonizes VEGF signaling. Pregestational Type 1 (T1DM) and Type 2 (T2DM) diabetes impact both systemic sNRP1 and the development of the human placenta. We hypothesized that these changes in sNRP1 are related to villous NRP1 expression, and that both are regulated by maternal and/or neonatal glycemic control.
Methods: Maternal and umbilical cord blood (3 controls, 5 T1DM, 4 T2DM), and villous placental tissue (4 controls, 3 T1DM, 6 T2DM) were collected (approved by the University of Florida IRB-01). Plasma was analyzed for maternal Hemoglobin A1c (HbA1c) and neonatal insulin. Serum sNRP1 and villous NRP1 expression were analyzed by ELISA and Western blotting, respectively. Gestational age, fetal sex, and intrapartum maternal glucose levels were recorded. Groupwise comparisons were conducted using Mann-Whitney and Kruskall-Wallis tests. Mann-Whitney tests and linear regressions were performed to assess the effects of clinical and laboratory parameters on NRP1 and sNRP1 levels. Paired serum comparisons were performed using Wilcoxon Signed-Rank tests. Significance was set as $p<0.05$.
Results: sNRP1 was significantly higher in cord serum than maternal serum in pregestational diabetes ($p=0.0156$), but not in controls ($p=0.25$). Maternal, but not umbilical cord, serum sNRP1 was negatively correlated with average intrapartum maternal glucose in T1DM ($p<0.0478$), but not in T2DM ($p<0.26$). Villous NRP1 expression trended towards a negative association with maternal HbA1c in T2DM ($p<0.0672$), but not T1DM. There were no effects of fetal sex or gestational age.
Conclusion: In pregestational diabetes, sNRP1 is preferentially secreted into the fetoplacental circulation, potentially secondary to effects on the villous microvasculature. Maternal sNRP1 is regulated by short-term glucose control, while transmembrane NRP1 may be regulated by longer-term control - potentially a function of maternal insulinemia.

P1.91.
PREECLAMPTIC PLACENTAL EXTRACELLULAR VESICLES PERMANENTLY ALTER CARDIOVASCULAR FUNCTION IN SPONTANEOUSLY HYPERTENSIVE RATS.

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Objectives: Risk of premature cardiovascular disease and death is doubled after a preeclamptic pregnancy. Why cardiovascular damage persists after delivery is presently unknown. Placental extracellular vesicles (EVs) from preeclamptic pregnancies may provide the mechanistic link between the placenta and adverse long-term maternal cardiovascular changes. This study aimed to identify the effects of placental EVs during pregnancy and long-term postpartum on the maternal cardiovascular system.
Methods: EVs isolated from human placental explants were injected into pregnant spontaneously hypertensive rats (SHRs) via a tail vein. SHRs received placental EVs from either normotensive (n=10), late-onset preeclamptic (n=10) or early-onset preeclamptic (n=10) placentae. Non-invasive blood pressure and echocardiography were performed pre-pregnancy, day 20.5 of pregnancy, and monthly until twelve months postpartum. Vasoactivity of third-order mesenteric vessels was assessed using wire myography twelve months postpartum.
Results: In comparison to pre-pregnancy, systolic blood pressure (SBP) reduced significantly during pregnancy in the normotensive (-22±21 mmHg, $p=0.0091$, as expected) and late-onset groups (-23±15 mmHg, $p=0.0005$), but not in the early onset group (-11±15 mmHg, $p=0.0519$). At one week postpartum, SBP had returned to pre-pregnancy baseline in all groups ($p>0.05$). Across the following twelve months postpartum, the early-onset and late-onset groups demonstrated a greater ΔSBP than the normotensive group ($p<0.0001$ and $p<0.0001$, respectively). Echocardiography showed no difference in any functional cardiac parameters between groups. Twelve months postpartum, mesenteric resistance arteries from early-onset and late-onset groups were more responsive to vasoconstrictors phenylephrine ($p=0.0066$ and $p<0.0001$ respectively) and endothelin-1 ($p<0.0001$ and $p<0.0001$ respectively) compared to the normotensive group.
Conclusion: Early onset preeclamptic EVs alone were capable of preventing the expected decrease in SBP during pregnancy. Long-term postpartum, both early-onset and late-onset preeclamptic EVs facilitated negative impacts on the cardiovascular system, suggesting these EVs may be the mechanistic link between preeclampsia and premature cardiovascular disease and mortality.

P1.92.
PLACENTAL LIPID MEDIATORS OF INFLAMMATION AND RESOLUTION DIFFER BY FETAL SEX IN GESTATIONAL DIABETES

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Objectives: Gestational diabetes mellitus (GDM) is a common pregnancy complication affecting about 1 in 5 pregnant women in Singapore. Lipid dysregulation is a key feature of placentas from GDM-affected women. Nevertheless, little is known about the placental expression of pro-inflammatory and pro-resolving lipid mediators, and how they are affected by GDM. This study aimed to characterise these bioactive lipid mediators in the placentas from women with and without GDM and to determine any fetal sex-specific effects.
Methods: Placental villous biopsies were collected from GDM pregnancies (n=28) and normoglycaemic controls (n=26) at term elective caesarean section. Placental samples were freeze-dried and homogenised in methanol. Pro-inflammatory (prostaglandin, thromboxane; PG,Tx) and pro-resolving (resolvin, protectin, maresin; Rv, PD, MaR) lipid mediators were determined by rigorous liquid-chromatography tandem-mass spectrometry (LC-MS/MS) metabololipidomics.
Results: Overall, GDM placentas had higher levels of pro-inflammatory lipid mediators (PGD_2, PGE_2, TxB_2) and lower levels of pro-resolving lipid mediators (PDX, MaR2) than controls. Following sex-stratification, among normoglycaemic pregnancies, female placentas had higher inflammatory lipid mediators and precursors (PGD_2, 5-HETE) and higher pro-resolving lipid mediators and intermediates (RvE2, RvE4, 15-HETE, 17-HDHA, 15-HEPE, 18-HEPE) than male placentas. With GDM, male placentas had a higher inflammatory lipid mediator (TxB_2) than normoglycaemic male placentas, while GDM female placentas had lower pro-resolving lipid mediators and intermediates (RvE4, PDX, MaR1, MaR2, 15-HEPE, 18-HEPE) than normoglycaemic female placentas.
Conclusion: GDM placentas had elevated inflammation and reduced pro-resolving capacities possibly contributing to increased maternity risk (e.g. pre-eclampsia, stillbirth). While female placentas had higher levels of pro-inflammatory lipid mediators than males among normoglycaemic controls, they also had higher pro-resolving capacity which may partly explain generally better perinatal outcomes among females. Nevertheless, such enhanced resolution capacity in female placentas is lost with GDM. This study highlights a novel mechanism of physiological inflammation-resolution axis in the placenta that is dysregulated in GDM.

P1.93.
DYSREGULATED TRYPTOPHAN METABOLISM IS ASSOCIATED WITH HIGHER RISK OF OFFSPRING ATOPIC DERMATITIS DEVELOPMENT

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Objectives: Atopic dermatitis (AD) is a chronic inflammatory skin disorder that particularly affects early childhood. Emerging evidence suggests that intrauterine immune dysregulation plays a pivotal role in AD pathogenesis, yet the precise mechanisms are poorly understood. The tryptophan metabolic pathway is increasingly recognised for its immunomodulatory role. However, the relationship between tryptophan metabolism and offspring AD risk remains underexplored. This study aimed to investigate the associations between cord blood tryptophan metabolite concentrations, as well as placental expression of tryptophan processing genes, and AD risk in offspring within the Growing Up in Singapore Towards healthy Outcomes (GUSTO) mother-offspring cohort.
Methods: Cord blood from 718 GUSTO participants was analysed by LCMS to quantify tryptophan metabolites and assess their relationship with AD development in the first year (at 3,6,9,12 months) by logistic regression with adjustment for key covariates. In a subset of 8 cases with persistent AD across the first year of life and 8 controls matched for gestational age, birthweight centiles, sex and maternal characteristics, placental protein expression of key tryptophan processing genes were analysed by immunoblotting.
Results: Higher cord blood kynurenine levels associated with greater AD risk [6-month-adjusted-odds-ratio (aOR):1.55(1.06-2.27), p=0.023; cumulative-12-month-aOR:1.42(1.08-1.89), p=0.013], while a reduced risk of infantile AD was seen with higher cord blood levels of 3-hydroxyanthranilic acid [3-month aOR:0.62(0.41-0.92), p=0.018], anthranilic acid [3-month-aOR:0.48(0.25-0.92), p=0.026], and picolinic acid [3-month-aOR:0.53(0.88-1.12), p=0.036; 6-month-aOR:0.66(0.44-0.99), p=0.046; 12-month-aOR:0.70(0.51-0.96), p=0.027; cumulative-12-month-aOR:0.63(0.46-0.86), p=0.003]. Expression of IDO1, which is involved in catabolising tryptophan to kynurenine, was ~39% lower (p=0.001) in placental tissue of AD-affected infants as compared with matched controls.
Conclusion: Dysregulated tryptophan metabolism is associated with offspring first-year AD trajectories, indicating a possible role of tryptophan metabolism in shaping fetal immune programming and AD susceptibility. Further studies are required to investigate the potential mechanisms that can be targeted for early-life AD prevention.

P1.94.
IMPACT OF MATERNAL ADIPOSITY AND WEIGHT GAIN ON MATERNAL AND FETAL GLUCOSE HOMEOSTASIS AND PLACENTAL GLUCOSE TRANSPORTER EXPRESSION

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Objectives: Maternal obesity and excess gestational weight gain (GWG) are associated with glucose dysregulation in the mother and offspring. Disturbances in maternal glucose homeostasis might alter placental nutrient transfer through glucose transporter expression with potential sex-specific neonatal effects. We investigated the impact of pre-pregnancy BMI, GWG, and maternal glucose regulation on placental GLUT-1/GLUT-3 expression and DNA methylation and neonatal outcomes, emphasizing sex-specific differences.
Methods: Prospective longitudinal study with 176 healthy singleton pregnancies were categorized according to pre-pregnancy body mass index (BMI) as adequate (control; 18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese ($\geq$30 kg/m^2). Maternal anthropometrics, weight trajectories and diet quality were assessed throughout gestation. Metabolic parameters —including insulin, glucose, HOMA-IR, and leptin—were measured throughout each trimester and at birth. Placental GLUT-1 and GLUT-3 mRNA levels and CpG methylation was assessed. Data were analyzed using multivariable regression (adjusted for age, gestational age, parity, smoking, ethnicity, infant sex, and macrosomia) and repeated measures ANOVA stratified by neonatal sex.
Results: Higher maternal BMI and excess GWG were associated with elevated serum insulin levels and HOMA-IR indices. Maternal serum leptin and insulin concentrations varied across BMI and GWG categories.

Placental GLUT-3 mRNA levels were increased in excessive GWG women. Altered CpG methylation was found in male placentas from obese BMI women. Diet quality in obese women with excess GWG in their first trimester was significantly healthier than pregravid but decreased towards the end of pregnancy.
Conclusion: Maternal obesity and excess GWG impair maternal glucose homeostasis and are linked to an upregulation and epigenetic modification of placental GLUT-3 expression. Sex-specific alterations may predispose to adverse metabolic programming. Targeted prenatal interventions that consider both maternal metabolic status and fetal sex may improve placental function and optimize long-term metabolic outcomes.

P1.95.
PLASMA PROTEOMICS CAN IDENTIFY ASSOCIATIONS BETWEEN PREGNANCY COMPLICATIONS AND FUTURE HEALTH RISKS.

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Objectives: Pregnancy is a physiological stress test requiring major adaptations of maternal organs. Epidemiological studies have identified associations between pregnancy complications, such as fetal growth restriction (FGR) and preeclampsia, and increased risk of long-term chronic disease. However, no blood tests can identify women at risk. SomaLogic uses aptamer-based proteomics to measure over 7,000 proteins. Using this platform, machine learning algorithms (SomaSignal tests) identified protein signatures associated with chronic disease risk.
Methods: We measured over 7,000 plasma proteins using the SomaLogic platform in samples collected at 36 weeks' gestation, before diagnosis of fetal growth restriction or term preeclampsia. SomaSignal tests were applied to two independent, prospective cohorts: An Australia cohort (n=115 <3rd birthweight centile/FGR n=92 preeclampsia, and n=177 cohort), and a United Kingdom cohort (UK, n=80 <3rd birthweight centile/FGR, and n=172 cohort). Protein signatures associated with chronic disease risk across 18 health indicators were assessed.
Results: In the Australian cohort, women who later delivered a fetal growth restricted infant showed protein signatures associated with reduced heart function (within 6 and 12 months, p=0.001), and increased risk of cardiovascular events (p=7.6x10^{-6}). These protein signatures were also associated with increased risk of mid-life dementia (p=1.5x10^{-9}), greater visceral fat (p=4.06 x10^{-6}) and body fat percentage (p=0.003). These findings were validated in the United Kingdom cohort.
Women who later developed term preeclampsia showed more pronounced disease-associated protein signatures than those with fetal growth restriction, supporting existing epidemiological data. Protein signatures associated with reduced heart function (within 6 and 12 months, p=0.001), and increased cardiovascular (p=5.54x10^{-6}), hepatic (p=0.001) and renal (p=0.0007) disease risk were observed. These patterns were also associated with increased mid-life dementia risk (p=1.61x10^{-8}), glucose intolerance (p=0.001), and visceral fat (p=5.35x10^{-10}).
Conclusion: This platform can detect protein signatures in maternal plasma associated with pregnancy complications and chronic disease risk, offering a window for early intervention strategies.

P1.96.
LONG-TERM CARDIOVASCULAR CONSEQUENCES OF PREGNANCY IN A RAT MODEL: A ROLE FOR PLACENTAL EXTRACELLULAR VESICLES

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Objectives: Women who experience preeclampsia, a hypertensive disorder of pregnancy, are at an elevated risk of experiencing premature cardiovascular mortality. Throughout pregnancy placental extracellular vesicles (EVs) are released from the placenta into the maternal circulation and are key in mediating cardiovascular adaptation during pregnancy. We hypothesised that placental EVs can induce long-term changes in the maternal cardiovascular system.
Methods: Placental EVs were isolated from human placenta, obtained from mothers who had experienced either normotensive, early-onset or late-onset preeclamptic pregnancies. The placental EVs were then injected into normotensive Wistar or Spontaneously Hypertensive rats (SHR) over 10 days. Systolic pressure was monitored using tail-cuff measurements before and until 12 months post-injection. Post-mortem vascular function of mesenteric arteries was assessed via wire myography.
Results: Pregnancy was associated with a decrease in systolic pressure in both Wistar rats and SHR that received control or late-onset placental EVs, however no decrease in pressure was observed in the rats that received the EVs from early-onset placenta. In the 12 months following administration of the placental EVs, the rats that received EVs from normotensive pregnancies showed signs of cardiovascular protection with lower systolic pressures and vascular responses favouring vasodilation. In contrast rats that received EVs from either late-onset or early-onset preeclamptic pregnancies had higher systolic pressures and vascular responses that favoured vasoconstriction. The effects of the vesicles were similar regardless of pre-existing hypertension.
Conclusion: Together our results suggest that placental EVs are key in mediating vascular responses during pregnancy and the maternal cardiovascular risk later in life.

P1.97.
PAVATENSION - INFLUENCE OF BLOOD PRESSURE CONTROL DURING PREGNANCY ON THE LONG-TERM COURSE OF HYPERTENSIVE DISEASE - ANALYSIS OF A FOLLOW-UP STUDY 15 YEARS AFTER PRE-ECLAMPSIA

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Objectives: Good blood pressure control before, during and after pregnancy is important for the pregnancy outcome of mother and child and is likely to have important consequences for the long-term cardiovascular health of women after hypertensive pregnancy disorders. Tight blood pressure control during pregnancy can prevent severe hypertension and have a positive effect on pregnancy outcome. However, it is still unclear how specific blood pressure control during pregnancy affects the long-term cardiovascular outcome of women after hypertensive pregnancy. In the following study, the influence of blood pressure control during pregnancy on the cardiovascular risk profile (diagnosis of hypertension) 15 years after pregnancy with pre-eclampsia and/or fetal growth restriction was investigated.
Methods: From 2019-2022, 53 women 15 years after a complicated pregnancy with pre-eclampsia and/or fetal growth restriction were examined. Women with blood pressure values of ≥ 140/90mmHg and/or antihypertensive medication were compared with normotensive women (RR <140/90mmHg and no RR medication) at the time of the study. Data on blood pressure control for each trimester in pregnancy were retrospectively extracted from hospital records.
Results: At the time of the study, 35 women were hypertensive and 18 women were normotensive. The groups did not differ regarding BMI and age at the study visit, frequency of chronic diseases, time since pregnancy and antihypertensive medication during pregnancy. In group comparison, blood pressure values were on average lower during pregnancy in later normotensive women; the difference was significant for systolic blood pressure in second trimester (155 mmHg vs. 140 mmHg, p = 0.027) and diastolic blood pressure in third trimester (96 mmHg vs. 87 mmHg, p = 0.026).
Conclusion: This retrospective study provides initial indications of the value of blood pressure control during pregnancy for the long-term

cardiovascular outcome of women after hypertensive pregnancy disorders. However, prospective studies are necessary.

P1.98.

ACUTE GESTATIONAL HYPOXIA ALTERS FOETAL KIDNEY GENE EXPRESSION IN A CIRCADIAN PHASE-DEPENDENT MANNER

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Objectives: Gestational hypoxia, a common complication during pregnancy, increases risk of hypertension and cardiorenal diseases. Kidneys are key in blood pressure control; impaired development may contribute to long-term dysfunction. Adaptive responses to hypoxia are regulated by circadian clock genes, thus, we hypothesise that exposure to hypoxia at different times of the day results in distinct effects. We analysed the impact of acute gestational hypoxia, induced during either the light or dark phase of the day, on the kidneys of male foetuses.
Methods: Pregnant Wistar rats were exposed to 10.5% O_2 hypoxia on gestational day 20, either during the 12-hour light or dark phase (n=5/group). Normoxic controls were included for both time phases (n=5/group). Male foetuses were collected 18 hours post-exposure; kidneys were isolated and processed for RNA sequencing (Novogene, UK). Genes with adjusted $p < 0.05$ and log2 fold change ≥ 1 were considered differentially expressed (DESeq2).
Results: In normoxic controls, we found circadian phase-dependent gene expression in foetal kidneys. During the dark (active) phase, genes indicating increased cellular activity (*Hyou1*, *Hspa5*, *Hsp90b1*) were upregulated in normoxic group. In contrast in normoxic group, inflammatory (*S100a9*, *Oasl2*), cell cycle (*Cdkn2c*, *Rap1gap*), and hypoxia-response genes (*Egln3*, *Ldh*a) were downregulated. No significant changes were observed when hypoxia occurred during the dark. However, light-phase hypoxia downregulated genes such as *Ogdhl* and *Ptger3*, suggesting disrupted nephron development and blood pressure regulation pathways in comparison with normoxia group.
Conclusion: Observed transcriptomic changes highlight a complex interplay between circadian timing, renal development, metabolic adaptation, and cardiovascular regulation during prenatal life. Our findings emphasise that the timing of prenatal insults such as hypoxia may differentially affect organ development and probably disease vulnerability.
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P1.99.

SEX-DEPENDENT EFFECTS OF MATERNAL EXPOSURE TO SUBCLINICAL INFECTION ON OFFSPRING DEVELOPMENT

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Objectives: Maternal environment influences fetal development and metabolism, and fetal growth deficiencies could result in susceptibility to developing chronic disease later in life. Previously we described that low-dose LPS-induced subclinical infection alters fetal growth in utero, the vascular processes associated with placentation, and the balance of pro-inflammatory cytokines and vascular mediators at the maternal-fetal interface.
Methods: To assess the effects of LPS-triggered subclinical infections on the progeny, we evaluated the offspring born to control or LPS-treated mothers. We measured weight, anogenital distance, and head size from post-natal day (PND) 4 to PND21. After weaning, pups were weighed weekly. Key maturation milestones and neurodevelopmental tests were recorded. Measurements were discriminated by sex.
Results: No significant differences were found in weight, anogenital distance, and head size between pups born to control or LPS mothers. However, on PND40, male pups from LPS-treated mothers weighed less than males from the control group. For female pups, no difference in weight was observed. Maturity parameters, such as fur growth, teeth eruption, ear canal opening and eye-opening, showed no significant differences between groups.
In the righting reflex test (PND4), female pups from LPS-treated mothers took longer to regain an upright position than the control group while male pups did not show significant differences. Regardless of sex, a significantly lower percentage of pups from the LPS group exhibited mature posture (PND12) compared to the control group. In the cliff aversion and gait tests, no differences were observed between the pups of both experimental groups. Additionally, all control and LPS pups perform forelimb and hindlimb grasping successfully.
Conclusion: Our results show that LPS-subclinical infections do not change offspring maturation milestones but affect neurodevelopment and weight gain in both female and male pups, with sex-dependent effects. These findings support the hypothesis that subclinical infections can impact ongoing pregnancy and have lasting effects on offspring.

P1.100.

MATERNAL HYPERINSULINEMIA INDEPENDENTLY ALTERS PLACENTAL IMMUNE CELL COMPOSITION AND EFFICIENCY

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Objectives: Nearly 50% of reproductive-aged women are affected by obesity, increasing their risk of diabetes with parity and age progression. Currently, strong evidence suggests maternal hyperglycemia and obesity can either independently or collectively act on the placenta to negatively impact offspring health and induce placental inflammation via increased proinflammatory immune cell infiltration. However, the independent effect of maternal hyperinsulinemia, which is often associated with obesity and gestational diabetes mellitus (GDM), remains elusive. We used a murine model of maternal hyperinsulinemia (RIP-βAkt Tg), which exhibits increased β-cell mass, to investigate whether maternal hyperinsulinemia is sufficient to potentiate immune cell changes at the maternal fetal interface, alter placenta function, and impact fetal health.
Methods: We phenotyped placentas and fetuses exposed to hyperinsulinemia in pregnancy (HIP) at embryonic days 14.5 (E14.5) and 17.5 (E17.5) and compared them to wild-type controls (CTRL). Additionally, flow cytometry was performed to assess changes in placental macrophage populations and western blotting to assess protein levels.
Results: Both male and female HIP E14.5 placentas exhibited decreased placental efficiency. A noticeable difference in E14.5 fetal body weight was observed in both male and female fetuses. However, HIP fetuses demonstrated catch-up growth from E14.5 to E17.5 in both sexes. We also observed an increased protein level of pro-apoptotic caspase-3 in E17.5 HIP placentas. Flow cytometry of E14.5 HIP placentas revealed a reduction in several decidual and placental macrophage subsets. Additionally, HIP E17.5 placentas demonstrated decreased gene expression of IL1-β.
Conclusion: Altogether, our data suggests that apoptotic stress signals induced by hyperinsulinemia within the placenta may attenuate placental inflammation by reducing macrophages and IL-1β production. This could potentially contribute to placental insufficiency and smaller fetuses at E14.5, followed by rapid catch-up growth.

P1.101.
SFLT1-INDUCED PREECLAMPSIA AND A HIGH-CALORIC DIET AFTER WEANING IN OFFSPRING INCREASES THE RISK OF DEVELOPING A METABOLIC SYNDROME EXCLUSIVELY IN MALES

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Objectives: Preeclampsia (PE), a placental disease affecting 3-7% of pregnant women, is characterized by elevated levels of anti-angiogenic soluble fms-like tyrosine kinase-1 (sFLT1), often leading to fetal growth restriction (FGR). Fetal adaptations to adverse intrauterine environment due to PE may negatively pre-program the metabolism of the fetus/offspring, resulting in long-term metabolic changes and influencing the metabolic response to additional stressors such as a high-caloric diet.
Methods: We used a human sFLT1 PE/FGR mouse model and examined the offspring metabolic response to a high-caloric Western-style diet (WD) versus standard diet (SD) starting after weaning. Insulin (ITT) and glucose tolerance tests (GTT) were performed throughout the postnatal development and metabolically relevant tissues (liver, pancreas, gonadal fat) were collected on postnatal days P5/P12/P28 and P90 to assess morphological changes and altered mRNA and protein levels of metabolic factors. Serum and tissue samples were analyzed for metabolites relevant to metabolic syndrome.
Results: We found evidence of obesity and pre-diabetes exclusively in male offspring resulting from an hsFLT1-induced PE pregnancy. Blood glucose level responses during ITT and GTT were impaired in PE-males fed a WD compared to controls, whereas females were unaffected by diet or PE-pregnancy. In addition, liver morphology and expression of metabolic and liver damage markers were impaired in PE-offspring, with males being stronger affected than females. PE-females exhibited a type-1-diabetes-like phenotype, identified by low levels of serum insulin and high reactivity during ITT that might be associated with an impairment of insulin-producing cells in pancreatic islands.
Conclusion: The results suggest that hsFLT1-induced PE/FGR in mice may increase the risk of developing a metabolic syndrome and (pre-)diabetes only in male offspring. These effects are aggravated after feeding a high-caloric diet, while PE-females seemed to be protected from harmful effects of this high-caloric diet. Subsequent analysis of involved mechanisms are in progress.

P1.102.
PLACENTAL EXTRACELLULAR VESICLES MODIFIES FUTURE CARDIOVASCULAR DISEASE DEVELOPMENT IN RODENTS

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Objectives: In parous women, the risk of cardiovascular disease (CVD) later in life is associated with maternal health during previous pregnancies – a healthy pregnancy is associated with decreased CVD risk and complicated pregnancies, such as preeclampsia, is associated with increased CVD risk. The mechanism underlying this association remains unexplored.
We hypothesized that extracellular vesicles (EVs) released from the placenta can induce long term changes in the maternal cardiovascular system, and may be part of the mechanism by which pregnancy alters maternal CVD risk later in life.
Methods: Placental EVs were isolated by centrifugation of conditioned media from human term placenta explant cultures from normal pregnancies or pregnancies complicated by early- (EOPE) or late- onset preeclampsia (LOPE). We used spontaneous hypertensive rats (SHR) to model women destined to develop CVD. 14-week old virgin female SHRs were injected intravenously with EOPE (n=7), LOPE (n=10), or normal placental EVs (n=10). The dosing regimen was five injections over 10 days, each EV injection containing 315μg protein. Systolic blood pressure (SBP) and cardiovascular function were monitored over 12 months via tail-cuff and high-frequency ultrasound.
Results: Injections of LOPE EVs significantly elevated SBP (normalized to baseline) in SHRs compared to EVs from normal pregnancies, which persisted for 9 months (p=0.03, 42.52±22.76 vs 20.28±20.83 mmHg). Higher elevated SBP was still persistent in the EOPE group at the end of the 12-month observation period compared to normal EVs group (p=0.01, 64.06±20.99, vs 35.26±14.51 mmHg). There were no differences in left ventricular function measured by echocardiography.
Conclusion: Placental EVs from pregnancies complicated by preeclampsia can induce long-term changes in the maternal cardiovascular system, exacerbating the cardiovascular damage naturally occurring in spontaneously hypertensive rats and may partially explain how maternal health during pregnancy can be associated with future CVD in women.

P1.103.
ECTONUCLEOTIDASES AND PURINERGIC SIGNALING MIGHT CONTRIBUTE TO IMMUNOSUPPRESSION IN NEWBORNS

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Objectives: Ectonucleotidases and purinergic receptors modulate inflammatory responses by balancing extracellular ATP and adenosine. While extracellular ATP promotes cellular activation and inflammation, adenosine induces immunosuppression. Newborns are more vulnerable to infections due to quantitative and qualitative immunological differences, and the contribution of extracellular nucleotides to this profile is not yet fully elucidated. In this study, we sought to investigate the potential role of purinergic signaling in shaping the immune responses in early life.
Methods: Umbilical cord blood was collected from clinically healthy parturients undergoing cesarean section at the São Paulo University Maternity Hospital and were compared to adult peripheral blood samples obtained from healthy donors. The expression of ectonucleotidases and ADO receptors was verified in the whole blood by real-time PCR and flow cytometry.
Results: We identified a reduction of CD4+ and T CD8+ T cells, CD19+ B lymphocytes, and CD14+ monocytes expressing the ectonucleotidase CD39 in cord blood compared to adults. A reduction of CD4+ T cells, NK cells, and B cells expressing CD73, a nucleotidase that generates adenosine, was also observed. However, a higher frequency of CD8+CD73+ cells was verified in the cord blood. Furthermore, increased expression of ADO receptors was observed in the umbilical cord blood.
Conclusion: The findings of this study suggest the prevalence of naïve and low activated cells in the cord blood. Additionally, our data indicate that CD8+CD73+ cells, together with a higher expression of adenosine receptors, might contribute to the generation of adenosine and the immunosuppression in newborns.

P1.104.
PLACENTAL METABOLIC PHENOTYPE IN THE BTBR MOUSE MODEL OF NEURODEVELOPMENTAL DISORDERS

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Objectives: Neurodevelopmental disorders (ND) originate during prenatal life, yet their etiopathogenesis remains largely unclear. The BTBR T^{+} $Itpr3^{tf}$/J (BTBR) mouse model of ND displays core autism-like behaviors and distinct metabolic traits. Given the growing evidence supporting a crucial role of the placenta in shaping neurodevelopment, this study aimed to characterize the placental phenotype of BTBR offspring compared to the C57BL/6J control strain and dissect the embryonic and maternal contributions to offspring growth, placental health, and postnatal behavior.
Methods: Fetal and placental growth, as well as placental histological architecture, vascularization, and lipids accumulation, were assessed in BTBR and controls. Placentas and fetal heads transcriptomes were analyzed by microarrays and Real-Time PCR. Reciprocal embryo transfer between BTBR and B6 mothers was employed to dissect embryonic and maternal contributions to placental development, fetal growth, and offspring behavior.
Results: BTBR conceptuses displayed fetal growth restriction, reduced placental weight, reduced glycogen reserves, and an enlarged labyrinth compared to B6. No significant differences were found in total labyrinth vessel volume between strains, whereas maternal blood vessel area was significantly higher in BTBR. Strain differences in transcriptomic profiles were more prominent in placentas as compared to fetal heads and were enriched for genes involved in metabolic pathways. Concurrently, BTBR trophoblast giant cells accumulate more lipid reserves compared to the control strain. Reciprocal embryo transfers between BTBR and B6 mothers reveal that although strain-specific maternal factors influence placental morphology and fetal growth, the characteristic autism-like behaviors of BTBR mice persist in offspring gestated and reared by C57BL/6J dams.
Conclusion: These insights advance our understanding of early-life determinants of autism and suggest placental metabolism as a potential target for early diagnosis of neurodevelopmental risk. This research was supported by the National Science Center, Poland (grant no. 2020/39/B/NZ4/02105).

P1.105.
UMBILICAL ENDOTHELIUM-DERIVED THROMBOSPONDIN-1 (TSP-1) OF PREECLAMPSIA DECREASES THE ANGIOGENIC CAPACITY OF BRAIN ENDOTHELIAL CELLS

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Objectives: Previous studies have shown reduced cerebral angiogenesis in offspring from preeclamptic pregnancies. Since the placental vasculature is continuous with fetal circulation, placental endothelium-derived circulating factors may reach the fetal brain and impair angiogenesis. Thrombospondin-1 (TSP-1), an antiangiogenic glycoprotein, is elevated in PE placentas, but its role in the placenta-brain communication axis remains unexplored.
To analyze the effect of secreted factors from umbilical endothelial cells, particularly TSP-1, on the angiogenic and invasive capacity of cerebral endothelial cells using an *in vitro* model.
Methods: Primary human umbilical vein endothelial cells (HUVECs) from normal pregnancies (NP, n=6) and PE (n=6) were used to obtain conditioned media (CM-HUVEC-NP or CM-HUVEC-PE, respectively), which were applied to human cerebral microvascular endothelial cells (hCMEC/D3). Tube formation, migration, invasion, and actin cytoskeleton remodeling were assessed, along with the expression of angiogenic markers. Proteomic analysis was also performed on umbilical cord plasma from NP and PE pregnancies (n=3 per group).
Results: Results showed that MC-HUVEC-PE significantly impaired tube formation, migration, and invasion of hCMEC/D3 cells, accompanied by cytoskeletal remodeling abnormalities, such as longer but fewer actin fibers and the formation of filopodia ($p<0.05$ in all cases). Proteomic profiling revealed 30 differentially expressed proteins in PE plasma, including a significant increase in TSP-1, confirmed by Western blot. TSP-1 was also ~3-fold elevated in MC-HUVEC-PE compared to MC-HUVEC-NP ($p<0.03$). Importantly, the antiangiogenic effects of MC-HUVEC-PE were reversed by a neutralizing anti-TSP-1 antibody.
Conclusion: TSP-1 secreted by umbilical endothelial cells in PE may contribute to reduced cerebral angiogenesis observed in the offspring. Our findings provide novel evidence of a placenta-brain communication pathway that impacts fetal neurovascular development.

P1.106.
MYELOID CELL – T CELL INTERACTIONS ARE PROMINENT IN PREGNANCIES WITH EXTREME FETAL-MATERNAL HLA INCOMPATIBILITY AND AN IMMUNE TOLERANT MICROENVIRONMENT

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Objectives: Healthy pregnancy requires local tolerogenic immune mechanisms. Pregnancies after oocyte donation (OD) are related to more fetal-maternal HLA mismatching and to elevated risk of pre-eclampsia. We hypothesize that in OD pregnancies with high HLA dissimilarity the immune response at the fetal-maternal interface (decidua basalis) is divergent to maintain a healthy state.
Methods: Fetal-maternal HLA genotypes were determined by high-resolution typing. Decidua basalis regions were subjected to multispectral imaging for study of spatial immune cell orientation, to gene expression analysis, and to pathologic assessment. Maternal HLA antibodies were assessed by Luminex. Maternal NK cell receptor (KIR) typing was performed by qPCR.
Results: Myeloid cells represented 65% of the immune cell population at the decidua, and encompassed twelve phenotypically distinct subclusters. Healthy pregnancies with extreme fetal-maternal HLA mismatching (fully-allogeneic group) displayed a higher frequency of $CD163^{+}HLA\text{-}DR^{+}$ myeloid cells in the microenvironment of $CD4^{+}$ T cells compared to both healthy pregnancies with moderate fetal-maternal HLA mismatches (semi-allogeneic group) and to pregnancies with pre-eclampsia ($p<0.05$). The fully-allogeneic-OD-healthy group also showed a high frequency of $FoxP3^{+}CD4^{+}$ regulatory T cells (Tregs) in the vicinity of other $CD4^{+}$ T cells, which was reflected by a decidual gene signature of Treg reinforcement and decreased inflammatory chemokine expression. In contrast, decidua during pre-eclampsia was characterized by enhanced chemokine expression and an imbalance in oxidative stress related genes. Interestingly, pregnancy outcome was not related to pathologic alterations of the decidua, maternal anti-fetus-HLA antibody status, and fetal HLA-C/maternal KIR haplotype combinations.
Conclusion: This study shows the prominent frequency and phenotypic diversity of decidual myeloid cells in OD pregnancies. By interacting with T cells, decidual myeloid cells might exert immune regulatory effects to compensate for the high fetal-maternal HLA mismatch load in OD pregnancies.

P1.107.
ASSESSMENT OF PLACENTAL TRANSFER OF IGG ANTIBODIES AND CYTOKINE PRODUCTION IN RESPONSE TO A VIRAL ENVIRONMENT

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Objectives: Despite success in preventing vertical HIV transmission globally, neonates exposed *in utero* (HEU) face an increase in morbidity and mortality. Reported immune alterations related to this include: altered IgG transfer, proinflammatory cytokine profile, and lower proportions of differentiated Th cells with less capacity to secrete cytokines. Placental response to the antiviral milieu is suspected to contribute to alter the developing fetal immune system. We hypothesize that maternal antiviral mediators could alter the IgG and cytokine transfer ratios. In this work we investigated this using a human *ex vivo* placenta perfusion model and a microfluidic-supported placenta-on-a-chip model (PoC).
Methods: Double-side *ex vivo* human placenta perfusion was performed. For PoC, BeWo cells were seeded on a biochip (Dynamic42) and connected to an ibidi Perfusion System. Maternal side of both models was exposed to 5 pg/mL of IFN-ɣ or vehicle. Total IgG was added in the PoC model at 0.1 μg/mL. Perfused samples of both, maternal and fetal side, were taken and analyzed for IgG subclasses (IgG1, IgG2, IgG3 and IgG4) and cytokines (IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p70, IL-17A, IL-18, IL-23, IL-33) by Legendplex arrays. Neonatal Fc receptor (FcRn) expression in BeWo cells was analyzed by immunofluorescence.
Results: In both models, maternal IFN-ɣ exposure led to reduced IgG1 and IgG3, transport to the fetal side. This was accompanied by increased FcRn expression as well as elevated concentrations of IL-1β and reduced of MCP-1 on both maternal and fetal side.
Conclusion: Our results suggest that viral infections can alter placenta barrier functions and its capacity to transport immune components to the fetal side including different IgG subclasses and inflammatory cytokines. This has the potential to induce alterations in the development of fetal immune responses.

P1.108.
CLINICOPATHOLOGICAL ANALYSIS OF 14 PREGNANCY-ASSOCIATED LISTERIOSIS CASES INCLUDING A FETAL AUTOPSY REPORT

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Objectives: To investigate the clinicopathological characteristics of pregnancy-associated listeriosis and establish histopathological evidence linking *Listeria monocytogenes* (*LM*) infection to adverse pregnancy outcomes
Methods: A retrospective review was conducted on 14 pregnant women diagnosed with listeriosis at the International Peace Maternity and Child Health Hospital (IPMCH) affilated to Shanghai Jiao Tong University School of Medicine, from January 2017 to December 2024. Clinical records and histopathological specimens from placental and fetal tissues were analyzed.
Results: The cohort (0.12 per 1000 of IPMCH deliveries) had a mean maternal age of 31.4 years (range: 28–36 years). Median gestational age at symptom onset was 30^{+3} weeks (range: 21^{+3}–38^{+6} weeks). All cases presented with maternal fever (14/14, 100%), frequently accompanied by abdominal pain (7/14, 50.0%) and abnormal fetal movements (5/14, 35.7%). Adverse outcomes included five late miscarriages/stillbirths (5/14, 35.7%). Among nine live births, neonatal outcomes included two cases of sepsis, four cases of sepsis with purulent meningitis, one pneumonia case, one healthy term infant, and one lost to follow-up. Histopathological evaluation demonstrated several maternal and fetal inflammatory responses in 13 cases of placentas/umbilical cords/fetal membranes. Postmortem examination of the stillborn revealed widespread severe acute inflammatory infiltrates with coagulative necrosis involving multiple organ systems. Gram-positive bacilli clusters were identified in placenta, umbilical cord, fetal membranes, and various fetal organs.
Conclusion: *LM* induces acute, severe inflammatory responses capable of compromising the placental barrier, leading to high fetal and neonatal mortality. The histopathological findings in placental and fetal tissues offer crucial morphological insights into the mechanisms underlying *LM*-associated complications at the maternal-fetal interface.

P1.109.
GBS INFECTION INDUCES CELL PYROPTOSIS IN FETAL MEMBRANES VIA ACTIVATED INFLAMMASOME

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Objectives: The established link between Group B Streptococcus (GBS) and adverse pregnancy outcomes - including preterm premature rupture of membranes (PPROM), preterm labor, and neonatal sepsis - prompted our investigation into the underlying biological mechanisms
Methods: Using fetal membrane explant models and amniotic epithelial cells, we sought to determine whether/how GBS-induced inflammation triggers pyroptosis, with the goal of identifying novel therapeutic interventions for GBS-related perinatal complications
Results: Key findings demonstrate: 1). GBS infection markedly elevates maternal serum IL-1β levels and upregulates Toll-like receptor 2 (TLR2) expression in fetal membranes; 2). GBS-activated caspase-8 simultaneously initiates both inflammatory signaling and pyroptotic pathways while inducing substantial reactive oxygen species (ROS) generation; 3). This ROS surge creates a feed-forward loop that amplifies both inflammation and pyroptosis; 4). Pharmacological inhibition using the antioxidant N-acetylcysteine (NAC) and NADPH oxidase inhibitor diphenyleneiodonium (DPI) effectively suppressed ROS production and IL-1β secretion.
Conclusion: Our data reveal that GBS drives pregnancy complications through a ROS-TLR2 signaling axis that promotes mixed lineage kinase 1/3 (MLK1/3)-dependent pyroptosis in fetal membranes. Critically, targeting either ROS generation through NADPH oxidase inhibition or ROS neutralization via antioxidant therapy preserves membrane integrity by disrupting this pathogenic cascade.

P1.110.
NEUTRALIZATION AND ASSESSMENT OF THE BINDING CAPACITY OF MATERNAL SERA AGAINST VARIOUS SARS-COV-2 VARIANTS

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Objectives: SARS-CoV-2 infections during pregnancy are associated with increased COVID19 severity and mortality as compared to the general population. Two methods were developed to evaluate the antibody response of ~700 pregnant women against the dominating SARS-CoV-2 variants in the Israeli pandemic.
Methods: I.In a neutralization assay using recombinant (rVSVdG) pseudoviruses, each expressing a SARS-CoV-2 spike variant on their membrane, HEK293 cells overexpressing the SARS-CoV-2 receptor hACE2 and the protease TMPRSS2 were infected with the four pseudoviruses (Wuhan – Hu-1 (wild type (WT)), Alpha (B.1.1.7), Delta (B.1.617.2) and Omicron (B.1.1.529), were exposed to maternal sera. The capacity of each serum to inhibit any of the four variants from infection was simultaneously quantified by fluorescent microscopy. II.In a Multiplex system, where eight different fluorescent beads were coupled to four SARS-CoV-2 spikes and four nucleocapsid variants, the bead mixture was incubated with individual sera, and the binding capacity was assessed using an anti-hIgG-PE on Luminex MAGPIX reader.

Results: Our data suggest that the SARS-CoV-2 evolution led to variants that with time, escaped antibody neutralization; while the WT variant was inhibited rather well in both COVID-positive sera and vaccinated sera, the Alpha, Delta, and Omicron variants were less inhibited, respectively. Moreover, the Omicron variant escaped neutralization unless first the woman was vaccinated and only later infected, mainly during the Omicron wave. Our data also correlate with the severity of the side effects during the time of the infection of each variant; higher antibody titers were found towards the spikes of the Alpha and Delta variants compared with those of the WT and Omicron variants.
Conclusion: The comparison between the two methods showed good correlations for all variants except for the Omicron. This fits with the notion that despite binding capacity, most sera could not neutralize Omicron unless they were exposed first to vaccination and then were infected with Omicron.

P1.111.
SARS-COV-2 DELTA AND OMICRON VARIANTS ALTER TROPHOBLAST CELL FUSION AND SYNCYTIOTROPHOBLAST DYNAMICS: NEW INSIGHTS INTO PLACENTAL VULNERABILITY

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Objectives: Pregnancy is considered as a risk factor for severe disease associated with COVID-19. In addition, SARS-CoV-2 infection during pregnancy is associated with increased risks of adverse obstetrical outcomes. Placental pathological changes have also been increasingly reported in both symptomatic and asymptomatic infected pregnant women. It was already shown that SARS-CoV-2 could infect and propagate in term placenta. However, it is still unknown whether the viral entry in placenta is depending on gestational age or variants. The overall objective of this study is to assess the susceptibility of the first trimester placenta to SARS-CoV-2 Delta and Omicron variants.
Methods: We infected first trimester cytotrophoblast (CTB) or syncytiotrophoblast (STB) with SARS-CoV-2 Delta and Omicron BA.1, BA.2, BA.5 variants. Viral load of SARS-CoV-2 infected placental cells was determined by RT-qPCR. The infection of these cells was further demonstrated by immunofluorescence. Cell fusion of CTB infected with Mock versus SARS-CoV-2 variants was evaluated by hemalum-eosin (HE) staining. The release of syncytial knots from SARS-CoV-2 positive placentae were also evaluated by HE staining.
Results: First trimester CTB and STB are permissive to SARS-CoV-2 in variant- and donor-dependent manner. Delta variant showed a higher efficiency of replication in STB and CTB compared to Omicron variants. In STB, despite a slight subsequent increase of type III IFN response, no correlation was observed between virus replication and the induction of the overall host response after infection. In CTB, virus replication significantly correlated with the increased level of trophoblast cell fusion. In line with increased STB formation *in vitro*, we observed an increase of syncytial knots release in early placenta infected by SARS-CoV-2 compared both to SARS-CoV-2- negative areas from the same placenta, and to age matched references.
Conclusion: Altogether, our data suggested that efficient replication of SARS-CoV-2 variants in placenta cells during early stage of pregnancy might alter STB turnover.

P1.112.
TROPHOBLASTS PERMISSIVENESS TO SARS-COV-2 AND SEX-DEPENDENT EFFECTS OF COVID-19 ON THE PLACENTA MULTIOMIC PROFILE

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Objectives: The effect of an infection by SARS-CoV-2 during pregnancy on the placenta remain poorly understood. Studies have shown the presence of SARS-CoV-2 in human placental tissues and canonical viral entry factors ACE2 and TMPRSS2 are expressed in villous trophoblasts. Inflammatory and structural abnormalities have been observed in placental tissues from infected pregnant persons, but the underlying mechanisms are still unknown. Maternal infection during pregnancy with SARS-CoV-2 alters the development and functions of the placenta. Our specific objectives are to characterize the permissiveness of villous trophoblasts to SARS-CoV-2 and to identify the placental mechanisms altered by maternal infection.
Methods: Human primary villous trophoblasts were isolated from 4 placentas delivered at term from healthy pregnancies and infected with multiple variants of SARS-CoV-2. Plaque assays and RT-qPCR were done to assess trophoblastic permissiveness. Treatment with TMPRSS2 and endosomal pathway inhibitors allowed to test the viral entry route. Placenta samples collected from 43 pregnant persons infected during pregnancy and 27 non-infected controls, at CHUSJ (Montreal, Canada) were used to study protein expression by mass spectrometry and gene expression by next-generation RNA-sequencing.
Results: Our data confirm that human trophoblasts are permissive to the ancestral SARS-CoV-2 strain (PreVOC) *in vitro* and the viral entry appear to be mainly endosomal while in a lesser extend TMPRSS2-mediated, depending on the donors. While the proteomic dataset did not identify significant differences, gene expression analysis of placental tissues detected about 22800 genes and identified 8 and 44 genes differentially expressed (padj<0.05; FC 1.5) respectively in XY and XX placentas. The pathway enrichment analysis notably revealed an alteration of the cytoskeleton organization and of the reproductive process in the XX placentas of the COVID+ group.
Conclusion: This study shed light on the mechanism of infection/entry of SARS-CoV-2 in trophoblasts and that maternal infection during pregnancy affect the placenta in a sex-dependent manner.

P1.113.
SARS-COV-2 INFECTION OF THE PLACENTA DISRUPTS SYNCYTIAL INTEGRITY, DYSREGULATES THE RENIN-ANGIOTENSIN SYSTEM, AND IMPAIRS TROPHOBLAST FUNCTION VIA ORF3A

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Objectives: SARS-CoV-2 infection during pregnancy increases risk of adverse pregnancy outcomes such as preeclampsia but the underlying mechanisms are largely unknown. Here, we investigate the mechanisms by which SARS-CoV-2 infection during pregnancy alters placental dynamics associated with preeclampsia, with a focus on the role of viral protein ORF3a.
Methods: Placental villous tissue was collected at delivery from unvaccinated patients who tested positive for SARS-CoV-2 during pregnancy, using two large prospective cohorts. Histopathological features known to be altered in PE were assessed including 1) syncytial knot formation, 2) alterations in renin-angiotensin system (RAS) components (PlGF, Flt-1), 3) endothelial integrity, and 4) placental barrier integrity using tight junction protein marker, Z0-1. We investigated viral mechanisms driving histopathological changes by infecting cultured human trophoblast cell line (Jeg-3) with live Delta variant of SARS-CoV-2.
Results: SARS-CoV-2 viral proteins spike, nucleocapsid, and ORF3a were observed in the syncytiotrophoblast layer and stroma of placental VT. SARS-CoV-2-infected placentas were associated with increased syncytial knots, which were positive for SARS-CoV-2 viral proteins, further

validating SARS-CoV-2 replication in the placenta. Syncytial nuclear aggregates, placental infarctions and fibrin deposits in infected placentas were observed, indicating placental damage. Infection was associated with reduced placental expression of PlGF and thus an elevated Flt-1/PlGF ratio. Infected placentas also showed a significant decrease in vimentin expression indicating dysregulated endothelial function. Infection of Jeg-3 trophoblast with SARS-CoV-2 induced mislocalization of ZO-1 through *direct* molecular interaction between ORF3a and ZO-1, leading to weakening of cell-cell junctions.

Conclusion: SARS-CoV-2 infection disrupts placental integrity in the form of increased syncytial knots, dysregulated RAS components, and endothelial damage. Additionally, our findings highlight a novel role for ORF3a in disrupting trophoblast function by impairing tight junctions during infection. These alterations, which resemble features observed in preeclampsia, suggest a potential mechanism by which SARS-CoV-2 infection during pregnancy may elevate the risk of a preeclampsia-like syndrome.

P1.114.
EXPOSURE TO ZIKA VIRAL PARTICLES IS SUFFICIENT TO INDUCE GSDME-MEDIATED SYNCYTIOTROPHOBLAST PYROPTOSIS

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Objectives: The maternal, blood-facing, surface of the human placenta consists of a single, massive, multi-nucleated cell known as the syncytiotrophoblast (ST). Previously, we discovered that this ST layer undergoes pyroptosis, a programmed cell death mechanism dependent on membrane pore-forming gasdermin-family proteins. Moreover, we identified that ST pyroptosis occurs through non-canonical mechanisms – with progression dependent on gasdermin-E downstream of TNFα stimulation. Zika virus is an emerging pathogen capable of infecting the human placenta and inducing congenital Zika virus syndrome (CZVS). Murine models of CZVS implicate TNFα-induced gasdermin-E-associated pyroptosis, and Zika virus infection can elicit GSDME-dependent pyroptosis in JEG-3 cells. We, therefore, sought to determine whether Zika infection or exposure would also be sufficient to induce GSDME-dependent pyroptosis in our floating explant culture model.

Methods: Human first-trimester explants (7-8 weeks gestation) were exposed to Zika virus (10^6 virions), or UV-inactivated Zika, for 24HRs. ST dextran uptake assays and western blots for active and total Gasdermin-E were used to assess the extent of pyroptotic activation. Anti-NS5 protein staining served to assess the degree of infection.

Results: Zika viral infection was limited and found in mononucleate trophoblasts. Both live and UV-inactivated Zika increased active and total Gasdermin-E expression. These correlated with 2 to 2.5-fold increases in regionalized ST dextran uptake akin to TNFα-induced ST pyroptosis. Interestingly, UV-inactivated Zika elicited responses trend higher than active infection.

Conclusion: Exposure to infective Zika virus and the infection of mononucleate trophoblasts, and UV-inactivated Zika virus, is sufficient to induce ST pyroptosis. The presence of Zika virus can therefore trigger infection-dependent and infection-independent pyroptotic responses.

P1.115.
MIRROR SYNDROME SECONDARY TO FETAL PARVOVIRUS INFECTION: A CASE REPORT

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Objectives: Maternal generalized edema and placental edema may occur secondary to fetal hydrops, a condition known as Mirror syndrome. We report a case of fetal hydrops caused by parvovirus B19 infection, which subsequently led to the development of Mirror syndrome.

Methods: A 35-year-old woman, gravida 1, para 0, received regular prenatal care at our hospital. The patient has no past medical history and no relevant family history. At 25 weeks of gestation, an ultrasound examination revealed hydrops fetalis, including pericardial effusion and ascites, as well as placentomegaly. The middle cerebral artery peak systolic velocity (MCA PSV) was within normal limits. Maternal serological testing was positive for parvovirus B19 IgM.

At 26 weeks of gestation, the patient developed significant lower limb edema, and Doppler ultrasound showed an MCA PSV compatible with severe fetal anemia, raising suspicion for Mirror syndrome. An emergency cesarean section was performed the same day due to the maternal condition. The neonate, with a birth weight of 1,078 g, had Apgar scores of 2 and 3 at 1 and 5 minutes, respectively. Severe anemia (Hb 1.3 g/dL) was detected, and intensive care including blood transfusion was initiated. Despite comprehensive management, the infant passed away on day 61 of life. The mother's condition improved postoperatively, and she was discharged on postoperative day seven.

Results: Pathological examination of the placenta revealed nucleated fragments around chorionic stromal vessels and cells containing intranuclear inclusion bodies, which was consistent with parvovirus B19 infection.

Conclusion: Mirror syndrome is a rare complication, occurring in approximately 0.9% of fetal hydrops cases. Its clinical presentation closely resembles that of preeclampsia but can progress to maternal heart failure and pulmonary edema. Careful monitoring of both fetal ultrasound findings and maternal physical status is essential for timely diagnosis and management.

P1.116.
THE PLACENTAL MICROBIOME: STILL NO SIGNAL BEYOND NOISE

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Objectives: The "sterile womb" hypothesis has been debated since the introduction of the concept of "in-utero colonization". Some DNA sequencing studies have detected microbial DNA in placental tissue, albeit in minimal quantities. These studies often lack rigorous negative controls, raising concerns about contamination. This pilot study aims to adapt and validate a protocol for investigating the presence of a placental microbiome by culture techniques using strict contamination controls.

Methods: Building on our recent study of the fetal microbiome and following the establishment of a rigorous protocol, placental samples from 20 healthy pregnant women were collected during cesarean sections and cultured under aerobic and anaerobic conditions. Negative controls (skin, amniotic fluid, uterine wall, instruments, room air, laboratory dishes etc.) were implemented at each experimental step to minimize false-positive results.

Results: 18 out of 20 placental core samples were culture-negative. In 2 positive samples we found *Cutibacterium acnes or Streptococcus mitis-*

oralis, Haemophilus parainfluenzae and Schaalia odontolytica, respectively. Contamination controls demonstrated positive culture findings in room air, surgical instruments, and amniotic fluid in 20% of samples. Amniotic fluid tested positive in 37% of cases, while skin samples from patients after disinfection and uterine surfaces were positive in 50% of cases. The most frequently identified species were *Staphylococcus epidermidis/capitis/lugdunensis (haemolyticus)*, *Cutibacterium acnes/avidum*, *Bacillus cereus*, and *Corynebacterium tuberculostearicum*. The sterile bench yielded culture-negative results in 100% of cases.

Conclusion: By employing a stringent, standardized, and contamination-controlled workflow, this study failed to demonstrate consistent positive microbial cultures in placental tissue speaking against reliable evidence of a placental microbiome. Therefore, we conclude that single culture-positive samples should be considered as contamination.

P1.117.
THE ROLE OF TRYPANOSOMA CRUZI-DERIVED EXOVESICLES ON TLR-2 EXPRESSION AND NF-κB ACTIVATION IN HUMAN PLACENTAL EXPLANTS

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Objectives: Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) and can be transmitted congenitally through the placenta. The parasite presents different virulence factors that can be secreted in exovesicles (TcEVs). Here, we determined the effect of TcEVs during *ex vivo* infection of *T. cruzi* trypomastigotes in human placental explants (HPEs) on: i) TLR-2 expression and activation of the NF-κB pathway.

Methods: HPEs were incubated in the presence of *T. cruzi* trypomastigotes (10^5 parasites/ml) and the presence and absence of active and heat-inactivated TcEVs. Pam3CSK4 was used as a positive control. TLR-2 mRNA and protein expression were determined, respectively, at 2 or 24 hours post-incubation by RT-qPCR and Western blotting (WB). Immunohistochemistry (IHQ) evaluated TLR-2 distribution in placental tissue at 24 hours. NF-κB activation was evaluated by detecting phosphorylation of IκB at 10 minutes post-exposure by Western blotting. A TLR-2 inhibitor (C29) was used to assess its impact on NF-κB activation.

Results: TcEVs increase, respectively, at 2 and 24 hours of TLR-2 mRNA and protein expression. Moreover, protein expression increases further after co-incubating *T. cruzi* and the TcEVs. The results were confirmed by immunohistochemistry, showing an increased expression of TLR-2 in the trophoblast. In addition, TcEVs activate the canonical NF-κB signaling pathway.

Conclusion: These findings suggest that *T. cruzi*-derived extracellular vesicles may modulate the expression of TLR-2 and influence the activation of the NF-κB pathway in HPEs, modulating the inflammatory response against *T. cruzi* infection within the placenta. Further analysis is needed to clarify the full extent of these parasite-placenta interactions.

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P1.118.
INVESTIGATING DIFFERENCES IN PLACENTAL MORPHOLOGY THROUGH MEASURES OF VILLOUS SURFACE DENSITY AND VOLUME BY HIV AND ANTIRETROVIRAL CLASS EXPOSURE

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Objectives: Many HIV antiretrovirals (ARVs) are recommended for use in pregnancy without extensive safety testing in pregnant women. ARVs have been associated with adverse birth outcomes, including small-for-gestational-age (SGA) births. Alterations to placental morphology, including reduced villous surface area, can influence fetal growth. The effects of different ARV drug classes (PI – protease inhibitors, NNRTI – non-nucleoside reverse transcriptase inhibitors, INSTI – integrase strand transfer inhibitors) on placental villous structure and associations with birth outcomes are not known. Here we compare villous surface density and volume by HIV and ARV-class exposure.

Methods: Random sections from FFPE full placental cores were stained with Masson's Trichrome. Villous surface density and volume was quantified using stereological techniques. Associations of drug class (PI, NNRTI, INSTI) and HIV-status (positive, negative) with villous surface density and volume were examined using linear models. Associations of villous surface density and volume with SGA were assessed using chi-squared test.

Results: Fifty term placentas (12 HIV-negative, 10 HIV+/INSTI, 10 HIV+/NNRTI, 18 HIV+/PI) from Canadian women participating in the AAPH cohort were included. HIV+ status was associated with lower birth weight centile (BWC). Placenta weight, villous volume, and surface density did not differ by HIV-status. Comparing by ARV class, placenta weight was significantly lower in the NNRTI group vs. HIV-negative. Villous volume was lowest in the NNRTI group ($p=0.026$ vs. HIV-negative), and highest in the INSTI group ($p=0.001$ vs. NNRTI, $p=0.017$ vs. PI). Villous surface density was highest in the INSTI group ($p=0.06$ vs. control, $p=0.019$ vs. NNRTI). Villous volume correlated significantly with BWC. Having a low villous volume (<5000) was significantly associated with an SGA birth.

Conclusion: Placental villous surface density and volume differed by ARV class, but not by HIV-status, suggesting different ARV classes have differential effects on placental morphology. These data can help inform best treatment choices for pregnant women with HIV.

P1.119.
GALECTIN PROFILE AT THE MATERNAL-FETAL IMMUNE INTERFACE IN PREGNANT WOMEN LIVING WITH HIV-1 AND THEIR HIV-EXPOSED NEWBORNS.

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Objectives: The aim of this study is to analyze the expression profile of gal-1, gal-3, gal-9, and gal-13 at the maternal-fetal immune interface in HIV-1-infected pregnant women. This research focuses on the immune response modulation, with particular attention to potential alterations caused by the virus, in both the mother and the newborn.

Methods: Samples were collected from HIV+ pregnant women (n=13) and healthy controls (n=15), including peripheral blood, umbilical cord blood, and placental tissue (decidua and villous). Informed consent was obtained from all participants, and the study was approved by the Ethics Committee (CAAE 31605314.3.0000.0068). The expression of galectins was evaluated by RT-qPCR, and serum levels of gal-1 were quantified by ELISA in samples from HIV+ mothers (n=10) and exposed newborns (n=8).

Results: The analysis revealed a significant increase in the expression of gal-3 and gal-13 in the decidua of HIV-1-infected pregnant women. However, the expression levels of gal-1, gal-8, and gal-9 did not differ significantly between HIV+ mothers and healthy controls. Additionally, serum levels of gal-1 were similar between HIV+ mothers and their exposed newborns.

Conclusion: Gal-3, in particular, may play a pivotal role in modulating the immune response at the maternal-fetal interface during HIV-1 infection. The increased expression of gal-3 and gal-13 in the placenta suggests that these galectins may be involved in regulating immune tolerance and inflammatory responses. Understanding how galectins modulate maternal immune activation could provide new insights into potential therapeutic

targets for HIV-1 and other infectious diseases during pregnancy. Furthermore, this research may identify biomarkers for maternal-fetal immune regulation, offering pathways for the development of strategies to prevent HIV transmission and improve pregnancy outcomes.

P1.120.
TRYPANOSOMA CRUZI EXOVESICLES ENHANCE GELATINOLYTIC ACTIVITY–MEDIATED CONNECTIVE TISSUE DEGRADATION IN A HUMAN PLACENTAL EXPLANT MODEL

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Objectives: Congenital Chagas disease, caused by the protozoan *Trypanosoma cruzi*, remains a major public health issue in Latin America and an emerging concern in non-endemic regions. The parasite crosses the placental barrier through multiple virulence mechanisms, including the release of extracellular vesicles (TcEVs), contributing to the parasite-induced tissue damage. This study aimed to investigate the role of TcEVs in the degradation of placental connective tissue, focusing on their potential to disrupt extracellular matrix integrity.
Methods: Human placental explants (HPEs) from term, uncomplicated cesarean deliveries were cultured and exposed for 24 hours to (i) *T. cruzi* trypomastigotes (1×10^5 parasites/mL), (ii) purified TcEVs, (iii) heat-inactivated TcEVs, or (iv) a combination of parasites and TcEVs. The gelatinolytic activity was assessed by in situ histoenzymatic assays; MMP-2 and MMP-9 expression was analyzed by western blot, and tissue architecture was evaluated via histological methods and transmission electron microscopy.
Results: Both *T. cruzi* and TcEVs, independently and in combination, induced marked villous stroma disorganization in HPEs, as evidenced by conventional and ultrastructural analyses. All active treatments increased gelatinase/collagenase activity. Notably, TcEVs alone did not induce MMP-2/9 upregulation, whereas the combined treatment with *T. cruzi* and TcEVs significantly enhanced their expression. These findings suggest that TcEVs contribute to connective tissue degradation primarily by potentiating gelatinolytic activity, which may facilitate parasite passage across the placental barrier.
Conclusion: *Trypanosoma cruzi* exovesicles actively promote extracellular matrix breakdown in the human placenta, potentially increasing the risk of congenital transmission. These findings highlight TcEVs as critical modulators of placental integrity and suggest new avenues for therapeutic intervention to prevent vertical transmission of Chagas disease.
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P1.121.
INFLUENCE OF VIRUS VARIANT ON STILLBIRTH RATE - DATA FROM THE CRONOS REGISTRY

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Objectives: During the COVID-19 pandemic, an increased incidence of stillbirths associated with maternal SARS-CoV-2 infection was reported. This study investigates the relationship between stillbirths, placental pathology, and specific viral variants.
Methods: This analysis draws on data from the multicenter, prospective CRONOS registry, comprising 8,032 pregnant women with confirmed SARS-CoV-2 infection. In cases of stillbirth, supplemental clinical information was obtained directly from the respective centers.
Results: Pregnancy outcome data were available for 7,224 women who gave birth at ≥22+0 weeks' gestation. Cases involving chromosomal abnormalities or major congenital anomalies were excluded. Stillbirth occurred in 45 pregnancies, yielding a rate of 0.6%. Distribution by viral variant was as follows: 13 during the wild-type phase, 11 each during Alpha and Delta phases, and 10 during the Omicron phase. Corresponding stillbirth rates were 0.6% (wild-type), 1.6% (Alpha), 0.8% (Delta), and 0.3% (Omicron), with a statistically significant difference across groups ($p = 0.002$). Placental pathology was available for 23 cases; of the 14 from the Alpha and Delta phases, 10 (71%) exhibited features consistent with SARS-CoV-2 placentitis. No such findings were observed in the wild-type or Omicron cases.
Conclusion: Data from the CRONOS registry suggest a variant-specific effect of SARS-CoV-2, particularly the Alpha and Delta strains, on placental integrity, with a potential causal link to stillbirth. These findings support the implementation of targeted fetal surveillance during infection, particularly in the acute phase, and highlight the importance of histologic placenta evaluation.

P1.122.
DETECTION OF SCHISTOSOMA EGGS FROM POTASSIUM HYDROXIDE (KOH) MACERATED PLACENTAL TISSUE AT ZOMBA CENTRAL HOSPITAL, MALAWI

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Objectives: Schistosomiasis, also known as bilharzia, is a prevalent parasitic disease in Malawi caused by *Schistosoma haematobium* or *Schistosoma mansoni*. During pregnancy, it can affect the placenta and may contribute to poor maternal and neonatal outcomes. Microscopic detection in urine samples lacks sensitivity and requires experienced and well-trained personnel. We aimed to detect Schistosoma eggs from placental tissue at Zomba Central Hospital (ZCH), Malawi using a previously described potassium hydroxide (KOH) based maceration technique.
Methods: The placenta sample used was residual material taken from the bin of labor ward. It was sectioned into six circular pieces (about 5cm in diameter), five from the peripheral regions and one from the central region. The sections were put in individual containers filled with 0,9% saline and transported to the laboratory. Each section was further cut into 1 cm pieces and transferred into a 50 mL tube. Each tube was filled with 4% KOH to a final volume of 45 mL and incubated at 37°C for 24 hours while loosely capped. After incubation, samples were centrifuged at 2,500 rpm for 10 minutes at room temperature. The supernatant was discarded, and the remaining pellet was used to make a wet mount that was examined under microscope at 10x, 20x, and 40x objectives for the presence of Schistosoma eggs.
Results: Wet mount microscopic examination showed a typical background with blood cell debris. No Schistosoma eggs were observed in any of the slides.
Conclusion: Maceration of placental tissue offers a simple and affordable method for the detection of Schistosoma eggs. Even though no eggs were detected in this intent, ongoing examination may yield positive findings. This method can complement existing diagnostic strategies and may help improve detection of schistosomiasis during pregnancy.

P1.123.
ZIKA VIRUS-LIKE PARTICLE VACCINE CHALLENGE: ASSESSING IMMUNOGENICITY AND PROTECTION IN AN EARLY PREGNANCY LOSS RHESUS MACAQUE MODEL

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Objectives: Zika virus (ZIKV) is an arthropod-borne flavivirus linked to the 2015 pandemic in Brazil where up to 46% of pregnancies resulted in congenital ZIKV, including a wide range of neurologic birth defects and up to 7.6% reported cases of miscarriage. With no current approved ZIKV vaccines, our group sought to evaluate a ZIKV virus-like particle (VLPs) vaccine candidate in rhesus macaques (RM).
Methods: Our VLPs were produced in mammalian cells expressing the pre-membrane-envelope region of the Asian-lineage ZIKV (PRVABC59) since this lineage has caused congenital outbreaks. To evaluate this vaccine's protection against congenital infection, 2 cohorts of female RM were vaccinated with ZIKV-VLP with adjuvant or adjuvant alone prior to mating. At gestational day (GD) 30, pregnancy confirmed animals were challenged with ZIKV-Dakar 41524, a strain shown to induce 1st-trimester fetal demise in 78% of RM, making it an ideal model for evaluating ZIKV vaccines.
Results: In vaccinated animals, protection was elicited with minimal adverse pregnancy outcomes, and no infectious virus was detected in placental tissues. Two animals reached our study endpoint with one experiencing an early post-infection placental bleed and another placenta previa. In the vaccinated cohort that made it endpoint, there was no detectable viremia within fetal tissues tested. In the unvaccinated cohort, two animals had severe adverse events, including preterm labor in one animal, and one developed hydrops fetalis with diffuse ZIKV-RNA detected via RNA scope and placental damage.
Conclusion: These findings confirm a significant risk for pregnancy loss associated with ZIKV-Dakar and highlight the complexities of ZIKV-induced placental damage. This model can be further used to understand placental immunological challenges that lead to stillbirth and miscarriage following infection. Our findings indicate that this ZIKV VLP vaccine candidate is both safe and effective and a promising countermeasure for the mitigation of ZIKV-induced miscarriage, although it requires additional adjuvants.

P1.124.
IS THE PLACENTA A SELECTIVE BOTTLENECK? MODULATION OF T. GONDII GENETIC DIVERSITY DURING VERTICAL TRANSMISSION

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Objectives: The most devastating consequences of toxoplasmosis are linked to the ability of *Toxoplasma gondii* to access and infect vital anatomical sites such as the placenta and the developing fetus. Infection during pregnancy can cause miscarriages, stillbirths, premature births, and babies born with severe neuropathies. Despite its importance, the mechanisms underlying transplacental transmission remain poorly understood.
In addition to crossing the placental barrier, *T. gondii* can also cross the blood-brain barrier. It was unexpectedly found that during the establishment of chronic toxoplasmosis in the brain, *T. gondii*'s population genetic complexity remains stable, indicating that the blood-brain barrier does not act as a filter on parasitic genetic variance. Whether transplacental transmission is equally permissive remains unknown. Here, we set out to study population dynamics and parasite genetic stability during vertical transmission.
Methods: To do this, we generated *T. gondii* populations bearing unique, fitness-neutral barcodes (one per cell), enabling the study of population bottlenecks in a mouse model of acute infection during pregnancy. By retrieving barcodes from various maternal and fetal tissues, we monitored population dynamics and genetic bottlenecks.
Results: Our results showed an approximately 90% reduction in genetic diversity between the initial inoculum and placental/fetal tissues, whereas 50% of the diversity was retained in the spleen.
Clinically, the probability of congenital toxoplasmosis increases with gestational age, with transmission more likely in the third trimester, though earlier infections lead to more severe outcomes. Notably, our model recapitulates this pattern: infections initiated in the third gestational week resulted in higher fetal infection rates than those in the second week. However, the reduction in barcode diversity was similarly severe at both stages.
Conclusion: These results suggest that while the likelihood of transmission increases with gestational age, the placental bottleneck in genetic diversity remains consistently stringent despite its developmental differences as pregnancy advances.

P1.125.
INFECTION OF PLACENTAL OVINE 3-DIMENSIONAL-(3D)-TROPHOBLAST SPHEROIDS TO ASSESS THE INTERACTION OF COXIELLA BURNETII WITH OVINE TROPHOBLASTS

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Objectives: Monolayer cultures are limited cell culture tools to analyze the interaction of *Coxiella burnetii (C. burnetii)* [Q-fever] for ovine trophoblasts as they do not resemble 3D-tissue *in vivo*. We established 3D-spheroid models deploying ovine AH-1 trophoblasts to investigate the interaction of *C. burnetii* in tissue-like culture models.
Methods: Spheroids were formed via hanging drops (termed: AH-1HD) or poly-HEMA (AH-1PH) and characterized by immunohistochemistry (IHC: cytoskeleton, cell turn over) and transmission electron microscopy [TEM]. Spheroids were infected with *C. burnetii* (MOI: 100, NMII wild type and *dotA*-mutant) for 5 and 14 days and spheroids were analyzed by *icd*-qPCR (*C. burnetii* load) and *C. burnetii* localization (IHC).
Results: Infected cultures were allowed to attach to adherent plates in order to asses AH-1 vitality. AH-1HD (diameter: 200μm) developed within 3 days and AH-1PH (1000μm) within 10 days. AH-1HD contained intact cells, whilst AH-1PH had an inner degenerative core surrounded by intact AH-1 cells. All cells of AH-1HD and the intact AH-1 cells of AH-1PH expressed cytokeratin 18, ezrin, SHMT2 and Ki-67. Active caspase-3 was detected in the core of AH-1PH. Quantification of *C. burnetii* load in spheroids detected significant differences in (adjusted P-value < 0.05). *C. burnetii* was localized by IHC. Following infection, *C. burnetii* spread rapidly through AH-1HD until 14 days post infection (dpi) at which all cells contained Coxiella-containing parasitophoric vacuoles (PV). Upon transfer of supernatants from infected AH-1HD at 14dpi to AH-1PH spheroids, newly infected cells were detected in AH-1PH within 3dpi. When *C. burnetii*-infected day 5 and day 14 spheroids were seeded, both attached and grew to monolayer confluence in 7-10 days. Ovine trophoblast spheroids showed typical *in vivo*-like lesions like intracellular *C. burnetii*-containing parasitophoric vacuoles.
Conclusion: The AH-1 trophoblast 3D-spheroids are promising culture systems to study the interaction of *C. burnetii* with ovine trophoblasts under controlled conditions.

P1.126.
HIGH-FIDELITY, MULTIMODAL DETECTION OF MICROCHIMERIC CELLS IN THEIR SPATIAL CONTEXT

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Objectives: In humans and other placental animals, microchimerism (MC) – the bidirectional exchange during pregnancy of a small number of cells between mother and offspring – appears to be a common phenomenon. Many studies have looked at the presence and relevance of MC, but are confined to select regions of interest or employ high-throughput methods at the expense of spatial context. We are generating a 3D mouse Microchiome Avatar to investigate the spatial distribution of MC within an individuum in the context of the host environment. To verify the microchimeric nature of these rare cells, we are improving the workflow for highly specific detection of target markers on whole mouse sections.
Methods: Wild-type pups harboring maternal MC cells were obtained by crossing tdTomato+/- females with wild-type (-/-) males. Whole newborn pups were shock-frozen using liquid nitrogen and cryosectioned. The sections were screened for tdTomato-fluorescence and subsequently validated experimentally targeting both the RNA and protein expression of the transgene using padlock probe-based *in situ* hybridization and immunofluorescence staining, respectively.
Results: We show that a single assay is prone to yield false positive results, and cross-validation of assays is crucial for rare target analysis. We developed a workflow that allows the assessment of multiple molecular layers including reporter-based fluorescence as well as RNA and protein expression on a single histological section.
Conclusion: A multimodal approach combining the information of transgenic fluorescence, RNA and protein expression allows detection and validation of targets which are generally low in quantity, and significantly reduces the risk of detecting false positive events.

P1.127.
DETECTION OF POTENTIAL MICROCHIMERIC MATERNAL CELLS IN HUMAN MESENCHYMAL STEM CELLS DERIVED FROM FETAL MEMBRANES USING MONOCLONAL HLA CLASS I SPECIFIC ANTIBODIES

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Objectives: Microchimerism (MC) is defined as the presence of a small genetically distinct cell population from another individual. During pregnancy, cells transfer across the feto-maternal interface, resulting in both maternal and fetal MC. MC cells have been shown to exhibit stem and progenitor cell-like characteristics, including pluripotency and migratory capacity, suggesting that cells with these properties may contribute to the establishment and persistence of long-term MC. However, the specific cellular mechanisms and trafficking pathways remain unclear. We propose that maternal cells enter the fetus via fetal membranes, potentially migrating from the decidua to the chorionic membrane and subsequently into fetal circulation and/or the amniotic cavity by crossing the amniotic membrane. We further propose these trafficking cells to be a subset of mesenchymal stem cells (MSCs).
Methods: HLA-specific human monoclonal antibodies (HuMoAbs) of IgG1 isotypes were generated from transformed B lymphocytes in-house at LUMC. We tested and validated HuMoAbs that allow for the distinction between maternal and fetal cells in a sex-unbiased manner targeting HLA-A (*e.g.*: A2) and -B (*e.g.*: B51/B35). Furthermore, we implemented MSC-specific markers to verify their phenotype. To validate the maternal origin of the detected cells, we performed qPCR assays targeting maternal-specific alleles. By assembling and optimizing this panel, pre-enrichment of living maternal stem cells can be achieved.
Results: We developed a flow cytometry panel combining MSC-specific surface markers with HuMoAbs to distinguish maternal from fetal cells. This approach was validated through spiking experiments followed by qPCR using MSCs isolated from the amnion, chorion, and decidua, confirming the panel's ability to accurately identify and discriminate between maternal and fetal cells.
Conclusion: We established a robust method for a sex-unbiased approach to distinguish between maternal and fetal cells. This approach enables pre-enrichment and characterization of live maternal stem cells within fetal membranes, providing a valuable tool for further analysis of maternal cells.

P1.128.
EX VIVO HUMAN PLACENTAL PERFUSION STUDY OF T LYMPHOCYTE MIGRATION ACROSS THE PLACENTAL BARRIER USING NON-PREGNANT DONOR PBMCS

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Objectives: The aim of this study was to examine the migration and homing behavior of T lymphocytes, isolated from healthy non-pregnant female donors, within the human placenta. Unlike previous studies using autologous maternal cells, our approach investigates whether the placental barrier intrinsically recruits immune cells independent of prior pregnancy-specific priming or hormonal influences on the lymphocytes. An optimized *ex vivo* placental perfusion model was employed to reduce confounding factors related to gestational changes.
Methods: PBMCs were collected from healthy non-pregnant donors, fluorescently labeled, and then perfused through the maternal circulation of healthy term placentas using a one-side *ex vivo* perfusion system maintained for 6 hours. This extended perfusion period allowed sufficient time for cell-tissue interactions. CD3 immunofluorescence staining was subsequently performed to identify and precisely localize the perfused T lymphocytes across different placental compartments, including the syncytiotrophoblast, villous stroma, and fetal vasculature.
Results: Fluorescent T cells were consistently detected in all perfused placentas. Double fluorescence staining indicated their origin from the donor. The majority of $CD3^+$ T cells adhered to the syncytiotrophoblast, infiltrated the villous tissue, and, in several instances, entered the fetal vasculature. These observations suggest that the placenta possesses inherent mechanisms for recruiting T cells independent of their origin from an allogeneic donor without pregnancy-specific conditions.
Conclusion: Our findings demonstrate that T lymphocytes from non-pregnant donors can effectively migrate and home into human placental tissue. The novel use of non-autologous PBMCs combined with an advanced *ex vivo* placental perfusion model provides a robust platform for investigating maternal–fetal immune interactions and may yield new insights into the molecular mechanisms underlying microchimerism in pregnancy and placental immune tolerance.

P1.129.
SPATIAL DISTRIBUTION AND FUNCTIONAL IMPLICATIONS OF LYVE1 IN THE UMBILICAL CORD

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Objectives: Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) binds hyaluronic acid. Besides lymphatics, this receptor is expressed in tissue resident macrophages like Hofbauer cells and other cell types. Little is known about the presence and relevance of cells carrying this marker in

the umbilical cord. It has been reported that LYVE1 positive cells are present in the cord and share gene expression profiles with lymphatics. Nevertheless, it was reported that no classical lymphatic system is present in the placenta, and therefore likely not in the umbilical cord. In this work we aimed to study the morphological aspects of LYVE1 positive cells in the umbilical cord of healthy term pregnancies. We hypothesize that LYVE1-positive cells are present in a structured manner in the cord and may be spatially connected to LYVE1-positive structures in neighboring villous regions of the placenta.

Methods: We analyzed the distribution of LYVE1 positive cells in umbilical cord samples and placental tissue at the cord insertion area using in situ padlock hybridization, immunohistochemistry (IHC), immunofluorescence (IF) and light sheet microscopy.

Results: IHC and IF analysis revealed different cell types displaying immunoreactivity for LYVE1 in the cord. LYVE1 expression was confirmed by in situ padlock hybridization. Double positive cells expressing LYVE1 and macrophage marker CD14, as well as LYVE1 and smooth muscle cell marker desmin were identified. In the placental cord insertion area, LYVE1 positive structures resembling stroma channels known from placental immature intermediate villi were observed. The three-dimensional appearance of LYVE1 positive cells in the cord assessed by light sheet microscopy appeared more organized in 3D than visible in 2D.

Conclusion: Our preliminary data shows more than one LYVE1 positive cell population in the umbilical cord. These cells form a kind of loose network, indicating a more complex regulated functional aspect of these cells in the cord at the placental insertion region.

P1.130.
SOCIODEMOGRAPHIC AND CLINICAL PROFILES ASSOCIATED WITH PREECLAMPSIA: COMPARISON BETWEEN ARGENTINEAN AND MEXICAN WOMEN COHORTS

Jessica Barragán-Zúñiga [1], Mariano Reynoso [2], Martha Sosa-Macias [1], Laura Jazel Barragán-Zúñiga [3], Alicia E. Damiano [2,4], Carlos Galaviz-Hernández [1]. [1] *Instituto Politécnico Nacional, Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-Unidad Durango., Durango, Mexico;* [2] *Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Ciudad Autónoma de Buenos Aires, Argentina;* [3] *Centro Estatal de Cancerología de Durango, Durango, Mexico;* [4] *CONICET-Universidad de Buenos Aires, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Ciudad Autónoma de Buenos Aires, Argentina*

Objectives: Compare the sociodemographic and clinical profiles associated with preeclampsia between Argentinean and Mexican women cohorts.

Methods: Retrospective cross-sectional design to study pregnant women. A total of 387 pregnant women were included in the study, classified into two groups: those diagnosed with preeclampsia (PE, n = 84) and those without a diagnosis of preeclampsia (non-PE, n = 303). The comparison of quantitative variables utilized Student's t-test or Mann-Whitney U test while categorical variables received analysis through Chi-square or Fisher's exact test. A Multiple Correspondence Analysis was performed. Statistical analyses were performed in Python 3.11.12 using pandas, SciPy, and matplotlib. Statistical significance was set at $p<0.05$.

Results: The Mexican cohort demonstrated a younger age compared to Argentinean patients within the entire cohort as well as within non-PE groups and PE cases ($p<0.05$). The general comparison revealed Mexican cohort had lower maternal BMI ($p=0.001$) and higher teenage pregnancy rates ($p<0.0001$) and lower newborn birth weight ($p=0.0206$) and higher blood pressure levels ($p<0.05$). The analysis of women without a diagnosis of preeclampsia showed that Mexican cohort maintained lower BMI levels ($p=0.003$) and experienced higher cesarean delivery rates ($p=0.001$) but their birth weight and blood pressure measurements were equivalent. The analysis of PE cases showed Mexican cohort had lower final maternal weight ($p=0.019$). Previous abortions occurred more frequently among Mexican cohort during all analyses ($p<0.05$). Multiple Correspondence Analysis showed teenage pregnancy, primigravida status, Mexican cohort and PE diagnosis clustered together, while older maternal age and severe obesity appeared at the opposite end which indicates different sociodemographic patterns.

Conclusion: The Argentinean and Mexican cohorts showed notable differences in both demographic characteristics and clinical indicators. These findings underscore the need to consider sociodemographic and national variability when assessing preeclampsia risk in diverse populations.

P1.131.
HISTOPATH - NLP-BASED STRUCTURING OF HISTOPATHOLOGICAL PLACENTAL FINDINGS FOR RISK STRATIFICATION IN PREGNANCY PATHOLOGIES

Hanna Elena Gatzemeier [1], Nanae Sai [1], Silke Große [1], Ekkehard Schleußner [1], Friederike Weschenfelder [1], Tanja Groten [2], Anne Rüllich [1], Janine Zöllkau [1], Yvonne Heimann [1]. [1] *Universitätsklinikum Jena, Jena, Germany;* [2] *Universitätsklinikum Köln, Köln, Germany*

Objectives: Histopathological examination of the placenta plays a central role in the clinical and scientific assessment of pregnancy-associated pathologies. However, histopathological free-text reports have so far been limited in comparability due to a lack of standardization. The HISTOPATH project aims to use NLP-supported text analysis to automatically extract clinically relevant key terms (e.g., "signs of inflammation"). The developed model will be validated and aims to investigate systematic differences between these entities and establish standardized evaluation procedures.

Methods: The NLP model is initially trained using pattern recognition on the specific characteristics of Jena's placental pathology reports to extract clinically and scientifically relevant information. The identified patterns are validated through manual training control and iteratively optimized. The generated database of pathological features is linked with clinical pregnancy data. Subgroup analyses of various pregnancy pathologies and their comparison with healthy controls aim to identify systematic differences and derive new pathophysiological insights.

Results: Since 2010, 4,069 placental reports have been systematically archived. The cohort includes 312 twin and 11 triplet pregnancies, as well as pregnancies complicated by preeclampsia (267), diabetes (535), amniotic infection syndrome (300), or obstetric cholestasis (39), supplemented by approximately 1,060 healthy controls. Through secondary data utilization, the NLP tool enables the correlation of specific histopathological features (e.g., chronic inflammatory lesions, maternal-placental perfusion disorders) with clinical pregnancy outcomes. This structured database facilitates systematic analysis of the relationship between placental pathologies and perinatal complications, including FGR and preterm birth.

Conclusion: The HISTOPATH project aims to develop an automated system for histopathological report analysis that unlocks existing free-text data for scientific analytics through NLP-based pattern recognition. The project bridges basic research and clinical application—particularly for issues such as placental insufficiency. This approach provides access to medically relevant diagnostic structures even in the absence of pre-existing standardized databases, creating a foundation for systematic, data-driven investigations of pathophysiological relationships.

P1.132.
INFLUENCE OF CDCL2 ON LONG-TERM CULTURES OF HUMAN PLACENTAL EXPLANTS

Enora Flache. *Placenta-Labor UKJ, Jena, Germany*

Objectives: The placenta plays a vital role in mediating exchange between mother and foetus and in protecting the developing foetus from harmful substances. Due to species-specific differences in placental structure, toxicological studies using animal models are not reliably transferable to humans. Human placentas, which are readily available post-pregnancy, offer a practical alternative. Cultivating placental explants is a simple and effective method for studying toxicological effects in vitro.

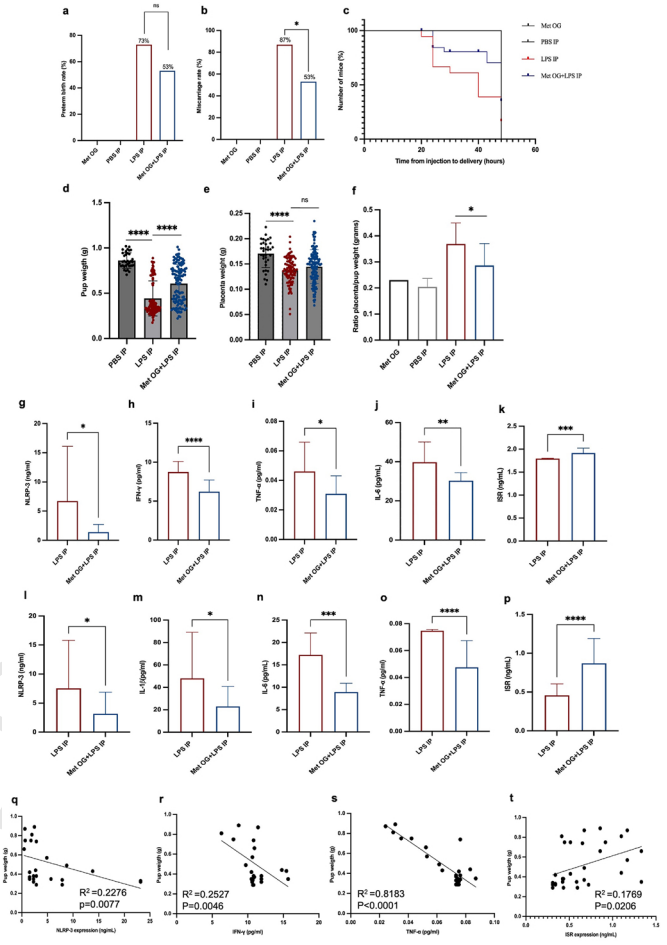
This study investigated the impact of cadmium chloride ($CdCl_2$) on human placental explants in long-term culture. The goal was to evaluate both the usefulness and limitations of the explant model for toxicological analysis.

Methods: Supernatants of the cultured explants were analysed for steroid hormones (estradiol and progesterone) and cytokines (IL-6, IL-8). In the tissue, immunohistochemical staining (for aromatase, β-HCG, ki-67) and in situ hybridisation (for miRNA-519d-3p) were performed. Mass spectrometry-based proteome analysis was also conducted.
Results: Results showed that the syncytiotrophoblast partially regenerated, as evidenced by hormone measurements and positive immunostaining, even after 14 days. Despite this, significant morphological alterations were observed, such as fusion and partial detachment of the outer villus layer. $CdCl_2$ exposure led to dose-dependent reductions in hormone production and increased IL-6 levels, aligning with previously known toxic effects. Proteomic analysis confirmed the upregulation of repair proteins like tenascin C, though fewer extracellular matrix proteins were upregulated in $CdCl_2$-treated samples.
Conclusion: In conclusion, placental explant are suitable for toxicological studies. However, the regenerative nature of the cultured tissue and its altered morphology compared to fresh tissue must be considered when interpreting results. Long-term cultures allow for additional parameters to be assessed, offering more comprehensive toxicological insights than short-term (e.g., 24-hour) models.

P1.133.
ORAL ADMINISTRATION OF METFORMIN PREVENTS MISCARRIAGE BY REDUCING AMNIOCHORIONIC AND PLACENTAL INFLAMMATION IN A MOUSE MODEL OF LIPOPOLYSACCHARIDE-INDUCED PRETERM BIRTH.

Marianna Onori [1], Giuliana Beneduce [1], Fabio Sannino [1], Nicoletta Di Simone [2,3], Tullio Ghi [1,4], Chiara Tersigni [4]. [1] *Catholic University of Sacred Heart of Rome, Rome, Italy;* [2] *Department of Biomedical Sciences, Humanitas University, Milan, Italy;* [3] *IRCCS Humanitas Research Hospital, Milan, Italy;* [4] *Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*

Objectives: Intra-amniotic infection/inflammation is a trigger mechanism leading to preterm birth (PTB). Abnormal glucose tolerance may contribute to its etiopathogenesis by amplifying tissue inflammation and increasing susceptibility to infections. Metformin is largely used in glucose metabolism disorders of pregnancy for its insulin-sensitizing effect, but its anti-inflammatory properties have been poorly investigated. The aims of this study were to evaluate the effects of metformin in a mouse model of PTB in terms of: a) amniochorionic membranes, placental and serum inflammation; b) prevention of miscarriage and PTB.
Methods: Swiss CD1 pregnant mice were treated with intra-peritoneally injection of lipopolysaccharide (LPS) (14.5 days post coitum-dpc) with (group 1; n=15) or without (group 2: n=15) 200mg/Kg of metformin administered by oral gavages (8.5/10.5/12.5/14.5 dpc). Control groups were treated with PBS (n=15) or metformin (n=15) only. The expression of the inflammasome NLRP-3, IL-1β, IL-6, IFN-γ, TNF-α and insulin receptor (ISR) were quantified by ELISA and Western blot in amniochorionic membranes, placentas and maternal sera. Data were analyzed by Student's t-test, Mann-Whitney U test or Pearson correlation.
Results: Reduced miscarriage rate and higher pups' weight were observed in group 1 compared to group 2 ($p<0.05$ and $p<0.0001$, respectively). Decreased placental expression of NLRP-3 ($p<0.05$), IFN-γ ($p<0.0001$) and TNF-α ($p<0.05$) and reduced expression of NLRP-3 ($p<0.05$), IL-1β ($p<0.05$), IL-6 ($p<0.001$), and TNF-α ($p<0.0001$) in amniochorionic membranes were observed in group 1 compared to group 2. Metformin significantly increased ISR levels in amniotic membranes and sera ($p<0.0001$ and $p<0.001$, respectively) and decreased serum IL-6 levels ($p<0.01$).



(a-b) Histograms showing preterm birth and miscarriage rate among the 4 experimental groups. **(c)** Kaplan-Meier curve showing the time from LPS+/-metformin treatment to delivery. **(d-f)** Graphs showing pups' **(d)** and placental weight **(e)** and placenta/pup's weight ratio **(f)**. **(g-k)** Placental levels of NLRP-3, IFN-γ and TNF-α and **(j-k)** serum levels of IL-6 and ISR in mice treated with metformin+LPS compared to mice treated with LPS only. **(l-p)** Levels of NLRP-3, IL-1β, IL-6, TNF-α and ISR in amniochorionic membranes of mice treated with metformin+LPS compared to mice treated with LPS only. **(q-t)** Scatter plots showing the correlation analysis between pups' weight and levels in amniochorionic membranes of NLRP-3, IFN-γ, TNF-α and ISR.
LPS: lipopolysaccharides; Met: metformin; OG: oral gavage; PBS: phosphate-buffered saline; IP: intra-peritoneum; NLRP-3: NLR family pyrin domain containing 3; IL-1β: interleukin 1β; IL-6: interleukin 6; IFN-γ: interferon gamma; TNF-α: tumor necrosis factor alfa; ISR: insulin receptor; *p<0.0.5; **p<0.01; *** p<0.001;****p<0.0001; ns=not significant. Data are showed as mean ± SD or percentage (%). N= 15 per group.

Conclusion: Oral administration of metformin in a mouse model of PTB is able to: a) decrease systemic, amniochorionic and placental inflammation; b) reduce miscarriage rate and increase pups' weight. This study highlights the anti-inflammatory properties of metformin and its potential clinical application in the prevention of miscarriage and PTB.

P2.134.
PLACENTA BANKING: A NOVEL APPROACH TO MESENCHYMAL STROMAL CELL PRESERVATION FOR REGENERATIVE MEDICINE

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Objectives: Developing and validating placenta banking as a regenerative medicine technique by cryopreserving placental tissue to preserve mesenchymal stromal cells (MSCs) for future therapeutic applications, leveraging the placenta's rich MSC content and building on established umbilical cord blood and tissue banking practices.
Methods: FamiCord's proprietary cryopreservation method was used to store specific stem cell-rich placental regions. MSCs were isolated from preserved tissue and cultured in explant systems. Cell viability, proliferation, surface marker expression (CD73, CD90, CD105, and absence of CD34, CD45), and genetic stability during expansion were assessed.
Results: Cryopreserved placental tissue yielded viable MSCs in explant cultures, expressing characteristic MSC markers (CD73, CD90, CD105) and

lacking hematopoietic markers (CD34, CD45). No clonal genetic abnormalities were observed during expansion, confirming pre-clinical safety.
Conclusion: Placenta banking offers a forward-thinking approach to stem cell preservation, providing a valuable resource for future medical needs. Early clinical trial data indicated therapeutic efficacy in neurological disorders, spinal cord injuries, cardiovascular regeneration, and orthopedic repair, highlighting the versatility of placenta-derived MSCs. FamiCord's method ensures high-quality MSC cryopreservation and isolation, with demonstrated safety and therapeutic potential. As research advances, placenta-derived MSCs hold significant promise for transforming regenerative medicine, enhancing personalized healthcare options.

P2.1.
ACTIVATION OF THE RAGE PATHWAY FOLLOWING PHOSPHORYLATION BY CK2 IN AN AMNIOTIC EPITHELIAL MODEL.

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Objectives: Throughout pregnancy, the fetal membranes protect the fetus. Their rupture at the end of the pregnancy is a physiological event leading to their fragilization at the cervix. It implicates various sterile inflammation processes whose pathway is partly under the dependence of the transmembrane receptor for advanced glycation endproducts (RAGE). Its activation mechanism would be based on its phosphorylation by an endogenous kinase. Recently published results by our team show that the CK2 protein kinase expression, also involved in inflammatory processes, is higher in amniotic cells compared to choriodecidua cells. We decide to explore the activation of RAGE signaling by CK2 using *in vitro* approaches in the amniotic epithelial cell line FL.
Methods: FL cells were transfected with RAGE-GFP and treated with its AGEs ligand for 24h. Mass spectrometry was used to identify phosphorylated Serine or Threonine throughout RAGE. *In vitro* RAGE phosphorylation by CK2 and the RAGE/CK2 interaction by Microscale Thermophoresis were performed. Site-directed mutagenesis was used to generate RAGE-GFP in which Serine was mutated to Alanine. Consequences of these mutations on RAGE signaling were studied (1) by human phospho-kinase array and (2) by their influence on the expression of mRNA (RT-qPCR) Connexin-43 RAGE target gene.
Results: CK2 interacts with RAGE and phosphorylates its Serine 400 in the RAGE intracellular domain. Ser400Ala mutation does not affect the CK2 affinity for RAGE but causes dysregulation of intracellular kinase signaling pathways. Furthermore, the disrupting of its phosphorylation cascade conduct to mRNA overexpression of Connexin-43.
Conclusion: Our work suggests that the Serine 400 phosphorylation has an important function in the RAGE signaling in response to AGEs ligands. It paves the way for future experiments regarding this regulation in primary amniocytes. Moreover, over the longer term, it may participate in the maintains integrity of fetal membranes and be involved in the pathophysiology of premature ruptures.

P2.2.
PREGCO: A DANISH BIRTH COHORT FOR MULTI-OMICS INVESTIGATION OF MATERNAL-PLACENTAL-FETAL SIGNALING

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Objectives: The placenta, an endocrine organ that acts as an interface between the mother and fetus, undergoes major genetic and structural changes throughout pregnancy, affecting maternal and fetal physiology. Our knowledge of the complex interplay between the placenta and maternal circulation is greatly lacking. To address this knowledge gap, we are conducting an extensive study of maternal-placental-fetal signaling. We aim to characterize placental contributions to changes in maternal circulation and to identify new targets for intervention.
Methods: PREGCO is a prospective cohort enrolled at Copenhagen University Hospital Hvidovre from April to July 2020. Participants were recruited either at the 2nd trimester malformation scan (mothers only) or birth (mother, partner, and neonate) from whom we collected blood samples. Moreover, we have access to serum samples collected as part of the 1st trimester risk assessment. For a subset of participants, multi-site placental biopsies were collected for molecular and histological analysis. All participants have been genotyped, and serum samples are currently being analyzed using the Olink Explore HT panel. Transcriptomics analysis of placental samples is ongoing.
Results: We have collected blood samples from 2531 pregnant women, 1247 partners, and 1258 neonates and placenta samples from 558 births. Of these, 1189 women had one or more pregnancy-related conditions. Using electronic health records, we have extracted extensive information on pregnancy and delivery, such as anthropometrics, biometrics, medication use, and longitudinal biochemical measurements. Histological examination has been performed by trained pathologists, and cell composition is being extracted using machine learning algorithms.
Conclusion: We have established a large, comprehensive multi-omics study in a prospective cohort of pregnant and birthing women, their partners, and their offspring. The data generated in the study enables an in-depth investigation of maternal-placental-fetal signaling.

P2.3.
OMICS INTEGRATION OF PLACENTAL CHORIONIC VILLI IN PREGNANT WOMEN WITH BREAST CANCER UNDERGOING CHEMOTHERAPY

Rafaella Scandiuzzi [1], Leisa Lopes-Aguiar [1], Amit Thakur [2], Fernanda Marqueto [1], Gabriela Mesquita [1], Guilherme Nobrega [3], Fernanda Surita [1], Maria Laura Nascimento [1], Maria Cristina Gomes-Marcondes [1], Olivier Pardo [2], Lais Viana [1]. [1] *University of Campinas, Campinas, Brazil;* [2] *Imperial College London, London, United Kingdom;* [3] *Icahn School of Medicine at Mount Sinai, New York, USA*

Objectives: Our aim was to elucidate the impacts of breast cancer and chemotherapy on the placenta through the integration of transcriptomic, proteomic, and metabolomic data from the chorionic villi of women diagnosed with breast cancer during pregnancy (PrBC) who underwent chemotherapy.
Methods: Samples from PrBC patients who received chemotherapy (case group, n=7) and from healthy participants during pregnancy (control group, n=8) were obtained through the Center for Integral Attention to Women's Health biobank of University of Campinas (CAAE: 65070122.0.0000.5404). Transcriptomic was assessed by RNA-Seq with DESeq2 used to identify Differentially Expressed Genes (DEGs). Proteomic was assessed with a 2D nano-UPLC-coupled Synapt G2-Si mass spectrometer, and Differentially Expressed Protein (DEPs) identified with Progenesis software. Metabolomic was analyzed via NMR and quantified with Chenomx software, and Differentially Expressed Metabolites

identified using MetaboAnalyst. Pathway enrichment was performed using Reactome FIViz (Cytoscape) and MetaboAnalyst. *P*-value < 0.05 (Bonferroni and/or FDR-adjustment), with a log fold-change cutoff of 0.58, was considered significant.

Results: A total of 811 DEGs were identified (321 downregulated and 490 upregulated), associated with 120 enriched pathways. Proteomic analysis revealed 54 DEPs (22 downregulated and 32 upregulated), linked to 58 functional pathways. Although no overlap was found between the transcriptomic and proteomic datasets, 15 shared pathways were identified, many related to vascularisation and immune processes (Figure 1A). Metabolomic analysis revealed 35 upregulated metabolites involved in 12 pathways. Multilayered data integration enabled the mapping of functional interactions using KEGG and HMDB resources, resulting in a network of 58 genes, 2 proteins, and 20 metabolites (Figure 1B), associated with 13 pathways, such as protein digestion and absorption, central carbon metabolism and aminoacyl-tRNA biosynthesis (Figure 1C).

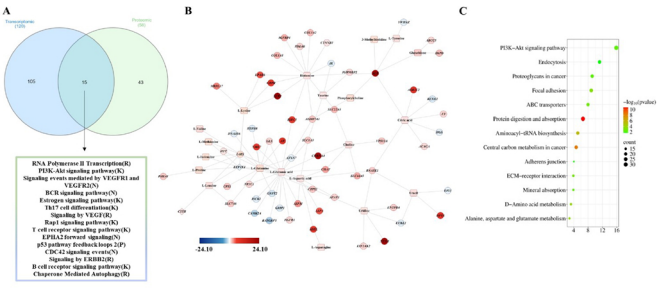


Figure 1. (A) Venn diagram showing the number of pathways present in the transcriptomic (in blue) and proteomic (in green) analyses, highlighting the 15 shared pathways. **(B)** Interaction network between genes (circles), proteins (diamonds), and metabolites (squares), constructed using MetaboAnalyst and Cytoscape. The color of the nodes represents the logFC (Fold Change) value, where blue indicates downregulated genes/proteins/metabolites and red indicates upregulated ones. **(C)** Bubble plot depicting 13 significantly enriched pathways identified through the integration of metabolites, genes, and protein.

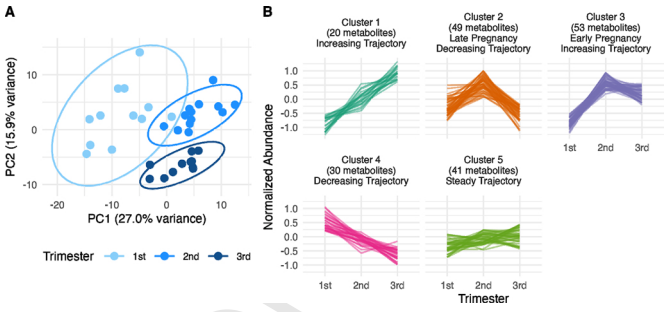
Conclusion: We revealed altered molecular pathways in the placenta of PrBC women who received chemotherapy that may enable future therapeutic intervention to mitigate the impact of breast cancer and chemotherapy on pregnancy.

P2.4.
DYNAMIC CHANGES IN PLACENTAL METABOLISM ACROSS PREGNANCY CHARACTERIZED BY DISTINCT METABOLITE TRAJECTORIES

Mariana Parenti [1], Sam Rosen [2], Daniel Raftery [3], Ian A. Glass [4], Birth Defects Research Laboratory (BDRL) [4], Alison G. Paquette [1,4], Stephen A. McCartney [2]. [1] *Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, USA;* [2] *Department of Obstetrics and Gynecology, University of Washington, Seattle, USA;* [3] *Department of Anesthesiology and Pain Medicine, University of Washington, Seattle, USA;* [4] *Department of Pediatrics, University of Washington, Seattle, USA*

Objectives: Metabolic demands of the developing conceptus are highly dynamic during pregnancy, however there is currently limited data defining how the placenta responds to changing requirements. We investigated the placental metabolome and metabolite trajectories across gestation in human pregnancy.

Methods: Targeted metabolomic profiling of 372 metabolites was conducted in placental samples collected in the first (n=12), second (n=13), and third (n=11) trimesters of normal pregnancy. Robust linear models identified differentially abundant metabolites (DAMs) across trimesters in models adjusted for sex and total protein concentrations. Metabolite trajectory analysis was conducted using hierarchical clustering. We conducted pathway over-representation analysis (ORA) using a human metabolic reconstruction to aid in biological interpretation.



Results: Samples clustered by trimester in principal component analysis (Figure A) and we identified 5 metabolite trajectories (Figure B). Out of 193 detectable metabolites, 144 (77%) differed by trimester (FDR<0.05). 95 DAMs had increased and 14 had decreased abundance in the second compared to first trimester. 70 DAMs had increased and 30 had decreased abundance in the third compared to first trimester. 17 DAMs had increased and 55 had decreased abundance in the third compared to second trimester. The 55 DAMs with decreased abundance in the third compared to the second trimester were enriched for the *aminoacyl-tRNA biosynthesis* (13/20 metabolites, FDR=0.009), *exchange/demand reaction* (13/21 metabolites, FDR=0.012), and *protein degradation* (13/20 metabolites, FDR=0.009) pathways.

Conclusion: Placental metabolite abundances change substantially across gestation and metabolites cluster into 5 distinct trajectories, providing insight into changing metabolic function during pregnancy. Notably, amino acids and their derivatives show a pattern of high abundance during the second trimester, which may be related to patterns of both fetal and placental growth. This metabolomic profiling can inform other molecular analyses of the placenta by providing enhanced resolution of changes across pregnancy.

P2.5.
METABOLOMIC ANALYSIS OF PLACENTAL CHORIONIC VILLI IN PREGNANT BREAST CANCER PATIENTS UNDERGOING CHEMOTHERAPY

Fernanda Marqueto [1], Gabriela Mesquita [1], Rafaella Scandiuzzi [1], Carla Salgado [1], Beatriz Talassi [1], Leisa Lopes-Aguiar [1], Maurício Sforça [2], Silvana Rocco [2], Gustavo Rodrigues da Silva [2], Guilherme Nobrega [3], Fernanda Surita [3], Maria Laura Costa [3], Maria Cristina Gomes-Marcondes [1], Lais Viana [1]. [1] *Institute of Biology of the State University of Campinas (Unicamp), Campinas, Brazil;* [2] *CNPEM - National Center for Research in Energy and Materials/ LNBio - National Laboratory of Biosciences, Campinas, Brazil;* [3] *CAISM - Prof. Dr. José Aristodemo Pinotti Women's Hospital (UNICAMP), Campinas, Brazil*

Objectives: Investigate the metabolomic profile of placental chorionic villi (CV) from patients who had breast cancer during pregnancy (PrBC) and underwent chemotherapy treatment.

Methods: Placentas were collected from patients who had breast cancer during pregnancy and underwent chemotherapy treatment (case group, N=6) and from healthy pregnant participants (control group, N=7). This study was approved by the ethical committee (CAAE: 65070122.0.0000.5404). Metabolites were extracted from the CV and analyzed using Nuclear Magnetic Resonance (NMR). The metabolites were identified and quantified using Chenomx software (Figure 1). Subsequently, multivariate statistical analyses were performed using MetaboAnalyst software. Adjusted P-value FDR < 0.05 were considered significant.

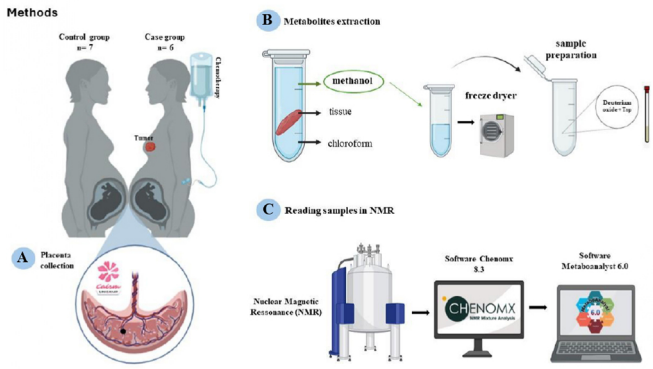


Figure 1. Overview of the project methods, consisting of three main steps: **A)** Placenta collection; **B)** Metabolite extraction; **C)** Placental sample analysis by Nuclear Magnetic Resonance (NMR). The first step consisted of collecting 6 from patients with breast cancer treated with chemotherapy (Case group) and 7 from patients with low-risk pregnancies (Control group) placentas from patients at the Women's Health Care Center of UNICAMP (CONEP registration B-056) (CAAE: 65070122.0.0000.5404). The region selected for this study was the chorionic villi, which plays the main role in gas and nutrient exchange between the mother and fetus, with great potential for promising results in metabolomic analyses. The second step involved the extraction of placental metabolites from the chorionic villi at the Brazilian Synchrotron Light Laboratory/National Center for Research in Energy and Materials, using homogenization with methanol and chloroform. polar metabolites were collected and lyophilized. After lyophilization, samples were prepared by adding 540 µL of deuterium oxide and 60 µL of triphosphate buffer, and the full content was transferred to a specific NMR tube. The third step consisted of reading the samples using the NMR equipment, identifying all metabolites present in the samples. These metabolites were identified and quantified using the Chenomx 8.3 software, and multivariate statistical analyses were performed using MetaboAnalyst6.0.

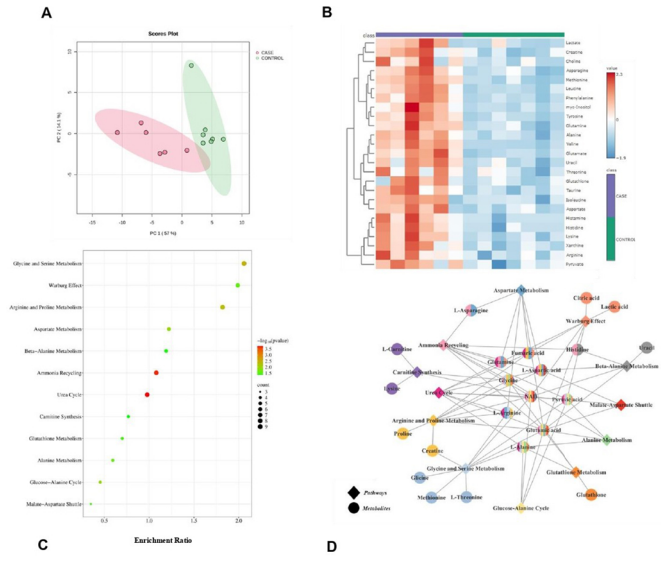


Figure 2. A) Principal component analysis (PCA) showing significant difference between the metabolomic profile of the case and control groups. **B)** Heatmap and variation of metabolites in samples from the case and control groups. **C)** Analysis of the 12 impacted metabolic pathways. **D)** Interaction network of pathways (diamonds) and metabolites (circles) constructed with MetaboAnalyst and Cytoscape. The color of the nodes represents the different pathways, where the common color of the metabolites reflects specificity in the pathway. Metabolites with a color gradient are related to the different pathways that are involved.

Results: 53 metabolites were identified, of which 35 showed increased concentration in the case group compared to the control group. Principal Component Analysis (PCA) showed a clear separation in the metabolomic profile (PC1: 57% and PC2: 14.1%) (Figure 2A), where they showed increased concentration in the case group compared to the control (Figure 2B). Enrichment analysis revealed 12 significantly altered metabolic pathways (Figure 2C), of which the metabolites participating in common pathways are L-asparagine, Histidine, L-arginine, Glutamine, Fumaric acid, L-alanine, Glycine, Pyruvic acid, L-aspartic acid, NAD, and Glutamic acid, on the other hand, the metabolites participating in specific pathways are Citric acid and Lactic acid (Warburg effect), Uracil (beta-alanine metabolism), L-carnitine and Lysine (carnitine synthesis), Proline and Creatine (arginine and proline metabolism), Glycine, Methionine, and L-threonine (glycine and serine), and Glutathione (glutathione metabolism) (Figure 2C).

Conclusion: The identified pathways reveal significant metabolic adaptation of PrBC in context. These changes may reflect protective remodeling to preserve function. Processes such as cell proliferation, amino acid metabolism, antioxidant defenses, and cellular signaling highlight the adaptive response of the placenta to maternal pathology and gestational demands. This insight strengthens the understanding of placental resilience in maternal and fetal health under challenging conditions.

P2.6.
MODELLING THE FUNCTION OF GLYCOSYLATION GENES EXPRESSED IN EARLY PLACENTAL DEVELOPMENT USING HUMAN SINGLE-CELL RNASEQ DATA AND EVIDENCE FOR EVOLUTIONARY IMPACT IN PLACENTAL MAMMALS AND HOMINIDS.

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Objectives: Glycosylation is established as an important post-translational modification at the feto-maternal interface. Focusing on trophoblast, this study models functional activity associated with glycogene expression by applying hypergraph analysis to measure coordination of gene expression, then refining the model using evolutionary impact in relation to mammalian phylogeny and recent hominid evolution.
Methods: Genes involved in glycosylation pathways (GG, n=135) were extracted from GlyCosmos and screened across early placental human single-cell RNA-seq datasets. A hypergraph-based prioritisation of GG assessed their coordination within the transcriptome (high activity >95th percentile of the number of related genes with correlated expression). Genetic constraint was used as a measure of purifying selection in the current human population (LOEUF score) and in mammals (GERP score). Genomic introgression from *Homo neanderthalensis* was assessed using

ArcSeqHub. Phylogenetic analysis was conducted in MEGA11.and MAFFT version 7.

Results: *MGAT5*, *CHSY1*, *CHST6* and *SIGLEC10* had evidence of high transcriptomic coordination in both early extravillous trophoblast [EVT] and syncytiotrophoblast [STB] ($p<1x10^{-7}$, odds ratio >9.0). *MGAT5* and *CHSY1* were both >97th percentile for gene expression coordination, consistent with functional importance in EVT/STB. *MGAT5* encodes an enzyme for the biosynthesis of N-glycans and regulates trophoblast invasion. *CHSY1* (chondroitin sulphate (CS) synthase 1) is expressed in villous syncytiotrophoblast, and CS acts as a receptor for import of malaria-infected erythrocytes. Both *MGAT5* and *CHSY1* are highly constrained at the level of the current human population (LOEUF <0.5) and over mammalian evolution (GERP >4.5) – showing impact of purifying selection. Introgression from *H. neanderthalensis* was demonstrated [*MGAT5* in 21% & *CHSY1* in 6% of the Asian population] demonstrating impact on recent hominid evolution. Phylogeny clusters these genes by type of embryo implantation (invasive versus superficial) rather than placental anatomy.

Conclusion: These data imply central functional roles for *MGAT5* and *CHSY1* in human placentation and highlight the impact of GG in recent hominid evolution.

P2.7.
IS PLACENTAL WEIGHT ASSOCIATED WITH ADVERSE PREGNANCY OUTCOMES? A SYSTEMATIC REVIEW AND META-ANALYSIS

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Objectives: Our aim was to systematically review studies with data that could be used to estimate the associations between placental weight (PW) or birthweight to PW ratio (BW:PW) with one or more adverse pregnancy outcomes (APOs) such as preeclampsia (PE), fetal growth restriction (FGR), and small for gestational age (SGA).

Methods: PUBMED, Cochrane Library, and EMBASE were searched for relevant studies and a random effects meta-analysis was conducted. Results are presented as the mean difference [95%CI] in PW or BW:PW between pregnancies with and without each APO.

Results: The pooled mean PW difference between pregnancies with and without PE was -111.60g ([-127.48g,-95.72g]; N studies=123;I^2=98.40%). The pooled mean BW:PW difference by PE was 0.33 ([-0.30,0.96]; N studies=7;I^2=96.35%;SD=0.85).

The pooled mean PW difference between pregnancies with and without FGR was -156.79g ([-175.99g,-137.52g]; N studies=64;I^2=96.11%). The pooled mean BW:PW difference by FGR was -0.60 ([-1.05,-0.15]; N studies=9;I^2=93.82%;SD=0.69).

The pooled mean PW difference between pregnancies with and without SGA was -141.20g ([-155.69g,-126.72g]; N studies=38;I^2=96.25%). The pooled mean BW:PW difference by SGA was -0.14 ([-0.45,0.18]; N studies=4;I^2=78.91%;SD=0.32).

Conclusion: All three APOs are associated with lower PW. Lower PW is also associated with proportionally lower BW in PE and SGA cases, resulting in no evidence of a difference in BW:PW; but in FGR cases BW is reduced more than PW, resulting in lower BW:PW. However, these findings should be treated with caution until we have explored the marked heterogeneity and controlled for gestational age.

P2.8.
ASSOCIATIONS BETWEEN BLOOD PRESSURE CHANGE ACROSS PREGNANCY AND PLACENTAL WEIGHT: THE AVON LONGITUDINAL STUDY OF PARENTS AND CHILDREN

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Objectives: To investigate associations of early pregnancy blood pressure and changes in blood pressure throughout gestation with placenta weight measured at delivery.

Methods: The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective birth cohort that recruited pregnant women resident in the Bristol area, with estimated delivery dates between April 1991 and December 1992. Here we used repeat antenatal blood pressure measurements for ~5500 women (median [interquartile range], 10 [9–11] measurements per woman) who had their placenta measured at birth. Bivariate linear spline models were used to relate blood pressure changes across pregnancy to placental weight and placental weight (as the outcome). We incrementally adjusted for maternal age, ethnicity, parity, pre-pregnancy BMI, smoking, education and offspring sex.

Results: There was no strong evidence of an association between systolic blood pressure (SBP) at 8 weeks or change between 8-18 weeks and placental weight after adjustment for confounders. A greater increase in SBP between 18-36 weeks of gestation was associated with lower placental weight in confounder-adjusted models. For example, each 1 mm Hg/week greater rise in SBP between 18 and 30 weeks was associated with a −16 g (95% CI: −39 to +6) lower placental weight, and between 30 and 36 weeks with a −15 g (95% CI: −26 to −6) lower placental weight. There was no strong evidence of an association between SBP change from 36 weeks onwards and placental weight (MD: 4 g; 95% CI: −5 to 12). Similar findings were observed for diastolic blood pressure and when analyses were restricted to normotensive pregnancies.

Conclusion: Greater increases in blood pressure, from ~18-weeks gestation, are related to reduced placental weight between 30 and 36 weeks, even in women whose blood pressure does not cross the threshold for hypertensive disorders of pregnancy.

P2.9.
N-ACETYL-ASPARTATE-MEDIATED INHIBITION OF TROPHOBLAST ACTIVITY VIA TARGETING PEROXIREDOXIN 1 IN POLYCYSTIC OVARY SYNDROME PREGNANCY

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Objectives: Polycystic ovary syndrome (PCOS) is a common endocrine-metabolic disorder in women of reproductive age, associated with increased risks of miscarriage, preeclampsia, gestational diabetes, and preterm birth during pregnancy. Despite improvements in assisted reproductive technologies, the molecular mechanisms underlying these adverse pregnancy outcomes remain unclear. Recently, elevated levels of the metabolite N-acetyl-aspartate (NAA) were identified in the placentas of PCOS patients. This study investigates how NAA contributes to adverse pregnancy outcomes in PCOS.

Methods: NAA levels in placental tissues and peripheral blood from PCOS patients and controls were quantified using metabolomic analysis and ELISA. In vitro assays, including colony formation, flow cytometry, and transmission electron microscopy, assessed NAA's effects on trophoblast cell viability, apoptosis, cell cycle progression, and autophagy. Integrated metabolomic and transcriptomic analyses identified affected signaling pathways. Mitochondrial function was evaluated by measuring ROS, mitochondrial membrane potential, and ATP synthesis. Molecular docking and other assays confirmed the interaction between NAA and Peroxiredoxin 1 (PRDX1). A placenta-targeted nanoparticle system was used for in vivo testing in mice.

Results: NAA levels were elevated in both placental tissues and serum of PCOS patients. In vitro, NAA inhibited trophoblast cell activity by inducing apoptosis, G2/M phase cell cycle arrest, and excessive autophagy. Metabolomic analysis revealed enriched pathways related to redox homeostasis and energy metabolism following NAA treatment. Transcriptomic analysis showed inhibition of the PI3K/AKT pathway. NAA exposure impaired mitochondrial function, evidenced by increased ROS production, reduced mitochondrial membrane potential, and decreased ATP synthesis. Interaction studies confirmed PRDX1 as the direct target of NAA. In vivo, placenta-targeted NAA delivery led to adverse pregnancy

outcomes, while PRDX1 overexpression alleviated placental abnormalities and improved pregnancy outcomes.
Conclusion: NAA inhibits PRDX1's antioxidant activity, causing ROS accumulation and mitochondrial dysfunction, which suppress trophoblast cell activity. Targeting the NAA-PRDX1 axis may provide new strategies to mitigate PCOS-related pregnancy complications.

P2.10.
OBESITY AND GDM DIFFERENTIALLY IMPACT PLACENTAL FATTY ACID β-OXIDATION IN AGA VS LGA FETUSES

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Objectives: Placental utilization of fatty acids (FA) via β-oxidation for energy generation potentially affects FA availability for fetal growth and development. Maternal obesity and GDM, both linked to hyperlipidemia, show increased rates of large for gestational age (LGA) births. We reported sex-specific placental β-oxidation differences in obesity and GDM with appropriate for gestational age (AGA) fetuses and now examine adaptations with LGA.
Methods: Term villous tissue was collected post C-section (no labor) from lean (LN, BMI 18.5-24.9 kg/m^2), obese (OB, BMI $\geq$30 kg/m^2) and OB + type A2GDM pregnancies delivering AGA (BW 3000-3500gm) or LGA, (BW$\geq$4000gm) babies (n=3-5/group/ sex). Expression of mitochondrial β-oxidation enzymes: acyl-CoA dehydrogenase for medium-chain (C6-12, ACADM), very-long-chain (C$\geq$22, ACADVL) and trifunctional enzyme subunit α (HADHA) was evaluated. Significance differences = $p<0.05$.
Results: BW was higher in all LGA groups vs AGA, with placental weight elevated only in LGA-LN, LGA-OB. Only LGA-GDM males had higher BW than females. In AGA, levels of ACADM and ACADVL –catalyze 1st step of β-oxidation were similar across groups. In LGA, OB placentas had higher ACADM and ACADVL vs LN, GDM, and AGA-OB. Sex-stratified analysis revealed no differences in AGA-LN vs LGA-LN whereas LGA-OB females had significantly higher levels vs LGA-OB males and AGA-OB males and female. In contrast, LGA-GDM females had lower expression of ACADM and ACADVL (p=0.07) vs AGA-GDM females. Levels of HADHA (generates acetyl-CoA for ATP production) were comparable in AGA groups but increased in LGA-OB vs LGA-LN. In sex-stratified analysis LGA-LN, LGA-GDM females had significantly lower HADHA vs AGA counterparts, but LGA-OB females had higher levels vs AGA-OB.
Conclusion: Maternal obesity may increase female placental capacity to metabolize FA via increased conversion of medium and very-long-chain FAs to acetyl-CoA, contributing to energy production. GDM might impact alternate metabolic pathways implying distinct, sex- and weight-dependent effects on placental metabolism.

P2.11.
TROPHOBLAST DYSFUNCTION IN GESTATIONAL DIABETES MELLITUS (GDM): A TRIAD OF MITOCHONDRIAL DYSFUNCTION, DISRUPTION OF AUTOPHAGY AND INCREASED SENESCENCE

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Objectives: We have reported altered mitochondrial function and senescence pathways in differentiating trophoblast from GDM placentas. Elevated senescence is associated with both mitochondrial dysfunction and altered autophagy. Here we determine how placental mitochondrial dysfunction, dysregulated senescence and autophagy are interlinked in GDM.
Methods: Primary human cytotrophoblast (CT) were isolated at term, following C-section from normoglycemic (NW) and obese + type A2GDM (GDM) women (n=6/group) and differentiated into syncytiotrophoblast (ST). We measured mitochondrial respiration at 24 (CT) and 72hrs (ST). Cell lysates were collected for western blotting analysis of proteins involved in mitochondrial homeostasis (LONP1), early autophagy (Beclin-1), late autophagy (P62, LC3-II) and senescence (p53, p16, Lamin A/C,). Significant differences were set at $p<0.05$.
Results: Basal and ATP production-linked respiration increased significantly as NW-CT differentiated to ST. This increase with differentiation was absent in GDM trophoblast. GDM-CT and ST had reduced basal respiration and ATP production-linked respiration (vs NW-CT, ST) emphasizing lower mitochondrial capacity in GDM-CT and an inability to increase respiration with differentiation. Levels of LONP1 involved in mitochondrial quality control were significantly lower in GDM-CT (vs NW-CT). Decreased LONP1 can lead to mitochondrial dysfunction and is associated with elevated senescence and dysregulation of autophagy. Consequently, we observed an increased accumulation of Beclin-1 (p=0.06) and p62 in GDM-CT (vs NW-CT). GDM-CT (vs NW-CT) and GDM-ST (vs NW-ST and GDM-CT) also had higher LC3-II accumulation. Upregulation of Beclin-1 suggests an induction of autophagy but an increase in p62 and LC3-II indicates overall dysregulation in autophagy. LONP1 mediated mitochondrial dysfunction can trigger senescence and we observed increased levels of activated p53, p16 and Lamin A/C – senescence markers in GDM-CT and ST vs corresponding NW cells.
Conclusion: LONP1 mediated mitochondrial dysfunction coupled with increased senescence and impaired autophagy suggests that GDM cytotrophoblast exhibit multifaceted cellular damage, ultimately resulting in dysfunctional ST following differentiation

P2.12.
MICRORNA TRENDS AFTER GDM DIAGNOSIS: A LONGITUDINAL STUDY OF GESTATIONAL DIABETES PROGRESSION

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Objectives: Gestational diabetes mellitus (GDM) is a common pregnancy complication, affecting 10–15% of all pregnancies, and is associated with long-term health consequences for GDM women. There are currently no early predictive biomarkers for GDM. A key challenge is that many candidate biomarkers fluctuate throughout pregnancy due to maternal physiological adaptations or gestational changes, making it difficult to distinguish disease-specific signals. A previous study with a single time point identified several miRNAs in early pregnancy that were associated with insulin sensitivity between 24 and 29 weeks of gestation. The imbalance of insulin sensitivity and secretion contributes to the development of GDM. However, the lack of longitudinal data limits our understanding of how these miRNAs behave over time in GDM. In this pilot study, we investigated the longitudinal expression patterns of ten miRNAs previously associated with insulin sensitivity following GDM onset.
Methods: Blood samples were collected from 18 GDM women and 18 healthy pregnancies at 4 time points (onset, 28 weeks, 32 weeks, and one day before delivery). The levels of four placenta-specific C19MC cluster miRNAs (miRNA-519d-5p, miRNA-512-3p, miRNA-516a-5p, and miRNA-517-5p) and six non-C19MC miRNAs (miRNA-141-3p, miRNA-143-3p, miRNA-218-5p, miRNA-221-3p, miRNA-483-5p, and miRNA-489-3p) were measured.
Results: Levels of C19MC cluster miRNAs were significantly elevated at GDM onset and continued to increase at 28 weeks, then declined at 32 weeks, then rose again before delivery, compared to controls. In contrast, levels of three non-C19MC miRNAs (miRNA-218-5p, miRNA-221-3p, miRNA-483-5p) were significantly reduced from onset through delivery compared to controls. Three miRNAs (miRNA-141-3p, miRNA-143-3p, and miRNA-489-3p) showed no significant changes over time compared to controls.
Conclusion: The fluctuating levels of C19MC cluster miRNAs during pregnancy may reflect placental and metabolic adaptations during GDM and could serve as biomarkers for disease progression. Persistently reduced non-C19MC miRNAs may indicate disrupted regulatory control of insulin sensitivity in GDM.

P2.13.
IMPAIRED CLEARANCE OF PLACENTAL EVS AFTER DELIVERY COULD BE A RISK FACTOR FOR DEVELOPING ENDOTHELIAL DYSFUNCTION IN WOMEN WITH A HISTORY PREECLAMPSIA

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Objectives: Placental extracellular vesicles (EVs) are released into the maternal circulation during pregnancy and are rapidly cleared by maternal cells, such as endothelial cells and immune cells. However, the clearance of placental EVs in maternal circulation has not been fully investigated in both physiological and pathological conditions. miRNAs clustered on chromosome 19 (C19MC) are unique to primate human placentae and can be detected in placental EVs. In this study, we investigated the clearance of placental EVs by measuring plasma levels of C19MC miRNAs.
Methods: We measured nine C19MC miRNAs (miR-526a-5p, miRNA-527, miRNA-1283, miRNA-498-5p, miRNA-520-3p, miRNA-519a-3p, miRNA-512-3p, miRNA-515-3p, and miRNA-516-3p) in maternal circulation three to five days before and three days after delivery in healthy pregnancies (n=20) and preeclampsia (n=10). We then compared the levels of these miRNAs before and after delivery.
Results: In healthy pregnancies, levels of all nine C19MC miRNAs in maternal circulation significantly decreased, while only four C19MC miRNAs significantly decreased after delivery in preeclampsia, compared to pre-delivery levels. Additionally, compared to healthy pregnancies, the plasma levels of the five C19MC miRNAs were significantly higher, and the rest of the four C19MC miRNAs remained unchanged after delivery in preeclampsia. Furthermore, transfection with individual or combination mimics of C19MC miRNAs (miR-526a-5p, miRNA-515-3p, and miRNA-516-3p) into endothelial cells significantly increased monocyte adhesion to endothelial cells.
Conclusion: Our findings suggest impaired clearance of placental EVs after delivery in maternal circulation in preeclampsia, and the impaired clearance may contribute to endothelial dysfunction and increase the risk of developing long-term cardiovascular diseases in women with a history of preeclampsia.

P2.14.
SPECIFIC ACTIVATION OF THE VASOPRESSIN TYPE 2 RECEPTOR (V2R) FROM THE 7TH DAY OF GESTATION IN RATS INDUCES PHENOTYPIC CHANGES ASSOCIATED WITH THE PATHOGENESIS OF PREECLAMPSIA

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Objectives: Preeclampsia (PE), characterized by hypertension and proteinuria, remains a leading cause of maternal and perinatal morbidity and mortality worldwide. Clinical studies indicate that arginine vasopressin (AVP) levels rise as early as six weeks of gestation, preceding hypertension and PE diagnosis, suggesting a role for AVP in PE pathogenesis. Experimental models support this hypothesis, showing that continuous AVP administration induces PE features. AVP acts through V1AR, V1BR, and V2R receptors, with V2R-blocking attenuating hypertension, glomerular damage, and *in utero* growth retardation, highlighting its potential role in PE, particularly in the placenta.
Methods: To confirm V2R involvement, we infused dDAVP, a V2R-specific agonist, into gravid rats from gestational day 7.5 to 19.5. We also examined V2R function in the rat and human trophoblast cell lines (rCHORIO, HTR-8/SVneo) to clarify the mechanisms leading to features of PE.
Results: Our results show that dDAVP increases systolic blood pressure and proteinuria in a dose-dependent manner, exclusively in gravid rats. Additionally, dDAVP infusion led to increased renal V2R RNA expression in gravid but not in non-gravid rats. Importantly, we demonstrated, for the first time, V2R RNA and protein expression in gravid rat placenta, trophoblast cell lines, and PE-affected human placenta. We showed an overexpression of V2R in women with PE compared to age-matched controls of 26-31 weeks of gestation. Furthermore, dDAVP infusion reduced placental vascularization and induced glomerular lesions in gravid rats. Finally, our data showed that specific V2R-activation decreases cellular proliferation in rCHORIO and HTR-8/SVneo trophoblasts by activating the phosphoERK signaling pathway.
Conclusion: In conclusion, specific V2R-activation induces PE-like symptoms in animals, supporting role of vasopressin and V2R in the pathology. The overexpression of V2R in PE-affected women further strengthens this hypothesis. By impairing trophoblast proliferation, V2R may contribute to PE onset. These findings pave the way for better understanding and potential therapeutic strategies for PE.

P2.15.
COMPONENTS AND PRODUCTS OF THE NLRP3 INFLAMMASOME ARE UPREGULATED IN PLACENTAL EXPLANTS EXPOSED TO HYPOXIA

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Objectives: Placental hypoxia and upregulation of the NLRP3 inflammasome are implicated in pregnancy complications, including pre-eclampsia (PE) and fetal growth restriction (FGR). The NLRP3 inflammasome, a protein complex that unites within cells, plays a role in inflammation, and if dysregulated can cause various pathologies. It can be activated by damage associated molecular patterns (DAMPs), inducing sterile inflammation. Hypoxia can trigger DAMP release and is theorised to perpetuate this pathway. This study aimed to assess whether components and products of the NLRP3 inflammasome are upregulated in placental explants cultured under hypoxic conditions, compared to unexposed controls.
Methods: Healthy term placental explants were exposed to hypoxia (1% oxygen) or 21% oxygen for 4 days, (n=10 per group). Immunohistochemistry was utilised to evaluate placental abundance of NLRP3 and ASC, as well as IL-18 and IL-1β, components and products of the NLRP3 inflammasome respectively. QuPath was used to quantify the area of positive staining, followed by statistical analysis (Mann-Whitney test).
Results: All proteins were present in control and hypoxia-exposed explants, although overall abundance was low in both groups. Abundance of NLRP3, ASC and IL-1β was significantly higher in placental explants exposed to hypoxia, compared with untreated controls. NLRP3, IL-18 and IL-1β expression was localised to Hofbauer cells, with IL-1β also present in villous stroma. ASC was expressed in the syncytiotrophoblast, cytotrophoblast and villous stroma.

Protein	Control		Hypoxia		P Value
	Median area of DAB staining (%)	IQR (%)	Median area of DAB staining (%)	IQR (%)	
NLRP3	0.04	0.04 - 0.01	0.06	0.08 - 0.05	0.0007
ASC	1.41	1.20 - 0.82	3.48	2.40 - 1.60	0.007
IL-18	0.03	0.07 - 0.01	0.05	0.09 - 0.02	0.29
IL-1β	0.07	0.10 - 0.05	0.16	0.35 - 0.12	0.006

Conclusion: This study demonstrates that expression of NLRP3, ASC and IL-1β are significantly higher in placental explants exposed to hypoxia, compared to untreated controls. These data suggest that hypoxia may induce sterile inflammation within the placenta via NLRP3 signalling, leading to the pathological inflammatory response often seen in PE and FGR. Further research is needed to establish whether this pathway plays a causal role in placental inflammation.

P2.16.

APELIN AND COPEPTIN : PROMISING BIOMARKERS FOR THE EARLY PREECLAMPSIA DETECTION

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Objectives: Preeclampsia is a heterogeneous disease with clinical signs appearing around mid-gestation, as early as 20 weeks, and worsening in severity, frequently leading to preterm delivery. Early diagnosis is essential due to the variability in symptoms and molecular mechanisms. Validated biomarkers of preeclampsia, soluble fms-like tyrosine kinase-1 (sFlt-1) and placental growth factor (PlGF), are unfortunately not predictive enough at an early stage. Apelin and Vasopressin are two neuropeptides synthesized in the same neurons, with opposite actions in the regulation of blood pressure and body fluid homeostasis through their interaction with their receptors: APJ for Apelin, and V1AR, V1BR and V2R for Vasopressin. Clinical studies showed lower plasma levels of Apelin in preeclamptic women. In contrast, Copeptin, co-secreted with Vasopressin with a higher plasma half-life, elevated in preeclampsia patient's plasma. These findings suggest that Apelin and Copeptin could serve as biomarkers for the early detection of preeclampsia.
Methods: We measured circulating Apelin and Copeptin, using ELISA kit, in a subset of the AngioPred cohort (NCT00695942) at 20, 24, 28, 32, and 36 weeks of gestation (48 control *versus* 24 preeclampsia patients). Additionally, we quantified by qPCR the expression of APJ, V1AR, V1BR, and V2R in 26-31 weeks control *versus* preeclamptic placentas.
Results: Our results showed a significant decrease in Apelin and increase in Copeptin levels in preeclamptic women as early as 20 weeks of gestation. Importantly, Apelin and Copeptin changes were higher during earlier stages of gestation, indicating their potential as early predictive biomarkers for preeclampsia. Regarding the expression of Apelin and Vasopressin receptors in placenta, our results reveal, for the first time, a significantly higher expression of V1AR and V2R in preeclampsia patients, while APJ expression remains unchanged.
Conclusion: Altogether, our results suggest that Apelin and Copeptin are promising biomarkers for early preeclampsia detection, offering insights into the molecular mechanisms underlying the disease.

P2.17.

STAGE-SPECIFIC REGULATION OF THE PLACENTAL NLRP3 INFLAMMASOME ACROSS GESTATION AND IN PRETERM BIRTH

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Objectives: The NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome, is a multiprotein complex and key mediator of sterile inflammation and immune responses. It is expressed in the human placenta, where it regulates proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18). While dysregulated inflammasome activity has been implicated in pregnancy disorders, including spontaneous preterm birth (PTB), its physiological regulation across gestation and in the placental tissue of PTB cases remains poorly defined. This study aimed to characterize NLRP3 inflammasome expression and activation in the placenta across pregnancy and in spontaneous PTB, stratified by gestational age.
Methods: Placental tissue samples were obtained from a pregnancy cohort, including first-trimester elective terminations (10-12 weeks), spontaneous PTB cases (early <32 weeks, moderate 32–34 weeks, and late 34–37 weeks), and term deliveries (39–40 weeks). Expression of inflammasome-related genes was analyzed via qPCR. Protein levels and cytokine secretion were assessed by Western blot and ELISA, respectively. Maternal and neonatal clinical variables were integrated with molecular findings.
Results: Preliminary analyses revealed robust inflammasome activation in early pregnancy placentas, evidenced by higher protein levels of cleaved caspase 1 (CASP1), NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC), and increased IL-1β secretion. Term placentas showed moderate upregulation of inflammasome gene transcripts but lower cytokine release, suggesting tightly regulated activity at later stages. In PTB placentas, altered inflammasome expression and activation patterns were observed, with ongoing analysis examining distinctions between early, moderate, and late PTB subgroups.
Conclusion: This study highlights dynamic, stage-specific regulation of the NLRP3 inflammasome in human placenta and suggests its dysregulation may contribute to the pathophysiology of preterm birth.
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P2.18.

INVESTIGATION OF THE MOLECULAR MECHANISMS OF THE EFFECT OF THE NO DONOR PENTAERYTHRITYL TETRANITRATE ON THE REGULATION OF CELLULAR STRESS

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Objectives: Pre-eclampsia (PE) is a pregnancy-specific disorder characterized by systemic maternal endothelial dysfunction leading to malperfusion of endorgans like liver, kidneys and also the placenta itself. Pentaerythrityl tetranitrate (PETN), a nitric oxide (NO) donor, has emerged as a promising therapeutic agent for PE prevention, demonstrating significant reduction in maternal hypertension and decreased prematurity rates in clinical trials. Beyond its NO-donating properties, our *in vitro* studies have revealed PETN's capacity to enhance heme oxygenase-1 (HO-1) expression and attenuate oxidative stress in endothelial cells. This study aims to elucidate the yet unknown precise molecular mechanisms underlying PETN's protective effects. The clarification of the mechanisms of action of PETN is the basis for the identification of further indications for the use of PETN in diseases associated with endothelial dysfunction in humans.
Methods: Human umbilical vein endothelial cells (HUVEC) were incubated for 24h with or without 50 μM PETN. Cell lysates underwent proteomic analysis to identify differentially expressed proteins. Western blot analysis was performed to verify proteomic findings and to assess protein phosphorylation status.
Results: Western Blot verification of proteomics data showed that PETN upregulates the expression of HO-1 and the transporter protein SLC7A11, while possibly reducing the G-protein RAC2. Further Western Blot analysis revealed PETN-induced phosphorylation of 5'AMP activated protein kinase (AMPK).
Conclusion: Our results show that PETN possibly reduces endothelial stress by interfering with different pathways involved in regulation of oxidative stress. On the one hand it increases the antioxidative HO-1 and on the other hand it possibly inhibits RAC2, which is a subunit of the ROS-producing NAPDH Oxidase 2 (NOX2). The upregulated SLC7A11 transports cysteine, essential for glutathione synthesis which regenerates the antioxidant enzyme glutathione peroxidase 4 (GPX4). Both GPX4 and AMPK are key factors in preventing ferroptosis, an iron-dependent lipid peroxidation cell death pathway recently implicated in preeclamptic endothelial dysfunction.

P2.19.
DECIPHERING THE ROLE OF PLACENTAL STRESS IN MODULATING FETAL GROWTH RESTRICTION AND MATERNAL MALADAPTATIONS TO PREGNANCY

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Objectives: Placental stress, including endoplasmic reticulum (ER) stress, is central to the pathophysiology of early-onset preeclampsia and fetal growth restriction (FGR). We recently reported that pathological placentas secrete mis-glycosylated proteins. This could potentially alter the activity and function of placentally-derived proteins, thereby compromising maternal glucose metabolism (Yung *et al.*, 2023, PMID: 36660474). Here, we provide further evidence from a spontaneous placental-specific ER stress transgenic model. ER stress mediated protein mis-glycosylation in placenta, not only compromises maternal glucose metabolism, but also induces FGR and placental pathology.
Methods: We generated a junctional zone (Jz)-specific ER stress mouse model, *Jz-Xbp1*$^{-/-}$ by crossing *Xbp1*$^{fl/fl}$ and *Tpbpa-Cre*$^{+/+}$ animals. Deletion of *Xbp1* reduces ER chaperones' expression and ER-assisted protein degradation capability, thereby sensitising cells to ER stress in response to high protein translation. For fetal and placental studies, we established a line with littermate control at 1:1 ratio between *Jz-Xbp1*$^{-/-}$ and wild-type (*Xbp1*$^{fl/fl}$). At E18.5, fetal and placental weights were measured and placental tissues were collected for stereological, histological and molecular analyses. To assess maternal physiology, we generated another line with females (*Xbp1*$^{fl/fl}$) carrying homogenous litters of either wild-type or *Jz-Xbp1*$^{-/-}$ placentas. At E16.5, maternal glucose metabolism was assessed by insulin and glucose tolerance tests.
Results: Characterization of the *Jz-Xbp1*$^{-/-}$ placentas confirmed ER stress activation exclusively in the Jz and the presence of mis-glycosylated protein deposits in the spongiotrophoblasts. Placental and fetal weight in *Jz-Xbp1*$^{-/-}$ mutants was reduced. Additionally, in *Jz-Xbp1*$^{-/-}$ placenta, the Jz volume was reduced, and the labyrinth had a higher number of calcification patches, relative to wild-type littermates. Finally, both glucose and insulin tolerance tests indicated abnormal maternal glucose metabolism in the females carrying a litter of *Jz-Xbp1*$^{-/-}$placentas.
Conclusion: These results further consolidate and reveal that an ER stress-mediated disruption of placental signals can play vital roles in FGR and maternal physiological maladaptation in complicated pregnancies.

P2.20.
SFLT-1 EXPRESSION AT THE MATERNAL-FETAL INTERFACE AND IN MATERNAL SERUM IS NOT COREGULATED AND SHOW DIVERGENT UPREGULATION IN PREECLAMPSIA SUBTYPES

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Objectives: Soluble FMS-like tyrosine kinase (sFlt-1) is central in preeclampsia (PE) pathophysiology and maternal serum sFlt-1 serves as a key biomarker in late pregnancy. While sFlt-1 is primarily expressed at the maternal-fetal interface, it remains unclear whether local expression in the decidua and placenta contributes to maternal serum sFlt-1 levels. In this study, we investigated potential coregulation of sFlt-1 expression at both sides of the maternal-fetal interface and its relationship to circulating levels in maternal serum.
Methods: Tissue samples were collected at caesarean delivery from 129 pregnancies: maternal serum (n=64), decidua (n=74), and placenta (n=111). The cohort included 65 uncomplicated pregnancies (controls), 42 cases of PE with fetal growth restriction (PE+FGR), and 22 cases of PE with normal fetal growth (PE-FGR). Serum sFlt-1 levels were measured by ELISA. Expression of sFlt-1 in decidual and placental tissues was assessed by immunohistochemistry and quantified using an automated image-analysis.
Results: Decidual and placental sFlt-1 expression was trophoblast dependent. Decidual sFlt-1 expression was higher in PE-FGR, placental sFlt-1 was higher in PE+FGR, while serum sFlt-1 was upregulated in both PE subgroups compared to controls ($p<0.001$, all comparisons).
High serum sFlt-1 levels (>245 ng/mL) were observed exclusively in PE cases (n=20), while all normal pregnancies (n=28) and 44% (n=16) of PE pregnancies exhibited low serum sFlt-1. Among PE cases, those with high serum sFlt-1 more frequently had concurrent FGR and showed weaker decidual sFlt-1 expression. sFlt-1 expression levels correlated between decidua and placenta – most strongly in PE – but not with serum sFlt-1 levels.
Conclusion: sFlt-1 at both sides of the maternal-fetal interface is correlated and trophoblast-dependent, showing distinct association with PE with or without FGR. Weak association between local sFlt-1 expression and maternal serum levels, along with a divergent maternal sFlt-1 response to PE, suggests a complex and context-specific regulation of this important biomarker in PE.

P2.21.
DECREASED PLACENTAL ACE2 ACTIVITY IS ASSOCIATED WITH DISRUPTED REDOX HOMEOSTASIS IN FETAL GROWTH RESTRICTION

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Objectives: Placental dysfunction linked to oxidative stress underlies up to 60% of fetal growth restriction (FGR) cases, a condition associated with increased fetal morbidity and mortality. We previously found that placental antioxidant angiotensin-converting enzyme 2 (ACE2) mRNA expression is decreased in FGR. We sought to characterise placental and circulating ACE2 levels and activity in FGR and map this to markers of placental oxidative stress.
Methods: Human placental villous tissue, maternal, and cord blood plasma were collected from control (n=35) and FGR (n=16) pregnancies. ELISAs, activity assays, and mass spectrometry were used to assess placental and circulating ACE2 levels and activity, along with placental oxidative stress markers.
Results: ACE2 activity was significantly decreased in FGR placentae compared with controls (p=0.006), which was accompanied by a significant increase in activity of the pro-oxidative xanthine oxidase (p=0.004). Placental protein expression of the cytosolic antioxidant SOD1 and mitochondrial SOD2 were downregulated 1.1- and 1.4-fold, respectively, in FGR compared with controls. Placental protein expression of the critical intracellular antioxidant, catalase, was downregulated 1.5-fold in FGR, a finding mirrored by a significant decrease in catalase activity (p=0.011). Furthermore, placental protein expression of the cytosolic (GPX1) and mitochondrial (GPX4) antioxidants was downregulated 1.1- and 1.3-fold, respectively, in FGR. Conversely, total GPX activity was significantly increased in FGR placentae compared with controls (p=0.0003). Placental alterations in redox-sensitive molecules were not accompanied by significant differences in maternal or cord blood plasma ACE2 levels or activity.
Conclusion: We show for the first time that placental ACE2 activity is decreased in FGR. Moreover, we demonstrate that individual placental

antioxidants exhibit distinct expression and activity patterns in FGR, suggesting each antioxidant is differentially affected by the pathological environment. We have also shown that changes to placental oxidative stress are not accompanied by changes to circulating ACE2, highlighting a novel aspect of ACE2 regulation in FGR.

P2.22.
IGF-1 SIGNAL TRANSDUCTION IN PLACENTAL MICROVASCULAR ENDOTHELIAL CELLS: A CRITICAL ROLE OF NF-κB?

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Objectives: Insulin-like growth factor 1 (IGF-1) is a critical regulator of placental vascular development and is essential for supporting fetal growth. Impaired IGF-1 signaling is strongly implicated in fetal growth restriction (FGR) due to placental insufficiency, and intraplacental IGF-1 gene therapy improves fetal outcomes in animal models by restoring microvascular density. NF-κB, a transcription factor traditionally associated with inflammatory gene regulation, has recently emerged as a critical modulator of angiogenic gene expression in fetal endothelial cells. The intersection of IGF-1 with NF-κB, however, remains unexplored. We postulate that IGF-1 promotes placental microvascular endothelial cell angiogenesis through NF-κB activation.
Methods: Human umbilical vein endothelial cells (HUVEC) and placental vascular endothelial cells (HPVEC) were treated with recombinant IGF-1 (500 ng/mL), with or without specific pathway inhibitors: BAY-11-7082 (IKK-β, key regulator of NF-κB), GDC-0068 (Akt), Rapamycin (mTOR), and LY294002 (PI3K). Tube formation was assessed on Geltrex matrix over 18 hours and analyzed using ImageJ software. Cell proliferation was measured via Ki-67 immunostaining and flow cytometry after 48 hours. One-way ANOVA with multiple pairwise comparisons was performed to determine statistical significance.
Results: Compared to negative controls, IGF-1 partially restored tube formation and proliferation in HUVECs and fully restored them in HPVECs (Figure 1A). While all inhibitors reduced IGF-1-induced angiogenesis and cell proliferation to some extent, BAY-11-7082 completely abolished these effects in HUVECs and HPVECs, reducing responses to levels comparable to the negative control (Figure 1B).

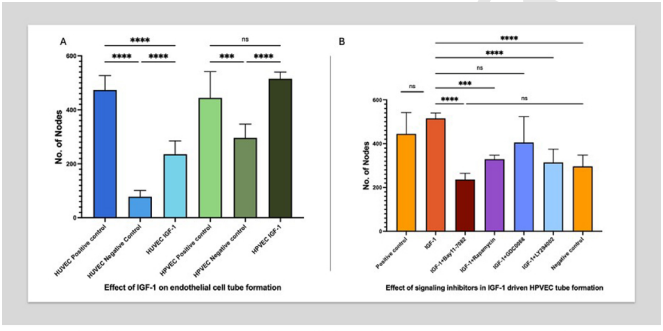

Figure 1: IGF-1-induced tube formation in HUVECs and HPVECs and the effect of pathway-specific inhibitors: Representative images and quantitative analysis of in vitro tube formation assays performed on Geltrex matrix. Human umbilical vein endothelial cells (HUVECs) and placental vascular endothelial cells (HPVECs) were treated with IGF-1 (500 ng/mL) in the presence or absence of signaling pathway inhibitors: BAY-11-7082 (IKK-β/NF-κB inhibitor), GDC-0068 (Akt inhibitor), Rapamycin (mTOR inhibitor), and LY294002 (PI3K inhibitor). Tube formation was assessed after 18 hours by quantifying the number of nodes using ImageJ angiogenesis analysis software. Data are presented as mean ± SD; statistical significance was determined using one-way ANOVA with multiple pairwise comparisons. ***p < 0.001, ****p < 0.0001, ns- non significant, n=8.

Conclusion: Placental microvascular endothelial cell angiogenesis and proliferation are critically dependent on IKK- β/NF-κB signaling. These results support a central role for NF-κB in mediating IGF-1-driven placental endothelial cell function, revealing a novel regulatory axis essential to placental microvascular development. Elucidating this pathway may provide novel insights into the pathophysiology of fetal growth restriction due to placental insufficiency.

P2.23.
NLRP3 INFLAMMASOME ACTIVATION AND LIPIDOMIC ALTERATIONS IN HUMAN PLACENTAL EXPLANTS EXPOSED TO GLUCOSE, LPS, AND METFORMIN

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Objectives: The placenta is increasingly recognized as an immunologically active organ, capable of responding to metabolic and microbial stress via innate immune pathways such as the NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome. Lipid species, beyond their metabolic roles, can act as activators or modulators of inflammasome signaling. This study investigates how glucose, lipopolysaccharide (LPS), and metformin influence NLRP3 inflammasome activation and lipidomic composition in human placental explants.
Methods: Term placental explants were treated with glucose (5–35 mM), LPS (0.1–10 μg/ml), and/or metformin (0.1–1000 μM) at various time points (3–48 hours). Expression of inflammasome-related genes was assessed by qPCR, while protein expression and cytokine release were measured via Western blot and ELISA, respectively. Lipidomic profiling of explant tissue and supernatants was performed using supercritical fluid chromatography-mass spectrometry (SFC/MS).
Results: High glucose induced low-grade inflammatory patterns and only minimal changes in the placental lipidome. In contrast, LPS robustly activated the inflammasome and triggered a distinct pro-inflammatory lipid signature, marked by elevated ceramides and moderate increases in sphingomyelins, fatty acids, diacylglycerols, and triglycerides. Metformin alone had modest effects on inflammasome activation but upregulated several lipid species associated with inflammatory signaling. Interestingly, when combined with LPS, metformin markedly amplified the inflammatory response, as evidenced by increased interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) release.
Conclusion: Our preliminary findings suggest a close interplay between placental immune signaling and placental lipid metabolism, with potential relevance for understanding inflammation-related pregnancy complications.
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P2.24.
DISSECTING CELL-TYPE-SPECIFIC ALTERATIONS IN FETAL GROWTH RESTRICTION THROUGH TRANSCRIPTOMIC DECONVOLUTION

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Objectives: Fetal Growth Restriction (FGR) and preeclampsia are both associated with placental dysfunction. While frequently co-occurring, FGR exhibits a distinct pathology beyond preeclampsia, which is not completely understood. Integrating placental bulk RNA sequencing data with computational deconvolution may elucidate cell-type-specific transcriptional dysregulation in FGR, with or without preeclampsia.
Methods: We performed bulk RNA sequencing on placental tissues from pregnancies delivering <34 weeks' gestation, including FGR (n=23, birthweight <3rd centile), FGR with concomitant preeclampsia (FGR+PE, n=24), and gestation matched preterm controls (n=21). Differential gene expression was analysed using DESeq2 in R. Functional enrichment analysis was performed using ClusterProfiler and protein-protein interaction networks were constructed using STRING. Network analyses was conducted in Cytoscape using ClueGO and MCODE.
Results: Differential gene expression analysis identified 239 dysregulated genes in FGR and 705 in FGR+PE versus preterm controls (adjusted p-value < 0.05, thresholds of $\log_2$FC > 1 and < -1). A smaller subset of 47 genes distinguished FGR from FGR+PE. Pathway enrichment and network analyses identified seven clusters (MCODE score $\geq$ 3) in FGR, enriched for cellular stress responses, protein degradation and innate immunity. Further enrichment in lysosomal transport and oxidative stress response supports known features of placental dysfunction. In FGR+PE, thirteen clusters were enriched in adaptive immunity, cytotoxic and regulatory immune responses. Interestingly, circadian regulation and hormone secretion pathways were enriched, suggesting neuroendocrine-immune crosstalk. Comparison of FGR and FGR+PE identified a single cluster enriched in immune pathways such as chemokine signalling and leukocyte chemotaxis. This suggests active immune cell recruitment in the placenta.
Conclusion: Our analysis reveals both shared and distinct molecular signatures in FGR with or without preeclampsia, with a common immune-associated gene network suggesting a unifying feature of placental dysfunction. Ongoing computational deconvolution using a single-nucleus placental atlas aims to connect molecular changes to specific cell types, potentially highlighting the pathophysiological mechanisms underlying FGR.

P2.25.
A NEW MOLECULAR MECHANISM INVOLVED IN HUMAN PREECLAMPSIA DEVELOPMENT

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Objectives: Preeclampsia (PE) is one of the most severe and complex pathologies of human pregnancy, causing significant morbidity and mortality in thousands of women and newborns worldwide. Although the sFLT-1/PlGF ratio is currently used as a predictive biomarker, the molecular basis of PE development remains largely elusive. We investigated the interaction between the amino acid transporter LAT1 and the transcription factor NRF2 and its impact on the sFLT-1/PlGF ratio.
Methods: The effects of impaired LAT1 activity, achieved through siRNA-mediated gene silencing and pharmacological inhibition with JPH203, on the sFTL-1/PlGF ratio were examined using the trophoblast cell line HTR-8/SVneo, primary human trophoblast cells (hTCs), and placental endothelial cells (PLECs) isolated from healthy and preeclamptic placentas. Additionally, PLECs were treated with conditioned medium from LAT1- or NRF2-depleted HTR-8/SVneo cells to investigate the effects on their viability and oxidative stress levels. Finally, key findings of the newly proposed mechanism were confirmed in an in vivo model using JPH203-treated wild-type C57BL/6J mice.
Results: We demonstrate that the elevated anti-angiogenic sFLT-1/PLGF ratio observed in PE is regulated by the amino acid transporter LAT1 and the key regulator of oxidative stress, NRF2. Any disruption of their mutual interaction ultimately results in a PE-like phenotype. Furthermore, we show that oxidative stress associated with PE is a secondary effect of reduced LAT1 and NRF2 activity.
Conclusion: PE is a multifactorial disease that cannot be attributed to a defect in a single protein. Perturbations in any step of the proposed circular mechanism can ultimately lead to a PE-like phenotype. A profound understanding of the molecular mechanisms underlying the development of this disease will pave the way for establishing innovative therapies.

P2.26.
QUANTIFYING HISTOPATHOLOGICAL FEATURES OF DELAYED VILLOUS MATURATION

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Objectives: Delayed villous maturation (DVM) describes a histopathological entity wherein the placenta does not mature adequately for gestational age. Presently, DVM is a qualitative diagnosis with high interobserver variation. This study aimed to determine if there is a noteworthy difference in syncytial knot (SK) counts and CD31 levels in DVM placentas compared to healthy controls to aid in the diagnosis of DVM.
Methods: 65 placentas complicated with DVM (34-41 weeks' gestation and 50 control placentas (37-41 weeks) were stained with haematoxylin and eosin (H&E) and underwent immunohistochemical staining for CD31. Ten regions of interest (ROI) were randomly chosen from tissue sections taken from the centre, middle and edge of each placenta (n=30 ROI/placenta) Dynamic image analysis scripts were used to quantify SKs in the H&E images and the percentage of CD31 positive area. Numbers of CD15 positive vessels were counted manually.
Results: The SK counts differed between control and DVM samples (mean of 51.19 SKs/10 ROIs and 40.07 SKs/10 ROIs respectively, p=0.004). The median SK count at each gestational week from 34 to 41 weeks in the DVM samples differed significantly (p=0.02). There was a significant relationship between gestation and SKs in the DVM cohort (r^2=0.0929, p=0.01). There was no significant difference in CD31 expression between control and DVM samples (median CD31 stained area of 16.0% and 15.45% respectively). There was a significant relationship between gestation and CD15 in the controls (r^2=0.2346, p=0.019). Preliminary CD15 data from the DVM cohort shows a trend towards increased CD15 positive vessels in DVM.
Conclusion: The significantly lower numbers of SKs in DVM compared to controls corresponds with previous research suggesting SKs as a marker for placental maturity. Overall, CD31 expression does not differ significantly in DVM, but there may differences in the type of or location of the vessels, which needs further study.

P2.27.
CYTOKINE-INDUCED EXPRESSION OF THE ANTIVIRAL PROTEIN GBP5 IMPAIRS PLACENTAL DEVELOPMENT

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Objectives: Pre-eclampsia (PE) and fetal growth restriction (FGR) are associated with abnormal immune activation and impaired placental

development. Syncytiotrophoblast formation depends on trophoblast fusion mediated by Syncytin-1 and Syncytin-2—two proteins derived from endogenous retroviruses and activated by the protease furin. GBP5, an antiviral protein and endogenous furin inhibitor, is elevated in PE and FGR, suggesting that dysregulated *GBP5* expression may disrupt syncytiotrophoblast formation. Furthermore, inflammatory mediators—including IFN-γ, IL-27, leptin, and CXCL10—are elevated in both PE and FGR. We therefore hypothesise that cytokine-induced upregulation of GBP5 impairs trophoblast fusion and thereby contributes to placental dysfunction.

Methods: RNA-sequencing data were analyzed to assess *GBP5* expression across placental cell types. *In vitro* experiments using BeWo cells and primary human trophoblasts, stimulated for 24 or 48 hours with 0-100 ng/mL of IFN-γ, IL-27, leptin, or CXCL10 were performed to evaluate *GBP5* induction. Plasma cytokine levels in women with PE, FGR or healthy controls were quantified using cytokine arrays and ELISA. Reporter assays investigated the activation of the retroviral LTR12C promoter upstream of *GBP5*. Functional studies assessed the effects of GBP5 overexpression or knockout on trophoblast fusion and syncytiotrophoblast formation under cytokine treatment.

Results: Single-cell RNA-sequencing revealed that *GBP5* expression is not detectable in healthy placentas, but elevated in PE and FGR. *In vitro*, IFN-γ significantly induced *GBP5* expression in BeWo cells and primary trophoblasts, and IL-27 significantly induced *GBP5* expression in primary trophoblasts. Leptin levels are elevated in PE plasma. Reporter assays demonstrated that the LTR12C promoter driving *GBP5* expression is activated by leptin and IFN-γ. Functionally, overexpression of GBP5 and cytokine stimulation inhibited trophoblast fusion, while GBP5 depletion significantly increased BeWo cell fusion.

Conclusion: Elevated levels of IFN-γ and IL-27 in pregnancy complications induce *GBP5* expression, disrupt trophoblast fusion and impair syncytiotrophoblast formation, suggesting a contribution to the pathogenesis of PE and FGR.

P2.28.
IS CIRCULATING PLASMA RENIN CONCENTRATION DYSREGULATED IN THE INTERACTION OF PREECLAMPSIA CO-MORBID WITH HIV INFECTION?

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Objectives: Preeclampsia is one of the main direct cause of maternal mortality. Although research has been undertaken extensively, there is currently no cure for the pregnant mother and thus, remains a challenge. The aim of our study was to determine the concentration levels of renin in the synergy of HIV co-morbid with PE in women stratified by their pregnancy type (normotensive vs PE) and HIV status (HIV-negative vs HIV-positive) using an immunoassay procedure.

Methods: The study population (76) was stratified according to their pregnancy type (normotensive *vs* preeclamptic) and HIV status (positive *vs* negative). Evaluation of the plasma renin concentration levels was performed using the Bioplex immunoassay procedure.

Results: There was a significant downregulation ($p = 0.0472$) in plasma renin concentration levels in the PE pregnant group (16.24ng/ml) compared to the normotensive (19.59ng/ml) group, irrespective of HIV status. There was no significant difference in plasma renin concentration levels between HIV-positive and HIV-negative groups, regardless of pregnancy type and across all four study groups (HIV-negative normotensive, HIV-positive normotensive, HIV-negative preeclamptic and HIV-positive preeclamptic).

Conclusion: The current study confirms that renin is downregulated in PE, regardless of HIV status. This finding possibly emanates from the body's physiological response to the hypertensive milieu during PE, thus reducing the expression of renin. We also demonstrate that plasma renin concentration was not affected by HIV status, irrespective of pregnancy type. This could be attributed to the similarity in renin's structure to HIV-1 protease and immune reconstitution post-ART administration. We also report no significant difference in renin concentration between all four groups, hence no effect in the synergy of HIV infection and preeclampsia. This could be attributed to compensatory effects of the RAAS during different physiological and pathological conditions of preeclampsia, HIV infection and to the effects of ART.

P2.29.
CHARACTERIZATION OF PROKINETICIN 1 CONCENTRATIONS IN AMNIOTIC FLUID: IMPLICATIONS FOR SPONTANEOUS PRETERM BIRTH AND HYDRAMNIOS

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Objectives: Over the past two decades, the protein Prokineticin 1 (PROK1), also known as EG-VEGF, has been recognized as a key factor in the female reproductive system. We demonstrated that PROK1 plays a crucial role in the success of pregnancy, from implantation to birth. It specifically acts as a central regulator throughout human pregnancy. PROK1 circulating concentrations have been shown to increase in pregnancy complications such as preeclampsia, fetal growth restriction, and more recently, in spontaneous preterm birth (sPTB). Additionally, we have found that PROK1 is highly expressed in fetal membranes (FM), suggesting its presence in amniotic fluid (AF), with a potential role in regulating pregnancy outcomes.

Methods: Two cohorts of AF were analyzed: The first cohort included samples from non-laboring women enrolled between 14–16 weeks ($n = 15$), 16–26 weeks ($n = 35$), and 27–34 weeks ($n = 15$) of gestation (wg). The second cohort was collected from laboring women at term ($n = 20$) and women with sPTB ($n = 30$)—15 with, and 15 without, infection. Additionally, two other sample sets were included: One from women with hydramnios, a possible trigger of sPTB ($n = 11$), and a second composed of serum samples collected between 14–20 wg ($n = 10$).

Results: Our results revealed that PROK1 concentrations were, i) six times higher in AF compared to serum, ii) Highest during the second trimester in non-laboring women, iii) Significantly elevated in sPTB compared to term laboring women, iv) Higher in sPTB with infection compared to sPTB without infection, and v) showed a trend toward a reduction under hydramnios .

Conclusion: These findings provide the first characterization of PROK1 concentrations in amniotic fluid, highlighting its potential involvement in pregnancy complications such sPTB. Ongoing studies are further investigating the role of this key factor in AF in relation to pregnancy pathologies.

P2.30.
PHOSPHATIDYLINOSITOL DYSREGULATION IN FETOPLACENTAL ENDOTHELIUM: A LINK TO PREECLAMPSIA PATHOPHYSIOLOGY

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Objectives: Preeclampsia (PE) is a multisystem pregnancy disorder characterized by endothelial dysfunction in the placenta. While systemic dyslipidemia in PE is well recognized, alterations of the placental membrane lipids remain poorly understood. Plasma membrane (PM) lipids are essential for maintaining cellular integrity, and mediating signal transduction. Among them, phosphatidylinositol (PI) and its phosphorylated derivatives are central signalling molecules involved in nearly all aspects of membrane function and dynamics. This study aimed to compare the PM

lipid composition of fetoplacental endothelial cells (fpECs) from early-onset PE and preterm birth (PTB) pregnancies to understand its role in endothelial dysfunction in PE.
Methods: Primary fpECs were isolated from chorionic arteries of PE and PTB pregnancies (n=5/group). PM were isolated, and lipid classes were quantified using mass spectrometry. Bulk RNA sequencing of fpECs was conducted to assess differential gene expression of enzymes involved in PI metabolism. Metabolic pathway analysis was performed to identify group differences in PI-related gene pathways.
Results: No significant differences in ceramides, cholesterol, lysophosphatidylcholine, (phosphorylated)-phosphatidylethanolamine, phosphatidylcholine or sphingomyelin were found between groups. However, total PI was significantly reduced in PE (p=0.0462). PI species 36:1, 38:3, 38:4, and 38:5 showed a decreasing trend in PE. RNA sequencing revealed a significant upregulation of *PI3KCG* (p=0.0159), and a trend towards upregulation of *PLCE1* (p=0.0556) in PE, both involved in $PI(4,5)P_2$ metabolism. *PLCE1* is activated by heterotrimeric G-protein subunits encoded by *GNA12*, *GNA13* and *GNB1-GNG2* genes. Notably, *GNA13* was significantly upregulated in PE (p=0.0317). Pathway analysis indicated an increased trend (p=0.074) of *PIK3CA*, *PIK3CB*, *PIK3CG*, and *PIK3CD*, suggesting enhanced conversion of $PI(4,5)P_2$ to $PI(3,4,5)P_3$ in PE.
Conclusion: fpECs show reduced total PM PI levels and altered gene expression of enzymes involving $PI(4,5)P_2$ metabolism in PE. These findings suggest disrupted PI signaling may contribute to endothelial dysfunction in PE.

P2.31.
PROKR2 EXPRESSION IN HUMAN PREECLAMPTIC PLACENTAS: A POTENTIAL THERAPEUTIC TARGET IN MULTINUCLEATED TROPHOBLAST GIANT CELLS

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Objectives: Preeclampsia (PE) is one of the most threatening pathologies of human pregnancy, and its underlying mechanisms remain incompletely understood. Prokineticin-1 is the canonical member of the prokineticin (PROKs) family and exerts its effects through two receptors: PROKR1 and PROKR2. Recently, we demonstrated that PROKR2 is highly upregulated in the placenta of a mouse model of PE, and that its antagonization may represent a promising therapeutic strategy to alleviate PE symptoms. However, the expression of PROKR2 in human PE placentas, has not yet been investigated.
Methods: To further explore the relationship between the PROK system and PE, we conducted a comparative analysis of placental tissues collected from control and PE patients, focusing on both histological alterations and PROK expression. Embedded placental tissues, which included 25 pregnant women: 12 early-onset PE, 4 late-onset PE, and 9 age-matched controls were analyzed for known histological hallmarks of PE, such as villous infarcts, syncytial knots (SK), and the presence of multinucleated trophoblastic giant cells (MTGC) clusters; alongside immunohistochemical staining for PROK1, PROKR1, and PROKR2.
Results: Our results confirmed histological lesions in PE placentas, including increased infarcts, enlarged intervillous spaces, higher SK numbers, and maintenance of MTGCs clusters, which are typically absent at term. PE placentas exhibited a reduction in PROKR1 expression in extravillous trophoblasts and a decrease in PROKR2 in ST/SK. Importantly, PROKR1 and PROKR2 were strongly expressed in MTGCs compared to other cells types expressing the receptors.
Conclusion: Together, these findings suggest that PROKR2-mediated effects in PE may be driven by MTGCs, the cell type that is associated with inadequate spiral artery remodeling and exclusive to PE. Hence, targeting PROKR2 could mitigate their detrimental signaling within the placenta. Further studies are needed to elucidate how PROKR2 actions on MTGCs contribute to the pathophysiology of PE.

P2.32.
UNCOVERING A NEW ADAPTIVE MECHANISM: THE IMPACT OF GESTATIONAL HYPOXIA ON PLACENTAL-FETAL OXYGEN TRANSFER

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Objectives: Gestational hypoxia is linked to fetal growth restriction (FGR), increasing perinatal risk, but current growth-curve-based diagnostics often misclassify cases. This study explores FGR's molecular pathways, emphasizing hypoxia and sex-based differences.
Methods:
Animals – Pregnant mice inhaled 12.5% oxygen from E0.5 (chronic; n=8) or E11.5 (acute; n=8), while controls (n=7) inhaled 20%. At E16.5, mice were euthanized, blood collected, placental and fetal weights recorded, and DNA extracted for sexing.
In Vivo MRI – Pregnant mice were scanned with 15.2T MRI using coronal 3D-MGE at 10%, 20%, and 40% oxygen.
Ex Vivo MRI – Fixed SGA (n=15) and AGA (n=16) fetuses were scanned with 9.7T MRI using sagittal 3D-diffusion.
TG2 Activity – Placental cryosections were incubated with Biotin-T29 and Alexa647-streptavidin.
Density Analysis – A pixel-based classifier identified blood sinusoids and exchange layers in H&E-stained placenta sections.
Results: In the chronic group, mothers had elevated hematocrit and hemoglobin, with reduced fetal but normal placental weight. Lower fetus-to-placenta ratios (FPR), especially in females. SGA rates increased.
Oxygen exchange was evaluated by *in-vivo* MRI. Surprisingly, only the placenta demonstrated a significant alteration in deoxygenated hemoglobin (deoxyHb) across treatment groups. Linear correlations were found between the placenta and fetal organs deoxyHB in the control group, which were demolished in the chronic group. These suggest a placental active role in oxygen transfer.
To assess fetal organogenesis, *ex-vivo* MRI was employed. The fetal brain-to-body ratio was significantly higher in the SGA than the AGA fetuses, demonstrating the 'brain sparing' phenomenon.
Oxygen exchange was further investigated by density analysis. Chronically hypoxic placentas presented a significantly higher proportion of exchange layers, implying a reduced oxygen exchange capacity.
Finally, Placental-TG2 activity was found to be significantly elevated upon chronic hypoxia compared to control.
Conclusion: These results show the placenta's role in oxygen transfer and FGR, support MRI for assessing oxygen delivery, and reveal its molecular adaptation.

P2.33.
IRON-SULFUR CLUSTER ASSEMBLY IN MITOCHONDRIA OF THE PLACENTA: IMPLICATIONS FOR FETAL GROWTH RESTRICTION

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Objectives: Placental mitochondria generate ATP through oxidative phosphorylation, supporting transport processes such as nutrient, gas and waste exchange for fetal development. Iron-sulphur clusters (ISCs) serve as vital cofactors in the electron transport chain, facilitating electron transfer and the generation of the proton gradient. Mitochondrial ISC biogenesis occurs through two stages: initial formation of [2Fe-2S] clusters, followed by [4Fe-4S] cluster formation. The disruption of ISC

assembly causes mitochondrial dysfunction, evident in metabolic myopathies. In this study, we examined the role of Fe-S clusters in placental function and Fetal Growth Restriction (FGR).

Methods: Placental villous tissue was collected from healthy (n=19) and FGR (n=18) pregnancies. Gene and protein levels of mitochondrial ISC components were examined using RT-qPCR and liquid chromatography-mass spectrometry. All protein levels are reported as linear fold changes relative to controls.

Results: We observed that ISCU, involved in [2Fe-2S] assembly, had significantly decreased mRNA expression ($p<0.05$) in FGR placentae. However, ISCU protein levels were 1.29-fold higher in FGR compared to controls. A critical stabiliser of initial ISC scaffold proteins, NDUFAB1, showed no change in mRNA expression between control and FGR placental tissue; however, protein levels were 1.08-fold lower in FGR placentae. In FGR placentae, we observed significantly decreased FDX2 mRNA expression ($p<0.05$), while FDXR protein levels were lower (2.11-fold), both of which are essential for [2Fe-2S] to [4Fe-4S] cluster conversion.

Conclusion: Our research indicates significant disruptions in the ISC assembly pathway in FGR placentae. We observed alterations in ISCU and NDUFAB1 mRNA expression and protein levels, which are essential to the initiation of ISC assembly. Furthermore, we observed reduced mRNA expression of FDX2 and lower protein levels of FDXR, which may prevent the formation of [4Fe-4S] and are critical for electron transport in the ETC. These molecular alterations may underpin ISC disassembly, impairing mitochondrial function, contributing to placental insufficiency and, ultimately, FGR.

P2.34.
REDUCED COL1A2 EXPRESSION IN EARLY PREGNANCY PLACENTA FROM TERM PREECLAMPSIA IS ASSOCIATED WITH DISRUPTED EXTRACELLULAR MATRIX FUNCTION

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Objectives: Term preeclampsia (>37 weeks) accounts for ~ 80% of all preeclampsia cases in developed countries, yet its precise etiology remains unclear. Despite placental dysfunction being central to its pathogenesis, the underlying mechanisms are poorly defined. We performed proteomics analyses rare chorionic villus sampling (CVS) samples collected from ongoing pregnancies that later developed term preeclampsia or remained normotensive (n=6-8 per group; 11-13 weeks gestation). Proteomics identified Collagen Type I Alpha 2 Chain (COL1A2) as one of the most downregulated proteins in term preeclamptic group ($p<0.05$, >1.5 fold). Here, we explored the expression and function of COL1A2 in the placenta.

Methods: Placental COL1A2 production was quantified by immunohistochemistry (scored by two independent observers) in CVS (n=8/group) and placental villous from all three trimesters (n=6/group). Primary human placental fibroblasts were isolated from 1st trimester placenta (n=4). COL1A2 was silenced using siRNA and the impact on fibroblast function assessed by xCELLigence (proliferation, adhesion, migration) and proteomics (n=6/group).

Results: COL1A2 immunolocalized to placental fibroblasts, endothelial and Hofbauer cells. Immunostaining was markedly diminished in CVS samples from term preeclamptic pregnancies compared to normotensive controls (2-fold; $p<0.05$). Moreover, COL1A2 staining intensity in placental villi was significantly lower at term compared to the first trimester (1st trimester vs term; 3-fold; $p<0.05$). Silencing COL1A2 in placental fibroblasts caused dysregulation of proteins (1.2-fold cut-off; 39 upregulated, 35 downregulated) which were associated with extracellular matrix (ECM), wound healing and fibroblast activation. COL1A2 knockdown in placental fibroblasts significantly reduced fibroblast migration and adhesion (1.3-fold; $p<0.05$).

Conclusion: In conclusion, COL1A2 was dysregulated in the early pregnancy placental stroma in pregnancies that later developed term preeclampsia, highlighting a potential link to abnormal fibroblast activation and disrupted ECM function and remodelling essential for normal placental development. These findings offer critical insights into early pregnancy placental dysfunction associated with term preeclampsia.

P2.35.
THE CHARACTERISTIC HISTOPATHOLOGICAL FINDINGS SUGGESTIVE OF PLACENTAL VASCUIAR MALPERFUSION OBSERVED IN MECONIUM OBSTRUCTION OF PREMATURITY IN THE CONTEXT OF EARLY PRETERM FETAL GROWTH RESTRICTION; KNOB-LIKE SYNCYTIAL KNOTS AND EXTREME DISTAL VILLOUS HYPOPLASIA

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Objectives: There is a paucity of information about the role of placental-pathology in the development of meconium-obstruction of prematurity (MOP), a rare-form of neonatal intestinal-obstruction, in the context of fetal-growth-restriction(FGR). We examined whether specific placental-lesions in placental-vascular-malperfusion(PVM) are associated with MOP in the context of early-preterm FGR.

Methods: This study is a secondary-analysis of a prospectively-conducted study investigating the association between prenatal and/or postnatal Doppler-ultrasound findings and MOP in pregnancies complicated by FGR ($<10^{th}$ percentile). Study-population included 27 preterm-FGR neonates(i.e., 7neonates with MOP vs. 20neonates without MOP) delivered before GA 33.9weeks from May 2018 until Jan 2022 in SNU Hospital. We analyzed placental-histopathology using the standard 'Amsterdam-Placental-Workshop-Group-criteria', with additional-assessment of lesions such as extreme-distal-villous-hypoplasia(EDVH, defined in the presence of sparse, slender terminal-villi occupying >50% of the lower two-thirds of the parenchyma) and knob-like syncytial-knots(KSK, defined in the presence of $\geq$3 clusters showing 'a hobnail-like configuration, featuring a narrow-neck and often exhibiting a bulbous-protrusion from the villous-surface' per high-power field[HPF] in at-least three distinct-foci) according-to the presence or absence of MOP. MOP was diagnosed based-on clinical-signs of bowel-obstruction and feeding-intolerance, with resolution following the passage of inspissated-meconium after a contrast-enema or surgical-intervention.

Results: The standard histopathological-features of whole maternal-vascular-malperfusion(MVM, G2) and fetal-vascular-malperfusion(FVM, G3) as defined-by 'Amsterdam-Placental-Workshop-Group-criteria' were not significantly different between placentas with and without MOP. However, targeted-analysis revealed that EDVH(85.7%[6/7]vs25.0%[5/20], $p=0.009$) and KSK(100%[7/7]vs50%[10/20], $p=0.026$) were significantly more-common in the placentas with MOP than those without MOP.

Conclusion: In early-preterm neonates with FGR, MOP was associated with specific-lesions(i.e., EDVH and KSK) in PVM, which are not identified by the standard 'Amsterdam-Placental-Workshop-Group criteria'. This finding suggests that targeted-analysis of EDVH and KSK, alongside the standard 'Amsterdam-Placental-Workshop-Group-criteria' may improve the prediction of MOP-development in the setting of early-preterm FGR.

P2.36.
THE ROLE OF LYVE1 HOFBAUER CELLS IN PREECLAMPSIA

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Objectives: Preeclampsia (PE) poses a severe health risk during pregnancy, is potentially deadly, and can lead to long-term negative effects on the health of mother and child. While the causes of PE are not well understood, recent studies suggest significant differences in placental Hofbauer cells (HBCs) in cases of PE. HBCs have been shown to express LYVE1, a marker commonly associated with lymphatic endothelial cells and tissue-resident macrophages, which facilitates the binding and uptake of hyaluronic acid (HA). Here we aimed to study the role of LYVE1 positive HBCs in placental tissue from first trimester, healthy term, and preeclampsia diagnosed pregnancies. We hypothesize that a changed number and function of HBCs, especially in regard to expression of the HA receptor LYVE1, may lead to changes in tissue integrity, and therefore could contribute to the development of PE.
Methods: We analyzed the number and distribution of LYVE1 positive HBCs in placental tissue samples using immunohistochemistry (IHC) and light sheet microscopy. LYVE1 expression in HBCs was confirmed by in situ padlock hybridization. Placental total LYVE1 expression was analyzed by RNA sequencing, qPCR and immunoblot analysis. Additionally, the interactions between macrophages and HA of different molecular weight were investigated.
Results: IHC analysis revealed a reduced number of LYVE1 HBCs in placentas from PE patients, when compared to age-matched healthy controls. The three-dimensional appearance of LYVE1 positive HBCs was detected in placental tissue by light sheet microscopy. The total LYVE1 expression was reduced in placental tissue from PE patients, as shown by qPCR. Immunoblotting showed that HAs of different molecular weight affected the LYVE 1 expression in macrophages.
Conclusion: Our preliminary data show that PE is associated with a reduced number of LYVE1 positive HBCs, which may reflect an altered HA remodeling and an HA profile that promotes a pro-inflammatory microenvironment within the villous stroma.

P2.37.
FIRST TRIMESTER PREDICTION OF GDM AND ITS COMPLICATIONS: A POTENTIAL WINDOW FOR EARLY RISK STRATIFICATION.

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Objectives: To investigate whether first trimester placental biomarkers, including PAPP-A, PlGF, placental volume and placental vascularity are associated with subsequent development of GDM and its complications. To assess whether these biomarkers improve the predictive performance of early screening models when combined with maternal risk factors to risk stratify for GDM in the first trimester.
Methods: A preliminary retrospective cohort study, including 266 women who developed GDM and 2608 normoglycaemic controls. Comparisons between the groups were performed using Wilcoxon rank-sum test due to non-normal distribution of biomarker levels. Receiver operator characteristic (ROC) curve analysis was used to assess predictive performance. Placental volume data was evaluated with further analysis underway on vascularity.
Results: Women who were later diagnosed with GDM had significantly lower first trimester PAPP-A levels (mean ± SD: 2.61 ± 2.20) compared to controls (3.00 ± 2.54; $p = 0.0008$). In contrast, PlGF levels were higher in the GDM group (32.55 ± 17.49) than in controls (30.59 ± 16.82; $p = 0.012$). ROC curve analysis showed that a model incorporating age, BMI and ethnicity yielded an AUC of 0.6985. The addition of PAPP-A and PlGF increased AUC to 0.709 indicating a significant improvement in detection ($p = 0.028$). No significant difference in placental volume centiles was observed ($p = 0.1899$) though, further work is ongoing to assess to role of abnormal placental vascularity in GDM pathophysiology.
Conclusion: Our initial findings indicate that low PAPP-A and elevated PlGF are associated with later GDM development. These biomarkers, both available from routine first trimester screening, may reflect early placental pathology present in GDM. Their incorporation into a model with age, BMI and ethnicity significantly improved its performance at detecting GDM, suggesting that these have utility in early risk stratification.

P2.38.
SFLT1 OVEREXPRESSION IN THE PLACENTA PROMOTES PLACENTAL THROMBO-INFLAMMATION

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Objectives: Preeclampsia (PE) is a thrombo-inflammatory gestational vascular complication associated with high levels of anti-angiogenic factors including soluble fms-like tyrosine kinase-1 (sFlt1). This causes endothelial dysfunction and PE-symptoms in mothers. PE has limited therapeutic options and may cause long-term consequences for maternal and child health. In-vivo systemic human (hsFLT1) overexpression has been shown to promote placental insufficiency, fetal growth restriction and PE-like syndrome in mice. Platelet activation and placental thrombo-inflammation are associated with PE. However, whether sFLT1 is associated with platelet activation and thrombo-inflammation remains unknown. We aim at evaluating the effect of sFLT1 overexpression on placental thrombo-inflammation.
Methods: hsFLT1/rtTA-transgenic mice with doxycycline induced systemic (maternal or maternal/fetoplacental) human sFLT1 (hsFLT1) overexpression since 10.5 days post-conception (dpc) were used. In these mice, hsFLT1 is ubiquitously overexpressed during pregnancy in mothers and according to the genetics in hsFLT1/rtTA homozygous and heterozygous fetuses (and placentae). Placenta sampled at 14.5 dpc was evaluated using immunoblotting and immunostaining.
Results: hsFLT1/rtTA double transgenic mice were bred to obtain PE heterozygous (hsFLT1+/+; rtTA+/-) and PE wt (hsFLT1+/+; rtTA-/-) fetuses (and placentae). PE homozygous (hsFLT1+/+; rtTA+/+) do not survive until term. Accordingly, placentae were defined as hsFLT1-placental (PE het, expresses both in placenta and mother) and hsFLT1-maternal (PE wt, expressed exclusively in mother and not in placenta). Elevated expression of cleaved IL-1β, cleaved caspase-1 and PAD4 and reduced expression of thrombomodulin were observed in both hsFLT1-placental and hsFLT1-maternal placentae. Increased activated platelets (CD62P) and NETs (H3Cit, MPO) were observed in both hsFlt-1 groups compared to controls.
Conclusion: These findings suggest that while placental sFLT1 overexpression promotes platelet activation and placental thrombo-inflammation, circulating maternal hsFLT1 expression was sufficient to cause these effects. Further studies including sequencing analysis are required to evaluate mechanisms by which sFlt-1 promotes thrombo-inflammation. These insights will allow us to evaluate the long-term effects in mother and offspring.

P2.39.
DIFFERENTIAL PLATELET ACTIVATION AND THROMBO-INFLAMMATORY MECHANISMS IN EARLY ONSET AND LATE ONSET PREECLAMPSIA

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Objectives: Preeclampsia (PE) is a thrombo-inflammatory gestational vascular complication with limited therapeutic options. Based on its onset preclampsia can be categorized into early (EOPE) or late (LOPE) onset preeclampsia. However, whether platelet activation and associated inflammation are differentially regulated in EOPE and LOPE remains unknown. We aim to study whether platelet activation and inflammation are differentially regulated in EOPE and LOPE.
Methods: Whole blood and plasma from patients with EOPE, LOPE and gestational age-matched healthy pregnancies were studied for platelet activation (flow cytometry for CD41, CD62P and αIIbβ3 integrin expression), markers of inflammation (IL-1β) and endothelial dysfunction (sVCAM1). Bulk-RNAseq data in whole blood and placenta and single-nucleus(sn) RNAseq from placenta was studied.
Results: Maternal platelet activation was increased in both EOPE and LOPE pregnancies compared to gestational age-matched controls and was overall higher in LOPE compared to EOPE. IL-1β and sVCAM-1 were increased in both EOPE and LOPE but correlated with platelet activation only in LOPE pregnancies. Whole blood Bulk RNAseq analysis showed pathways corresponding to metabolism, ageing and immune-activation in EOPE but that of platelet activation, inflammation and infection in LOPE pregnancies. Placental Bulk RNAseq showed several pathways (angiogenesis, hypoxia and inflammation) regulated in EOPE pregnancies. snRNA seq showed that these pathways were regulated within trophoblast cells and immune cells. LOPE placenta had only one pathway (KRAS signaling, bulkseq) regulated with a limited inflammatory phenotype in immune cells and trophoblast clusters (snRNAseq). However, endothelial cells within LOPE placentae showed several differentially regulated pathways corresponding to coagulation, apoptosis and mitochondrial dysfunction.
Conclusion: These findings suggest that platelet activation and endothelial dysfunction are differentially regulated in LOPE vs EOPE. This supports the role of maternal factors involved in LOPE, which ultimately results in placental dysfunction. On the other hand, EOPE is suggestive to be of placental origin eventually resulting in deficits in maternal health.

P2.40.
THE ROLE OF RELAXIN AND CORPUS LUTEUM ABSENCE IN HUMAN ENDOMETRIAL STROMAL CELL DECIDUALIZATION: INSIGHTS INTO PREECLAMPSIA PATHOPHYSIOLOGY

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Objectives: Absence of the corpus luteum has been linked to a higher risk of preeclampsia, potentially due a lack of relaxin-2 (RLX), a hormone involved in endometrial remodelling and decidualization. This study aimed to assess the effects of RLX on pro- and antiangiogenic factors in human endometrial stromal cells (hESC).
Methods: Primary HESC were decidualized *in vitro* using either 0.5 mM cyclic AMP (cAMP) or a combination of 10 nM estradiol, 1 μM progesterone, and 0.5 mM cAMP (EPC), with added RLX at 0, 0.3 or 1 ng/ml for 12 days. Expression levels of prolactin (PRL), insuline-like growth factor binding protein 1 (IGFBP1), vascular endothelial growth factor (VEGF), placental growth factor (PIGF), soluble fms-like tyrosine kinase 1 (sFlt-1) and endoglin (ENG) were measured using quantitative real-time PCR (qRT-PCR).
Results: Decidualization significantly increased PRL and IGFBP1, with stronger induction seen in the EPC group. RLX treatment enhanced VEGF, PRL, and ENG expression in EPC-treated cells. Specifically, 1 ng/ml RLX significantly increased PRL and ENG levels compared to lower doses ($p < 0.0001$). VEGF expression also rose with 1 ng/ml RLX ($p = 0.002$). IGFBP1 levels were unaffected by RLX. PIGF expression increased with cAMP alone, but not with relaxin. No significant changes in sFlt1 expression were observed.
Conclusion: RLX enhances the expression of key angiogenic and decidualization markers (VEGF, PRL, ENG) in decidualizing hESC, suggesting it may support endometrial angiogenesis and function. These findings highlight a potential mechanistic link between RLX and successful implantation and placentation. Further research is needed to explore RLX's regulatory role in shaping the angiogenic profile of the decidual endometrium.

P2.41.
DOES THE CORRELATION BETWEEN PLACENTAL MATRIX METALLOPROTEINASE-9 (MMP-9) AND TUMOR NECROSIS FACTOR ALPHA (TNF-A) PROTEINS DIFFER BETWEEN NORMAL HUMAN PREGNANCIES AND PREECLAMPSIA?

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Objectives: In this study we examined whether the correlation between MMP-9 and TNF-a proteins differs between normal pregnancies and preeclampsia.
Methods: Placentas were collected from elective termination of pregnancy or term delivery from normotensive women and from women diagnosed with preeclampsia, according to ACOG's criteria. Chorionic villi (CV) were isolated. Expressions of the two proteins were analyzed in 254 CV samples using ELISA kits that used monoclonal antibody to either human MMP-9 or TNF-a protein as capture antibody (R&D Systems, Minneapolis, MN). Independent t-test and Spearman's correlation were performed. P<.05 was considered significant.

Groups	N	MMP-9 (ng/100 mg tissue)		TNF-a (pg/100 mg tissue)	
		Mean	Std. Dev	Mean	Std. Dev
1st Trimester	51	21.19	12.66	53.39	41.94
2nd Trimester	55	23.16	14.70	89.09	96.03
3rd Trimester	119	27.29	14.33	34.75	45.44
Preeclampsia	29	31.92	12.54	42.51	36.25

Results: Expressions of the two proteins were highest in preeclamptic tissues. A positive correlation between these two proteins, in normal pregnancy, was only seen in the first trimester (r^2=.397, p=.004). In preeclamptic placentas, the correlation was negative (r^2=-0.480, p=0.008). Furthermore, in normal pregnancy, the correlation of the two proteins with gestational age (GA) differed as well. While MMP-9 was positively correlated with GA (r^2=.197, p=.003), the correlation between TNF-a and GA was negative (r^2=-.340, p<.001).
Conclusion: The positive correlation between MMP-9 and TNF-α protein seen only in the first trimester validates the involvement of TNF-a protein in MMP-9 activity during early pregnancy. In preeclamptic placentas, the correlation between MMP-9 and TNF-a protein showed a significant negative correlation. The data highlights more complex and potentially opposing roles of the two proteins in preeclampsia. Research in understanding the complex interactions between these two proteins in preeclamptic placentas may help identify agents in preventing the disease. Based on our findings it may additionally be suggested that in preeclampsia, the failure in the normal decrease of TNF-a in the third trimester, could be contributing to the observed complications.

P2.42.
HEALING THE FAILING PLACENTA: A REGENERATIVE THERAPY FOR FGR

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Objectives: Placental insufficiency drives complications such as fetal growth restriction (FGR) and preeclampsia. Current treatments offer limited benefit. We explored YAP activation, a master regulator of tissue growth and repair, as a regenerative strategy to restore placental function.

Methods: Human placental samples from preterm FGR, preeclampsia +FGR (≤34 weeks), and gestationally matched controls were collected (n=20/cohort), alongside first trimester and term and preterm FGR explants (n=5/treatment). YAP activation was measured via qPCR, Western Blots, and proteomics, and its effects on cellular proliferation (Ki67), apoptosis (cl-Caspase 3), and placental cell markers (cytotrophoblasts - Integrinβ4, syncytiotrophoblasts - CK7, and endothelial cells - CD31) were assessed. Primary Human Umbilical Vein Endothelial Cells (HUVECs) were exposed to normoxic and hypoxic conditions to mimic FGR, and functional outcomes such as cell viability, wound healing, and proliferation were evaluated (n=5/experiment). A novel xenograft model was employed to study the effects of YAP activation on human placental tissue implanted in mice (n=3/treatment).
Results: YAP was found to be significantly dysregulated in early preterm (<34 weeks) FGR placentas both alone ($p \leq 0.001$) and in combination with preeclampsia ($p \leq 0.01$). Treatment with compounds restored YAP activity in both term ($p \leq 0.01$) and FGR placental explants ($p \leq 0.01$). In explants, YAP activation led to a significant increase in endothelial cell (CD31) ($p \leq 0.001$ in term, $p \leq 0.01$ in FGR) and cytotrophoblast marker staining expression ($p \leq 0.01$ in FGR). Angiogenic gene expression and key pathways involved in cell cycle regulation and DNA replication were upregulated in proteomic analysis. HUVECs treated with YAP-activating compounds showed enhanced viability, faster wound healing ($p<0.05$).
Conclusion: YAP activation restores endothelial and trophoblast populations in FGR placental explants, enhances angiogenic and proliferative pathways, and improves endothelial cell function under hypoxia. It maintains tissue viability and vascularisation in xenografts. These data support YAP as a regenerative therapeutic to improve placental function and early pregnancy success.

P2.43.
INCREASED AQP9 EXPRESSION AND DISRUPTED MITOCHONDRIAL DYNAMICS IN TROPHOBLAST CELLS FROM PREECLAMPTIC PLACENTAS

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Objectives: We previously reported increased Aquaporin-9 (AQP9) expression in trophoblast cells from preeclamptic placentas. Subsequently, we observed AQP9 localization within trophoblast mitochondria, particularly enriched in the heavy/large mitochondrial fraction in normal placentas. In this study, we aimed to characterize AQP9 mitochondrial localization in normal and preeclamptic placentas and explore potential disruptions in mitochondrial dynamics.
Methods: This study was approved by the Ethics Committee of Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (RES CD-2023-836). Placentas were obtained from healthy term (n=9) and preeclamptic (n=8) pregnancies. Co-localization of AQP9 and cytochrome C was assessed using double immunofluorescence and confocal microscopy. Mitochondrial fractions were isolated from placental tissue by differential centrifugation and characterized by flow cytometry. Mitoplasts and the outer mitochondrial membrane were obtained using digitonin. AQP9 expression was analyzed by Western blot. Mitochondrial content and the expression of mitochondrial dynamic proteins were studied using qPCR.
Results: AQP9 expression was significantly increased in both microsomal and heavy/large mitochondrial fractions ($p<0.05$) from preeclamptic placentas compared to normal ones. Interestingly, we detected a higher AQP9 expression in the light/small mitochondrial fraction from preeclamptic placentas ($p<0.001$). Sub-fractionation revealed strong localization of AQP9 in the inner mitochondrial membrane. Mitochondrial DNA content (mtDNA/nDNA ratio) was increased in preeclamptic placentas ($p<0.05$). Preeclamptic samples exhibited increased Drp1 expression and reduced Mfn2 and BNIP3 levels ($p<0.05$), indicating altered mitochondrial dynamics.
Conclusion: AQP9 is predominantly localized to the inner mitochondrial membrane and is upregulated in both mitochondrial and plasma membranes of trophoblast cells in preeclampsia. These changes are associated with increased mitochondrial content and dysregulated expression of fission and fusion proteins, suggesting disruptions in mitochondrial dynamics. The altered AQP9 distribution may reflect impairments in mitochondrial differentiation during syncytialization, potentially contributing to placental dysfunction in preeclampsia.

P2.44.
DOES LOW-DOSE ASPIRIN FAIL TO IMPROVE NEWBORN WEIGHT IN OBESE WOMEN WITH PREECLAMPSIA?

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Objectives: Low-dose aspirin (LDA) is proposed as a preventive strategy for preeclampsia (PE), but its effectiveness remains controversial. This study assesses the impact of LDA on neonatal weight in obese and non-obese pregnant women with both normotensive pregnancies (NP) and those complicated by PE.
Methods: A retrospective study was conducted at the "Prof. Alejandro Posadas" National Hospital with 310 pregnant women. Participants were classified into eight groups based on body mass index (BMI) [non-obese (BMI < 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2)], LDA administration (150 mg/day from gestational week 16 to 36), and pregnancy outcome (NP or PE). The effect of LDA on neonatal weight was assessed using an ANCOVA model adjusted for gestational age. Post hoc comparisons were performed using Dunnett's test (untreated normotensive women as control) and Bonferroni's test (significance level 0.05).
Results: In non-obese women, neonatal weight was significantly lower in untreated PE cases compared to the control group (2908 g vs. 3244 g, $p = 0.0012$). However, there were no significant differences between PE-treated women and the control group (3163 g vs. 3244 g, $p = 0.9075$).
In obese women, neonatal weight was not significantly different between untreated PE cases and the control group (3158 g vs. 3468 g, $p = 0.3223$). However, in PE-treated obese women, neonatal weight was significantly lower compared to both the control group (2748 g vs. 3468 g, $p < 0.0001$) and untreated PE women (2748 g vs. 3158 g, $p = 0.0493$).
Conclusion: In non-obese women with preeclampsia, LDA improved neonatal weight. However, in obese women, LDA was ineffective in improving birth weight and was instead associated with a significant reduction, suggesting the need to reconsider its use in this group.

P2.45.
URIC ACID-INDUCED INFLAMMATION SUPPRESSES AQP3 EXPRESSION IN HUMAN PLACENTA: IMPLICATIONS FOR PREECLAMPSIA PATHOGENESIS

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Objectives: Preeclampsia is marked by placental dysfunction resulting from impaired trophoblast function, oxidative stress, and inflammation. Elevated plasma uric acid levels, commonly associated with systemic inflammation and endothelial dysfunction, rise before the onset of clinical

symptoms and are thought to contribute to the pathogenesis of the disease. Aquaporin-3 (AQP3), a membrane protein critical for an appropriate placentation, is significantly downregulated in preeclamptic placentas. Here, we aimed to investigate whether elevated uric acid levels affect AQP3 expression.
Methods: This study was approved by the Ethics Committee of the Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (RES CD-2023-836). Explants from Normal human placentas (n=7) were cultured in DMEM/F12 medium in the presence of increasing concentrations of uric acid (0, 3, 5, and 7 mg/dL) for 6 hours. Cell viability was assessed using the MTT assay. Gene expression levels of *NLRP3*, *TNF-α*, *IL-1β*, and *AQP3* were measured by real-time PCR, and AQP3 protein expression was evaluated by Western blot. Statistical significance was defined as $p < 0.05$.
Results: No changes in explant viability were observed under the experimental conditions. Incubation with uric acid induced an increase in *TNF-α*, *IL-1β*, and *NLRP3* expression. Under these conditions, both gene and protein expression levels of *AQP3* were significantly reduced ($p < 0.05$).
Conclusion: Our findings show that elevated uric acid levels induce an inflammatory response in human placental explants, characterized by increased expression of *TNF-α*, *IL-1β*, and *NLRP3*. This pro-inflammatory environment is associated with a significant downregulation of *AQP3* at both gene and protein levels. As previously demonstrated, *TNF-α* negatively regulates *AQP3*, these results suggest that uric acid may contribute to impaired placentation in preeclampsia through an inflammation-mediated suppression of *AQP3* expression.

P2.46.
DIVERGENT PATTERNS OF ZKSCAN1 EXPRESSION IN PREECLAMPSIA SUBTYPES: IMPLICATIONS IN PATHOGENESIS OF EARLY AND LATE-ONSET PREECLAMPSIA

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Objectives: Preeclampsia, a hypertensive disorder of pregnancy is associated with both immediate and long-term maternal complications, subcategorized to early-onset (GA< 34weeks) and late-onset (GA≥34weeks) on the basis of time of disease onset and they represent distinct clinical and pathophysiological entities. Failure in early placental development and spiral artery remodelling processes are proposed to be prominently disrupted in EOPE than in LOPE. Spiral artery transformation is facilitated by the invasive phenotype of extravillous trophoblast The lack of significant changes in early placental development in LOPE suggest that other maternal or systemic factors may play a more dominant role in its pathogenesis.
ZKSCAN1 gene encodes a zinc finger protein with KRAB and SCAN domains. Recent studies suggest that ZKSCAN1 may have a context-dependent function, acting either as tumour suppressor or promoting oncogenic activity in certain cancers. This study aimed to evaluate whether ZKSCAN1 is differentially expressed in early and late-onset preeclampsia, and to consider its possible implication in trophoblast cell invasion
Methods: Placentae were collected from pregnancies complicated with EOPE(n=10), LOPE(n=10) and normotensive, non-proteinuric controls (n=20), analyzed for ZKSCAN1 expression by immunohistochemistry and immunoblotting. Serum ZKSCAN1 levels were also assessed by ELISA
Results: Placental ZKSCAN1 expression was elevated in EOPE compared to LOPE. However, the expression levels between EOPE cases and controls were comparable. The serum ZKSCAN1 levels were significantly higher in EOPE cases compared to gestational age-matched controls. Conversely, women with LOPE exhibited significantly lower serum ZKSCAN1 levels than their matched controls
Conclusion: The differential ZKSCAN1 expression in serum and placental tissues underscores distinct pathophysiological mechanisms underlying these two preeclamptic subtypes. Elevated ZKSCAN1 in EOPE supports its role in inhibiting trophoblast invasion and disrupting spiral artery remodelling, processes crucial during early gestation. The molecular implications of ZKSCAN1 in preeclampsia and its potential indicator as a biomarker or therapeutic target requires more research

P2.47.
PRMT1 AND CSE EXPRESSION DYNAMICS IN EARLY AND LATE ONSET PREECLAMPSIA: A NEW PERSPECTIVE ON PLACENTAL REGULATION

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Objectives: Protein arginine methyltransferase 1 (PRMT1) plays a pivotal role in vascular dysfunction through the production of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, and through activation of NF-κB signaling, which influences placental inflammation, angiogenesis, and hypoxia adaptation. Cycles of hypoxia–reoxygenation (H/R), frequently occurring in preeclamptic placentas, intensify oxidative stress and inflammatory cascades. In contrast, hydrogen sulfide (H_2S), synthesized by cystathionine γ-lyase (CSE), exhibits vasoprotective, anti-inflammatory properties and has been shown to attenuate ischemia/reperfusion injury. This study investigates the interplay between PRMT1 and H_2S signaling under hypoxic stress and evaluates PRMT1 expression in subtypes of preeclampsia.
Methods: Placental expression of PRMT1 and CSE was analyzed in women with early-onset (EOPE) and late-onset preeclampsia (LOPE) (n=13 and 12, respectively) and compared to gestational age-matched normotensive controls (n=25) using immunohistochemistry, western blotting, and double immunofluorescence. Serum levels were measured via sandwich ELISA in preeclamptic (n=40) and healthy pregnant women (n=40). PRMT1 expression in HTR-8/SVneo trophoblast cells was assessed under H/R injury and H_2S supplementation using western blot and immunofluorescence.
Results: EOPE placentae exhibited significantly elevated PRMT1 and decreased CSE expression compared to controls and LOPE cases. LOPE and control groups showed no significant difference. Circulating PRMT1 was markedly increased in EOPE but not in LOPE; however, CSE levels were reduced in both subtypes. H/R stress induced PRMT1 expression in trophoblast cells, which was mitigated by H_2S treatment.
Conclusion: Our findings reveal an inverse regulatory axis between PRMT1 and H_2S in preeclampsia, with more pronounced dysregulation in EOPE. The ability of H_2S to downregulate PRMT1 suggests a potential therapeutic avenue targeting this pathway to mitigate early-onset placental dysfunction.

P2.48.
THE CELLULAR AND MOLECULAR RESPONSES TO TRIPHENYL PHOSPHATE IN HUMAN UTERINE MYOMETRIUM: IMPLICATIONS ON PRETERM LABOR

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Objectives: Triphenyl phosphate (TPhP) is commonly used as organophosphate flame retardant or an additive plasticizer in consumer goods including nail polish. TPhP tends to migrate easily into the environment without chemically binding to the end products, chronically exposing the majority of the general population. Animal models suggest exposure to TPhP could impact child growth, especially during early life, yet impacts on human pregnancy outcomes are understudied. Therefore, we aimed to examine the in vitro effects of TPhP to human uterine myometrial cells, to define how exposure to TPhP affects human birth outcomes.
Methods: Human myometrial cells were obtained from premenopausal women who underwent hysterectomy. We treated myometrial cells with lipopolysaccharides (LPS), Progesterone, and TPhP (0.1, 1, and 10 M). We determined whether the exposure to TPhP triggers abnormal changes in intracellular calcium signals, the ultimate factor in early uterine contractions that result in preterm labor (PTL). We examined whether the

exposure to TPhP upregulates the expression profile of contraction-associated protein (CAP) genes in uterine smooth muscle cells. To investigate that TPhP mediates inflammatory imbalance through the ERK signaling pathway, ERK expression was evaluated.
Results: Prenatal TPhP led to an increase in the expression levels of oxytocin receptor (OTR) and connexin 43 (Cx-43). Exposure to TPhP could release various inflammatory cytokines, such as TNF-, IL-6, and IL-1 mainly via the MAPK/ERK kinase signaling pathways. These signaling molecules induced nuclear translocation of NF- kB in human myometrial cells.
Conclusion: Inflammatory imbalance plays a major role in the pathogenesis of PTL. In the present study, we investigated that maternal exposure to TPhP induced the expression of CAPs and involved NF-kB activation mediated by MAPK/ERK signaling pathways, providing insights into the association between endocrine-disrupting chemicals exposure and obstetric complications.

P2.49.
REDUCED NOTCH1 SIGNALING IN PLACENTAS FROM CASES OF CONGENITAL HEART DEFECTS

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Objectives: Congenital heart defects (CHDs) are the most common birth anomalies, accounting for approximately 260,000 deaths annually. Using two publicly available single-cell sequencing databases we identified NOTCH1 commonly expressed between first-trimester cardiac and placental endothelial cells. NOTCH1 knockout mice demonstrates embryonic lethality and failure of blood vessel formation in the labyrinth region, suggesting a critical role in placental angiogenesis. However, whether NOTCH1 signaling is disrupted in CHD placentas and how it impacts microvascular development remains unknown. We hypothesize that NOTCH1 signaling is downregulated in CHD placentas, and that reduction of NOTCH1 expression in human placental endothelial cells (HPMVECs) would impair cellular proliferation and survival.
Methods: Under IRB approval (IRB 202101799), placental samples were collected from CHD cases (n=12) and uncomplicated controls (n=8). RNA was isolated to assess NOTCH1, HES1, and HEY1 expression by quantitative PCR (qPCR). HPMVECs were cultured in DMEM supplemented with 20% fetal bovine serum and 10% penicillin-streptomycin. Upon reaching 60% confluency, Lipofectamine-mediated siRNA knockdown was performed targeting NOTCH1, alongside GAPDH, non-targeting scramble, and untreated controls. Forty-eight hours post-transfection, cells were harvested for qPCR analysis. Cell proliferation was evaluated using crystal violet staining, while cell viability was determined by trypan blue exclusion. Statistical significance was assessed using Student's t-test.
Results: NOTCH1 expression was not altered in CHD vs control placentas. HES1 expression was significantly reduced in all CHD subtypes (n=12; p=0.03), including septal (n=3; p=0.05) and conotruncal (n=3; p=0.01) defects. HEY1 expression was significantly reduced in septal defects ($p<0.05$) but not in other subgroups. In HPMVECs, NOTCH1 expression was reduced by 56% ($p<0.0001$, n=5 passages) following siRNA treatment. However, cell proliferation and viability remained unchanged (n=3; ns).
Conclusion: These findings demonstrate the functional activity of NOTCH1 may be impaired in CHD placentas. Furthermore, we successfully achieved NOTCH1 knockdown in HPMVECs giving us a model to define its role in the placental endothelium.

P2.50.
UNCOVERING THE ROLE OF PLACENTAL SERINE HYDROLASES IN LIPID SIGNALING: INSIGHTS INTO ENDOTHELIAL DYSFUNCTION IN PREECLAMPSIA

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Objectives: Preeclampsia (PE) is a hypertensive pregnancy disorder marked by systemic and placental endothelial dysfunction. Lipid mediators like arachidonic acid (AA) and its precursor 2-arachidonoylglycerol (2-AG) are central to vascular regulation and placental function. Our prior RNA-sequencing of human fetoplacental arterial endothelial cells (fpECAs) from PE pregnancies revealed downregulation of genes encoding serine hydrolases involved in 2-AG/AA metabolism. Here, we aimed to characterize activity profiles of these enzymes in fpECAs from PE, preterm birth (PTB), and term (TERM) pregnancies.
Methods: fpECAs were isolated from PE (n=5), PTB (n=5), and TERM (n=5) placentas. Cytosolic and membrane fractions were analyzed for serine hydrolase activity, including diacylglycerol lipases (DAGLs), monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH), and α/β-hydrolase domain-containing proteins (ABHDs), using activity-based protein profiling (ABPP) with a fluorophosphonate-TAMRA probe. MAGL activity was confirmed with fluorometric assay.
Results: Serine hydrolase activities were detected in fpECAs across all samples. MAGL exhibited the highest enzymatic activity, appearing as two active isoforms (~33 and 35 kDa) in both cytosolic and membrane fractions. FAAH activity was localized in the cytosol, while DAGLα was detected in the membrane. In contrast, DAGLβ, ABHD6, and ABHD12 showed negligible activity. Although overall enzyme activity profiles were similar among TERM, PTB, and PE samples, PE-derived fpECAs showed a trend toward reduced MAGL and increased DAGLα activity compared to gestational age-matched PTB. This trend was further supported by MAGL-specific fluorometric assay.
Conclusion: This study provides for the first time a map of metabolically active serine hydrolases in fpECAs, suggesting an active cellular 2-AG/AA metabolism. By moving beyond gene expression to direct enzymatic profiling, we established a foundation for understanding endocannabinoid and AA signaling dynamics in placental endothelial cells. Finally, the optimized ABPP technique in the placenta demonstrates its feasibility, reproducibility, and potential applicability to profiling enzyme activity in different physiological and pathophysiological conditions.

P2.51.
CANNABINOID EXPOSURE REDUCES PRO-INFLAMMATORY GENE AND CYTOKINE EXPRESSION IN HUMAN PLACENTAL EXPLANTS

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Objectives: Growing legalization and promotion of Cannabis sativa have increased its use during pregnancy, raising concerns about effects on placental and fetal development. Cannabinoids may exert pro- or anti-inflammatory effects, prompting investigation into their role in immune regulation during pregnancy. Given the placenta's critical immunomodulatory role mediated through cytokine and chemokine signaling, this study aimed to assess the impact of different cannabinoids—Δ9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabivarin (CBV), and cannabigerol (CBG)—on placental inflammatory responses in human placental explants. To mimic an inflammatory environment, explants were treated with lipopolysaccharide (LPS) to trigger a controlled immune response.
Methods: Villous explants were obtained from healthy term placentas (n=6) following elective cesarean sections. Explants were cultured with THC, CBG, CBN, or CBV at 2.5, 10, and 20 μg/mL for 48 hours, then exposed to LPS (1 μg/mL) for 4 hours. Metabolic activity, membrane integrity, and endocrine function were assessed via MTT, LDH, and hCG secretion assay, respectively. Expression of inflammatory genes (*IL6, TNF, IL1B, IL18, NFKB1, NLRP3, CASP1, TLR4, PTGS2*) was measured using RT-qPCR. Cytokine levels (IL-6, TNF-α, IL-1β) were quantified in culture medium via ELISA.
Results: Cannabinoid treatment had no significant impact on the metabolic activity, membrane integrity, or hCG secretion of placental villous explants. Notably, exposure to THC, CBG, CBN, and CBV resulted in a marked reduction in the expression of several pro-inflammatory genes, specifically *IL6, TNF, IL1B, IL18, NFKB1, NLRP3*, and *PTGS2*, with the most pronounced effects observed at the highest concentrations. In contrast,

CASP1 and *TLR4* expression remained unaffected. Observed gene expression patterns were reflected at the protein level, with decreased secretion of IL-6, IL-1β, and TNF-α into culture media.
Conclusion: These findings indicate that cannabinoids can attenuate LPS-induced inflammatory responses in human placental tissue, potentially impacting maternal-fetal immune interactions and the regulation of placental immune homeostasis.

P2.52.
PLACENTAL TRANSFER OF THE NOVEL ENDOTHELIN-1 RECEPTOR ANTAGONIST APROCITENTAN: A MODEL CONSIDERING ITS PRESENCE IN THE INTERVILLOUS SPACE

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Objectives: The vasoconstrictor endothelin-1 is elevated in preeclampsia, making endothelin receptor antagonists (ERAs) a logical therapeutic strategy. Here we evaluated the placental transfer of the novel ERA aprocitentan, which has recently been approved for hypertension treatment.
Methods: Dual-sided cotyledon perfusion was used to study transfer of aprocitentan, added maternally at a clinically relevant concentration of 150 ng/mL. Subsequently, these placentas were fetally exposed to endothelin-1. Antipyrine, which freely transfers to the fetal compartment, and fluorescein isothiocyanate [FITC]-dextran, which does not transfer, were used as comparator substances.
Results: After 3 hours of perfusion, the fetal-to-maternal ratio for total (free+protein-bound) aprocitentan was 0.25±0.04, while for free aprocitentan it was 0.42±0.08. The fetal aprocitentan concentrations were too low to block the contractile effects of endothelin-1 in the fetal compartment. After perfusion, 50-60% of aprocitentan was recovered in the maternal and fetal fluid compartments. Around 20-30% was present in tissue, where it had partially been hydrolyzed to its hydrolysis product ACT-080803. Since the recovery of maternally applied antipyrine and FITC-dextran was 85-90%, while fetally applied FITC-dextran was fully recovered, the missing 10-15% is likely present in the intervillous space.
Conclusion: Aprocitentan transfers across the human placental barrier, although its fetal levels following maternal application of 150 ng/mL were insufficient to block endothelin-1. Drug studies in isolated cotyledons should consider the intervillous space when calculating recovery.

P2.53.
RAPID OXIDATION OF THE CANNABIS COMPONENT CANNABIDIOL PREVENTS THE STUDY OF ITS PLACENTAL TRANSFER

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Objectives: Cannabis use is increasing worldwide, also among pregnant women. Cannabis use during pregnancy associates with pregnancy complications like fetal growth restriction and preeclampsia. While most research focuses on tetrahydrocannabinol, many pregnant women use cannabidiol (CBD)-containing products like CBD-oil. CBD is a non-psychoactive component of cannabis. It is unknown whether CBD is transferred across the placenta.
Methods: CBD transfer was studied using the *ex vivo* dual sided placenta perfusion set-up, where the maternal and fetal side are separately perfused with Krebs-Henseleit buffer, aerated with 95%O_2/5%CO_2 at 37°C. Placentas were obtained from women with uncomplicated singleton pregnancies. After a wash-out (45 minutes), CBD was added to the maternal buffer (200 mL) to 200 ng/mL. Subsequently, maternal and fetal samples were obtained every 30 minutes for 3 hours. Post-perfusion biopsies of the perfused cotelydon were taken to measure tissue accumulation. CBD concentrations were measured using LC-MS/MS.
Results: CBD concentrations in the maternal buffer dropped by >80% within 30 minutes and remained undetectable in fetal samples. Maximally 20% of the total CBD was recovered in tissue. When performing the experiments in the presence of 3 g/L bovine serum albumin, the drop in maternal CBD was still 43% after 30 minutes. Yet, the drop also occurred when performing the perfusion without a placenta, and it was absent when not aerating the buffer. This indicates that it was due to rapid oxidation in a placenta-independent manner. Reducing the oxygen percentage to 5% did not alter the oxidation pattern, nor did reducing the temperature to 20°C. Yet, the anti-oxidant vitamin C (15 mg/mL) could prevent CBD oxidation in aerated Krebs-Henseleit buffer by 95%.
Conclusion: Cannabinoids are prone to rapid oxidation, also in the presence of albumin, and thus their placental transfer can only be studied by adding an anti-oxidant to the perfusion buffer.

P2.54.
ANTI-INFLAMMATORY ACTIONS OF CANNABIDIOL IN HUMAN PLACENTAL MODELS

Ramon Portillo [1], Tetiana Synova [1], Mohammad Rida Ghadar [1], Mia Al souki [1], Eliska Sankova [1], Fiona Kumnova [1], Miloslav Machácek [2], Rona Karahoda [1], Cilia Abad [1], Frantisek Staud [1]. [1] *Department of Pharmacology and Toxicology, Charles University, Faculty of Pharmacy, Hradec Kralove, Czech Republic;* [2] *Department of Biochemical Sciences, Charles University, Faculty of Pharmacy, Hradec Kralove, Czech Republic*

Objectives: This study aimed to characterize the effects of cannabidiol (CBD) on lipopolysaccharide (LPS)-induced inflammation in human placenta-derived models. We assessed the impact of CBD on cytokine expression and secretion in both villous explants and primary trophoblasts, evaluated NF-κB activation in trophoblasts, and explored the potential involvement of canonical cannabinoid receptors.
Methods: Human placental explants (n=10) and primary trophoblast cells (n=6) isolated from term placentas were cultured and treated with CBD (0.1–40 μM) followed by LPS stimulation (0.5–1 μg/mL). Cell and tissue viability were confirmed using MTT and LDH assays. Cytokine expression (IL6, TNF, IL1B) was analyzed by RT-qPCR and ELISA. In primary trophoblasts, NF-κB p65 nuclear translocation was assessed by immunofluorescence. To evaluate receptor involvement, cells were pre-treated with antagonists for CB1 (rimonabant), CB2 (SR144528), and TRPV1 prior to CBD exposure.
Results: CBD significantly reduced LPS-induced expression and secretion of IL-6, TNF-α, and IL-1β in both placental explants and primary trophoblasts, without affecting viability. In trophoblasts, CBD also attenuated NF-κB p65 nuclear translocation. The anti-inflammatory effects were not reversed by CB1, CB2, or TRPV1 antagonists.
Conclusion: CBD exerts anti-inflammatory effects in human placental models, including reduced expression of pro-inflammatory cytokines and inhibition of NF-κB nuclear translocation in trophoblasts. As these effects were not reversed by CB1, CB2, or TRPV1 antagonists, they may involve alternative, non-canonical pathways. The observed NF-κB modulation could partially explain the downregulation of inflammatory mediators. Further studies are warranted to clarify the underlying mechanisms and assess the relevance of these findings for pregnancy.
This study was supported by the Czech Science Foundation (23-07094S), the National Institute for Neurological Research (EXCELES, LX22NPO5107 – funded by the EU Next Generation), and the Grant Agency of Charles University (GAUK 336322).

P2.55.
COMPARATIVE ANALYSIS OF HHC AND THC EFFECTS ON VILLOUS AND EXTRAVILLOUS TROPHOBLAST CELL LINES: IMPLICATIONS FOR REDOX SIGNALING AND PLACENTAL FUNCTIONS

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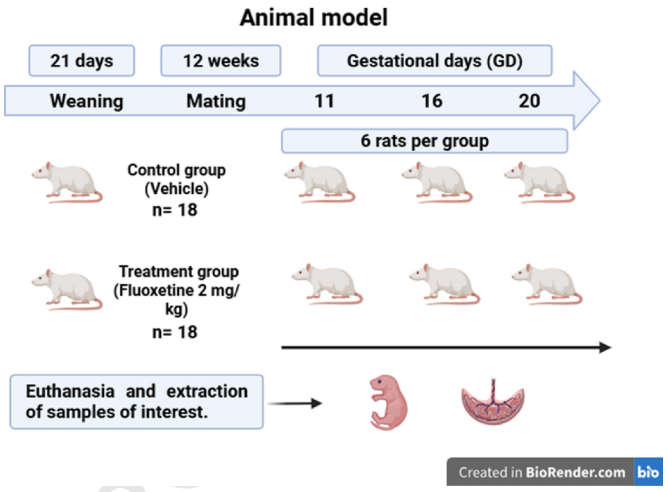
Objectives: Hexahydrocannabinols (HHCs) are semi-synthetic cannabinoids gaining popularity due to their ambiguous legal status. Often marketed with health-related claims, HHCs may be perceived as safe alternatives to natural cannabinoids, posing a potential risk to pregnant women. Despite increasing use, no data exist on their effects during pregnancy. This study aims to evaluate the impact of HHC, in comparison to Δ9-tetrahydrocannabinol (THC), on cytotoxicity, proliferation, and gene expression in placental cell models.
Methods: We utilized three trophoblast cell lines, HTR-8/SVneo (extravillous), Bewo (villous) with and without Forskolin and ACH-3P (extravillous), as well as primary trophoblast cells derived from term placenta in cytotrophoblast and syncytiotrophoblast stages. Cells were treated with two isomers of HHC, (9R)-HHC and (9S)-HHC, and THC at concentrations of 0.1, 0.5,1, 5, 10 and 20 μM. Cytotoxicity and viability were assessed via lactate dehydrogenase (LDH) release, MTT, and ATP content. Cellular proliferation was assessed using Image cytometer for high-content multifactor analysis (Olympus/EVIDENT), while gene expression of markers associated with trophoblast differentiation, invasion, and apoptosis was analyzed via qRT-PCR. ROS activation was evaluated using CM-H2-DCFDA. Additionally, syncytialization was monitored through the assessment of cell fusion markers.
Results: HHC modulates key aspects of trophoblast biology, including proliferation, cytotoxicity, and gene expression profiles related to differentiation and placental function. Given preliminary observations and structural differences, HHC may exert distinct or more pronounced effects compared to THC. Ongoing analyses will help clarify potential risks associated with semi-synthetic cannabinoid exposure during early placental development.
Conclusion: Our findings highlight similar effects of HHC and THC on trophoblast physiology, emphasizing the need for further research to clarify the safety profile of synthetic cannabinoids during pregnancy. Understanding their impacts is crucial for assessing potential reproductive risks.
Acknowledgment: This project was funded by the Grant Agency of the Czech Republic (23-07094S).

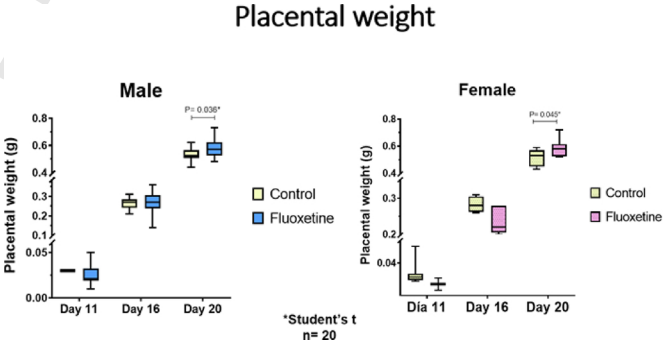
P2.56.
SEX-SPECIFIC EFFECT OF PRENATAL FLUOXETINE ADMINISTRATION ON THE EXPRESSION OF GENES INVOLVED IN PLACENTAL SEROTONIN REGULATION.

Jorge Eduardo Sifuentes-García [1], Laura Jazel Barragán-Zúñiga [2], Carlos Galaviz-Hernández [1], Claudia Ivette Gamboa-Gómez [3], Martha Guadalupe Sosa-Macías [1]. [1] *Instituto Politécnico Nacional-CIIDIR, unidad Durango., Durango, Mexico;* [2] *Centro Estatal de Cancerología, Secretaría de Salud Durango., Durango, Mexico;* [3] *Instituto Mexicano del Seguro Social-Unidad de Investigación Biomédica, Durango., Durango, Mexico*

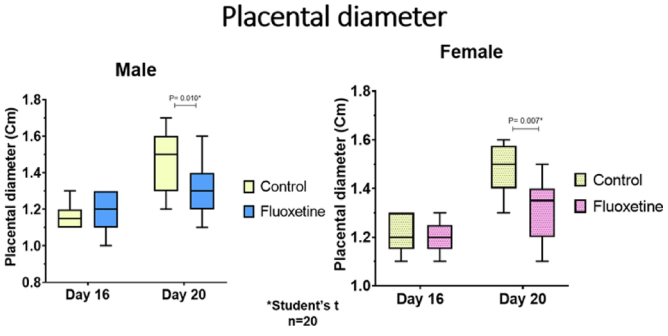
Objectives: This study aimed to evaluate the effects of prenatal fluoxetine (FLX) exposure on placental mRNA expression of *Tph1, Mao-a, Sert, Oct3,* and *Lat1*, considering fetal sex, and to assess correlations with fetal and placental somatometric parameters in pregnant rats.
Methods: The study was approved by the National Scientific Research Committee of the Instituto Mexicano del Seguro Social (R-2024-785-072). Pregnant rats were orally treated with 2.06 mg/kg of FLX or water every 24 h from gestational day (GD) 1. Evaluations were performed on GD 11,16, and 20.



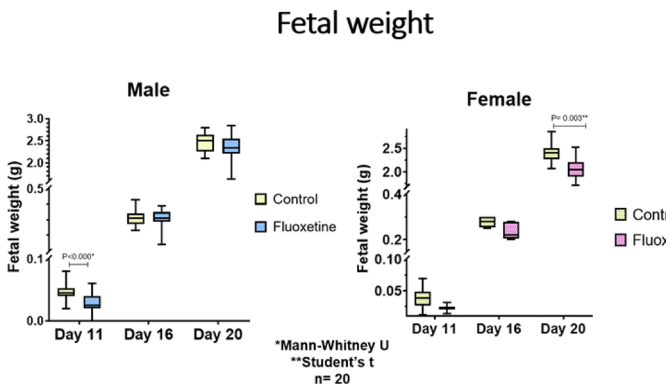
Fetal and placental somatometric parameters were analyzed and sex was identified in both by endpoint PCR. Placental gene expression was assessed by qPCR. The Mann-Whitney U test, Student's t test and correlation tests (Pearson and Spearman) were used to determine the statistical significance ($p < 0.05$).
Results: Placental weight was higher in the FLX-exposed groups of both sexes at DG20 ($p<0.05$).



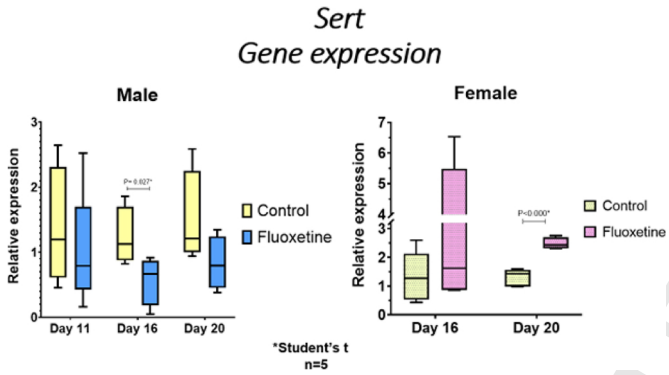
Placental diameter was smaller in the exposed groups of both sexes at DG20 ($p < 0.05$). While placental thickness was similar between the exposed and control groups.



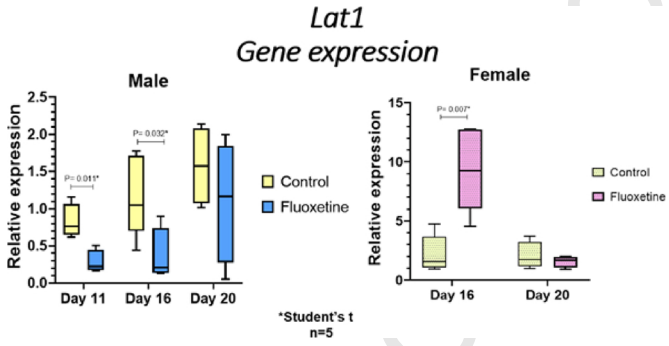
Fetal weight was lower in females exposed to FLX at GD20 and in males at GD11 ($p<0.05$).



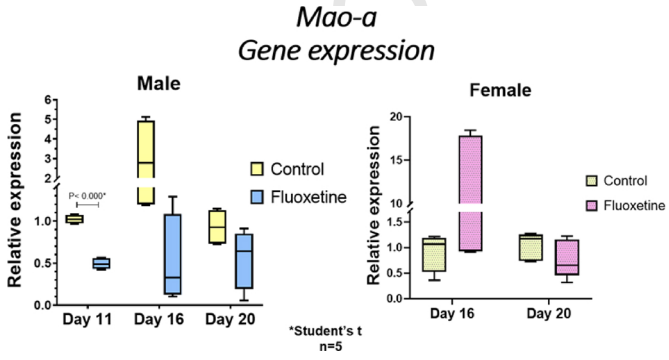
Sert expression decreased in exposed males at GD16 and increased in females at GD20 ($p < 0.05$).



Lat1 was downregulated in exposed males (GD11, GD16) and upregulated in exposed females (GD16) ($p < 0.05$).



Mao-a was downregulated in males exposed (DG11).



A positive correlation was found between fetal weight/*Mao-a* in exposed females at GD20. In exposed males at GD20, placental thickness positively correlated with *Lat1, Oct3*, and Sert expression, whereas in those exposed at GD16, *Oct3* showed a negative correlation with both placental weight and thickness.

Conclusion: Exposure to fluoxetine during pregnancy alters placental and fetal somatometry, as well as the expression of *Sert, Lat1, Mao-a,* with sex-dependent effects.

P2.57.
NUTRIENT-TOXIN INTERACTIONS AT THE PLACENTAL BARRIER: INTERFERENCE OF METHYLMERCURY WITH ESSENTIAL COPPER AND IRON HOMEOSTASIS

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Objectives: Mercury (Hg) from natural and anthropogenic sources accumulates as methyl mercury (MeHg) in the aquatic environment, leading to elevated levels in edible fish, which is still a present concern. Pregnant women and their fetuses are particularly at risk, as MeHg can cross the placental barrier, impairing fetal (neuro)development. Until today the effects of MeHg exposure on placental cells remains unclear, including its potential to alter copper (Cu) and iron (Fe) homeostasis – both essential for fetal development and healthy pregnancy outcomes.
Methods: Transfer experiments were conducted using the BeWo b30 *in vitro* model. MeHg chloride was applied one hour after Cu or Fe treatment on the apical (maternal) side for 6 and 24 h. Barrier tightness was assessed *via* transepithelial electrical resistance using the cellZscope device. Metal determination was executed by using inductively coupled plasma coupled to tandem mass spectrometry. Further toxicity-related endpoints like the formation of reactive oxygen species (ROS) or glutathione levels (GSH) were determined using *in vitro* dye assays or analytical instrumentation.
Results: Cu has a slight impact on Hg transfer, while physiological MeHg concentrations are leading to decreased Cu and Fe amounts on the basolateral compartment. All combinations of MeHg with Cu or Fe induced ROS formation 2 h after treatment which is partly supported by altered GSH and GSSG levels. In contrast, MeHg alone was only able to decrease GSH/GSSG ratio without evidence of ROS induction.
Conclusion: Data clearly demonstrate altered Cu and Fe transfer across the BeWo b30 cell layer, when MeHg is present, potentially involving oxidative stress mechanisms. Fetal development can be adversely affected by MeHg concentrations that remain asymptomatic in pregnant women. Therefore, a detailed understanding of MeHg-induced placental toxicity, also including its impact on essential trace element homeostasis is crucial for improving Hg risk assessment during pregnancy.

P2.58.
EVALUATION OF TRANSPLACENTAL TRANSFER AND PLACENTAL EFFECTS OF TWO RECOMMENDED MELANOMA TARGETED THERAPIES

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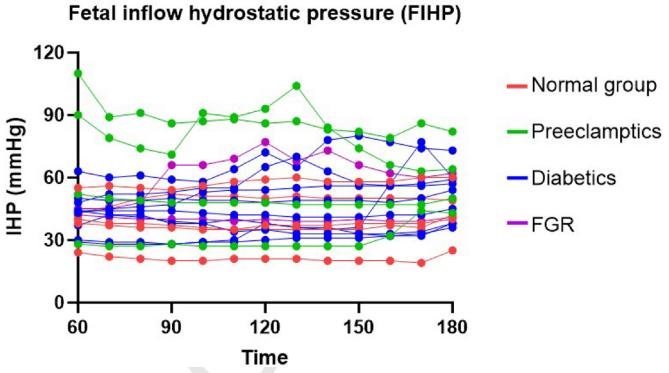
Objectives: To determine the fetal transfer rate and placental accumulation parameters of dabrafenib and trametinib.
To determine the potential effects of the two drugs on human trophoblastic cells.
Methods: Placentas from normal term pregnancies were collected immediately after delivery from donors having given a written consent.
Placental transfer and accumulation were assessed via ex vivo perfusion : Cotyledons were perfused in a double open circuit with a solution containing, on the maternal side, trametinib or dabrafenib and its metabolites

(hydroxy-dabrafenib and desmethyl-dabrafenib). Concentration of drugs in samples from the fetal side was evaluated by mass spectrometry. Antipyrine, a freely diffusing marker served as positive control.
The effects of the 2 drugs on trophoblastic cells survival, differentiation abilities and transport proteins expression were assessed on primary culture of villous cytotrophoblasts from third trimester placentas and on BeWo cell line.
After exposure to drugs at concentrations ranging from 100μM to 1 nM, cell survival was assessed after 24, 48 and 72h using the CellTiter Glo assay. IC50 were determined for both cell types.
After exposure to IC50 and to maximal plasmatic concentration (Cmax) based on litterature on phase I and II clinical trials, cell differentiation was assessed by HCG assay, microscopic evaluation of syncitization by immunocytochemistry and expression of syncitin and E-cadherin by RTqPCR.
Transport protein expression was assessed by RTqPCR.
Results: Both drugs moderately cross the placental barrier, with FTR 8.6 ± 3.1% for trametinib and 15.3 ± 5.4% for dabrafenib. They weakly accumulate in placental tissue, uptake ratios are respectively 7.5 ± 1.4% and 3.7 ± 2.2%.
Trametinib induces cell death at 72h at high concentrations. Dabrafenib does not decrease cell survival of primary culture or proliferation of BeWo cell line.
Conclusion: In vitro studies of the effect of these drugs on human trophoblastics cells are still ongoing and more detailed results will be available at the conference.

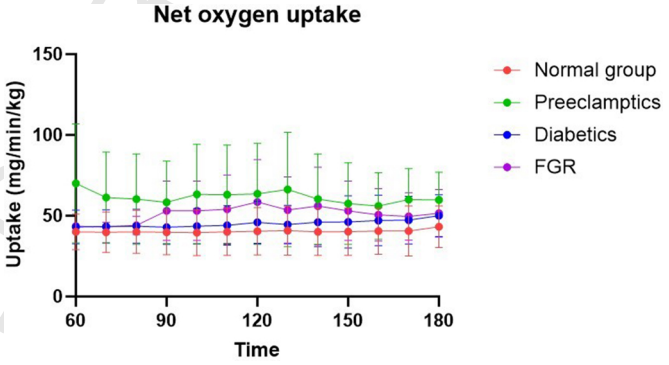
P2.59.
INVESTIGATION OF HUMAN PLACENTAL PHYSIOLOGY IN PREGNANCIES AT HIGH RISK OF STILLBIRTH TO IDENTIFY STRUCTURAL, FUNCTIONAL, AND MOLECULAR ALTERATIONS THAT MAY CONTRIBUTE TO ADVERSE FETAL OUTCOMES.

Angelos Evangelinos, Alexander Heazell, Igor Chernyavsky, Paul Brownbill. *The University of Manchester, Manchester, United Kingdom*

Objectives: The objectives of this project are to assess and understand net oxygen transfer, fetal inflow hydrostatic pressure, and metabolism using the *ex vivo* placental perfusion model, with the ultimate goal of comparing these findings to clinical metrics such as MRI modalities.
Additional ongoing work (not included in this abstract) involves the use of immunostaining to identify and evaluate pathophysiological changes—such as fibrin deposition and the associated expression of coagulation-related molecular factors—in pregnancy-related diseases compared to controls.
Methods: Peripheral human placental cotyledons obtained from uncomplicated and high-risk pregnancies were perfused in open-circuit configuration for 120 minutes, with maternal and fetal flow rates at 14 mL/min and 6 mL/min, respectively. Throughout perfusion, fetal-side inflow hydrostatic pressure (FIHP), perfusate partial pressure of oxygen, and maternofetal clearance of antipyrine, creatinine and FITC-inulin were assessed.
Results: In both preeclamptic and diabetic placentas an increasing trend in variability in the feto-placental pressure trace was observed, with the former group accompanied by unstable perfusion resistance tracings (Mann-Whitney, p>0.05) and net oxygen uptake per unit mass.



A comparison of mean net oxygen uptake per timepoint also showed an increase in the preeclamptic group vs. control group (Friedman's, p<0.0001).



The mean FITC-inulin:creatinine, FITC-inulin:antipyrine and creatinine:antipyrine ratios of disease groups showed no difference when compared to control.
Conclusion: Currently, no quantifiable differences are yet to be observed due to low power of the study, but more data will be added. Preliminary findings indicate impaired regulation of fetoplacental vascular tone in preeclamptics and some diabetic cases and mean net oxygen transfer in preeclamptics. The maternofetal transfer of molecular markers to understand the permeability and porosity of the cellular membrane showed no physiological changes in the groups affected by placental disease.
This research was supported by Wellcome LEAP under the *In Utero* program.

P2.60.
SHEAR STRESS MODULATES PFOS-INDUCED CYTOKINE RELEASE AND VIABILITY IN PLACENTAL EXPLANTS

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Objectives: Fluid shear stress is vital for placental function and perfusion of the placenta starts to establish as early as 6 weeks of gestation. Despite its importance, it is not commonly incorporated into *in vitro* placental toxicology research. This study aims to investigate the effects of Perfluorooctane sulfonic acid (PFOS) on cytokine release and tissue viability in human placental villi, comparing conventional static explant culture to conditions that simulate physiological shear stress.
Methods: Human term placental villous explants were cultured under static or laminar flow conditions for 24 hours and either treated with PFOS or a vehicle control. IL-6, IL-8, and TNF-α levels were quantified in supernatants. Tissue viability was measured using an LDH assay.
Results: Under static conditions, PFOS significantly reduced placental tissue viability and increased inflammatory IL-8, IL-6 and secretion. However, under flow conditions, tissue viability was largely preserved and

the PFOS-induced secretion of IL-8 and TNF-α was significantly suppressed, while IL-6 levels remained relatively high.
Conclusion: Under static conditions, PFOS significantly compromises placental tissue viability and induces inflammatory responses, whereas physiological flow conditions may help to reduce the adverse effects. Besides flow, the presence of a functional syncytiotrophoblast is proposed to affect toxicity outcomes of placental models.
In future, we aim to assess a panel of environmental chemicals/materials (incl. PFAS, (nano)pesticies, 2D materials) in different static and dynamic *in vitro* and *ex vivo* human placenta models in the EU-CHIASMA project, with the goal to establish a robust, accessible and predictive NAM (new approach methodology) for developmental toxicity testing in a regulatory context.

P2.61.
TRANSPLACENTAL PASSAGE OF AMPICILLIN: IMPLICATIONS FOR ANTENATAL THERAPY

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Objectives: Bacterial infections during pregnancy can endanger both mother and fetus, leading to premature rupture of membranes, chorioamnionitis, preterm birth, and neonatal sepsis. Effective antibiotic therapy must ensure sufficient exposure. Ampicillin is widely used in pregnancy, particularly against GBS and *E. coli*, but its pharmacokinetics, including placental transfer, are not fully understood. This study evaluated ampicillin's transplacental transfer using an *ex vivo* placenta dual-side perfusion model and assessed maternal serum concentrations in pregnant women undergoing therapy.
Methods: Six placentas were perfused *ex vivo* with 50 or 100 mg/L ampicillin. Ampicillin concentrations were assessed both in the maternal and fetal circuits every 30 minutes during a 4 hours. Additionally, maternal serum samples from six pregnant women (gestational age: 24+4 to 33+0) receiving 2 g ampicillin intravenously were analyzed every 30 minutes up to 4 hours post-administration and antibiotic concentrations were determined using LC-MS.
Results: Concentrations determined in the maternal perfusion circuit were 33.4 ± 15.9 and 41.2 ± 5.8 mg/L, as well as 15.4 ± 4.5 and 25.3 ± 19.9 mg/L in the fetal circuit. The transfer rate was about 25% after 4 hours of perfusion. Maternal serum concentrations at 30 minutes reached 54.1 ± 6.4 mg/L and declined to 2.3 ± 1.6 mg/L (range: 0.5 to 4.2 mg/L) at 4 hours.
Conclusion: This study using an *ex vivo* placenta perfusion model, revealed only moderate transplacental transfer of ampicillin. This finding, in the context of the retrieved serum concentrations, suggests that adjusted dosing may be required in severe conditions to optimize drug exposure, emphasizing the need for further pharmacokinetic research in pregnancy.

P2.62.
ASSESSMENT OF METHAMPHETAMINE TRANSFER ACROSS THE HUMAN PLACENTA USING A NEW SIMULTANEOUS LC-HRMS ANALYSIS OF METHAMPHETAMINE, AMPHETAMINE, AND TRANSFER CONTROLS IN PERFUSION MEDIA

Lea Dietrich [1], Jana Pastuschek [1], Frank Peters [2], Tanja Groten [3,1], Daniela Wissenbach [2]. [1] *Placenta Lab, Department of Obstetrics, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany;* [2] *Institute of Forensic Medicine, Jena University Hospital, Friedrich Schiller University Jena, Jena, Germany;* [3] *University Hospital Cologne, Cologne, Germany*

Objectives: Methamphetamine is a common drug of abuse, including during pregnancy, and its use has been associated with fetal growth restriction, preterm birth, intrauterine fetal death, and placenta abruption. Although limited animal studies have shown that methamphetamine crosses the placenta, its transfer and effects on the human placenta remain unclear. Therefore, this study aims to analyze the transfer of methamphetamine and its metabolite amphetamine using the human *ex vivo* placenta perfusion. To minimize time, cost, and sample volume, a simultaneous LC-HRMS method was developed for analyzing methamphetamine, amphetamine, and the transfer controls antipyrine and creatinine.
Methods: Healthy term placentas were perfused for four hours with methamphetamine and transfer controls. Samples were collected from maternal and fetal circulations at regular intervals. For LC-HRMS analysis, 50 µl samples underwent protein precipitation with 250 µl ACN:MeOH (4:1) containing internal standard. Supernatants (50 µl) were diluted with mobile phase. LC separation was performed using gradient elution on a Nucleoshell RP 18 plus column. Analytes were detected using HRMS after electrospray ionization in positive mode, with a mass accuracy of 20 ppm. A six-point calibration model and quality control samples were used.
Results: Preliminary data show that methamphetamine crosses the human placenta. The LC-HRMS method effectively separated and identified all analytes. Calibration curves were linear for methamphetamine/amphetamine in concentration ranges of 0.01-1 µg/mL, antipyrine between 5-125 µg/mL, creatinine 10-600 µg/mL..
Conclusion: The developed simultaneous analysis of methamphetamine, amphetamine, and transfer controls using LC-HRMS offers a valuable and more efficient approach to studying transfer of methamphetamine in human placenta perfusion. This is of clinical interest for a more in-depth investigation of methamphetamine abuse during pregnancy.

P2.63.
HUMAN PLACENTA EXPLANT CULTURE AS NEW APPROACH METHODOLOGY (NAM) FOR TOXICITY STUDIES

Emily Könnecke [1], Leon Philipp [1], Karthika Muthuraj [1], Aleksandra Kurova [1], Johanna Liesa Gollasch [1], Martin Westermann [2], Alexander Berndt [3], Dagmar Samsel [3], Michael Kiehntopf [4], Udo R. Markert [1], Astrid Schmidt [1]. [1] *Placenta Lab, Department of Obstetrics, Jena University Hospital, Jena, Germany;* [2] *Electron Microscopy Center, Jena University Hospital, Friedrich Schiller University, Jena, Germany;* [3] *Section Pathology, Institute of Legal Medicine, University Hospital Jena, Jena, Germany;* [4] *Department of Clinical Chemistry and Laboratory Medicine, Jena University Hospital, Jena, Germany*

Objectives: The human placenta is a unique organ, available daily and containing living tissue with diverse cell types even several hours post-delivery. It is an ideal model for toxicity testing of various substances on human tissue. Our objective was to establish a new approach method for the long-term culture of multiple placenta explants under different conditions. This included optimizing culture parameters such as medium composition and selecting appropriate sampling sites. As proof of concept, we used copper, a well-characterized heavy metal, to examine its toxic effects compared to untreated controls.
Methods: Culture conditions were systematically adjusted to extend explant viability, including testing various medium formulations and concentrations of human serum. Histological analyses, multiplex immunofluorescence, and transmission electron microscopy were used to monitor structural and cellular changes. Hormone secretion into the culture medium was assessed as an indicator of tissue functionality. Additionally, we evaluated the expression of senescence markers under toxic conditions using PCR.
Results: Optimal culture conditions included 5% human serum, which maintained explant viability for up to 14 days. Histological, immunofluorescence, and TEM analyses showed initial degeneration of the syncytiotrophoblast layer, followed by regeneration from the underlying cytotrophoblast. Hormone secretion was consistent with tissue viability and syncytiotrophoblast turnover. To compare explants from individual placentas, we established a historical control database from all group datasets. Copper exposure led to a significant decrease in hormone secretion, increased expression of senescence markers, and histological alterations, indicating a dose-dependent toxic response.

Conclusion: We established a placental explant culture system that maintains viability for 14 days, allowing comprehensive analysis using multiple methodologies. Copper exposure confirmed the model's capacity to detect dose-dependent toxic effects, supporting its use in high-throughput toxicity screening and contributing to the 3R principle by reducing reliance on animal testing in toxicological research.
Funding: URM, AS: BMBF 03VP08692, AIF; KK5360801NK1, CEPRE/ BMBF 01GR2305A; EK: IZKF Jena

P2.64.
SCORPION VENOM AND FETAL DEVELOPMENT: TERATOGENICITY IN ZEBRAFISH AND HUMAN PLACENTAL MODELS

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Objectives: Envenomations by venomous animals represent a global public health challenge. In Brazil, the scorpion *Tityus serrulatus* stands out as the most dangerous and widespread species, due to its parthenogenetic reproduction, potent venom, and adaptability to urban environments. Its venom contains low molecular weight toxins that act on voltage-gated ion channels, leading to excessive neurotransmitter release. Additionally, it can trigger a strong inflammatory response, increasing tissue damage. Evidence indicates that the venom may also induce uterine contractions, resulting in fetal malformations, intrauterine death, and spontaneous abortions. Despite advances, important gaps remain regarding the venom's effects in pregnant women and its ability to cross the placental barrier. This study assessed the teratogenic potential of *T. serrulatus* venom using the alternative zebrafish (*Danio rerio*) model, widely employed in embryogenesis and teratogenesis research.
Methods: Embryos were exposed to five venom concentrations and evaluated at four experimental time points. Teratogenic effects were analyzed based on embryo survival and the presence of developmental abnormalities, following OECD Test Guideline 236.
Results: The highest concentration (100 μg/mL) reduced survival to approximately 30% at 72 hpf. As concentrations decreased, a gradual increase in survival rates was observed, with the lowest concentration (6.8 μg/mL) maintaining rates comparable to the control groups (around 70%). Developmental abnormalities were also observed, including embryo coagulation, edema, lack of pigmentation, and incomplete formation of the eyes, head, and tail.
Conclusion: In future steps, *ex vivo* human placental perfusion models (unilateral and bilateral), along with a placenta-on-a-chip (PoC) system, will be used to investigate whether the venom can cross the human placental barrier. These models, including the PoC system, will provide dynamic and physiologically relevant conditions to analyze the transplacental passage of venom components and contribute to the understanding of the teratogenic risks associated with *T. serrulatus* envenomation during pregnancy and its potential impacts on fetal development.

P2.65.
EXPLORING HUMAN PLACENTAL EXPLANTS FOR IMMUNOTOXICOLOGICAL TESTING

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Objectives: The placental immune system significantly influences the course of pregnancy. Disruptions caused by xenobiotics may lead to adverse outcomes.
Toxicological studies regarding the placental immune system are commonly performed on animal models such as rodents, giving only limited information due to interspecies differences.
Human placental explants have shown promising results as an alternative model in toxicology, eliminating this issue. This study examines their potential in immunotoxicology aiming to provide a basis for a future test system.
Methods: The explants were prepared using villous tissue from healthy, term placentas (n=4) and cultured for 24 h using different immunostimulants. In order to cover a range of possible immune reactions they were stimulated with lipopolysaccharide (LPS), poly(I:C) or phytohemagglutinin (PHA). A non-stimulated group served as control.
Analyses of the reactions comprised cytokine release by ELISA, proteome profiling by mass spectrometry and exploration of immunologically relevant microRNA expression using qPCR.
Results: LPS and PHA induced strong release of IL-6, IL-8, IL-1ß and TNF- α. Poly(I:C) triggered a similar cytokine pattern, but at lower levels, and additionally induced INF-ß release. Il-10 and CD163 remained largely unchanged. Proteome profiling allowed an identification of over 8000 proteins, altered through the different stimuli. Preliminary data suggests an upregulation of hsa-mir-517b and hsa-mir-146a in response to LPS, poly(I:C) and PHA.
While reproducible reaction patterns can be observed upon specific stimulation, explant responses also show inter- and intraplacental variation.
Conclusion: Interplacental variability may complicate the determination of threshold values but also offers the opportunity to detect rare side effects, reflecting the unique biology of each human placenta – unlike the uniformity of inbred animal strains. Overall, our results highlight the considerable value of human placental explants as a model in immunotoxicology. Further analyses are planned to strengthen this conclusion.

P2.66.
DEVELOPMENT OF METHOTREXATE-LOADED NANOPARTICLES FOR THE TREATMENT OF GESTATIONAL TROPHOBLASTIC NEOPLASIA

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Objectives: Gestational Trophoblastic Neoplasia (GTN) is group of rare malignancies usually treated with methotrexate (MTX) for low-risk cases. However, around 30% of patients develop resistance, requiring aggressive treatments that can harm fertility. To improve therapy, we developed MTX-loaded nanoparticles (NPs) that will be functionalized with an anti-PD-L1 antibody for targeted drug delivery, combining chemotherapy with immunotherapy to enhance efficacy and minimize off-target effects.
Methods: MTX was encapsulated into poly(L-lactide-co-glycolide) acid-based NPs using nanoprecipitation and emulsion-solvent evaporation techniques, varying polyvinyl alcohol (PVA) concentrations to optimize formulation. Nanoparticles size and polydispersity index (PDI) were measured using dynamic light scattering and scanning electron microscopy. Encapsulation efficiency and drug loading were evaluated by spectrophotometry and UPLC. Cellular uptake and cytotoxicity were assessed on choriocarcinoma cell lines (JEG-3, BeWo) using DiO-labeled NPs and MTT assays, respectively. Patient-derived organoids from invasive mole tissue were also established as disease-relevant models.
Results: NPs generated by nanoprecipitation with 0.4% PVA showed optimal characteristics: 170 nm size and PDI around 0.2. Emulsion-based method yielded larger NPs (250–300 nm) with lower PDI (0.03–0.09). Encapsulation rates averaged 60%, with a peak of 70% using emulsion with

0.2% PVA. Nanoprecipitated NPs exhibited rapid internalization (within 15 min) in both cell lines, while emulsion-based NPs were internalized by 30 min. MTX-loaded NPs had a lower IC50 than free MTX, and unloaded NPs showed no cytotoxicity.
Conclusion: Future work involves standardizing the nanoprecipitation method using microfluidics to improve consistency and further enhance MTX encapsulation. These optimized NPs will be conjugated with anti-PD-L1 antibodies and evaluated in co-cultures of organoids and patient-derived immune cells to assess therapeutic potential.

P2.67.
PLACENTAL MIR-181A-5P MAY NEGATIVELY MODULATE DEVELOPMENT, CELLULAR DIFFERENTIATION, AND HOMEOSTASIS BY DIRECTLY TARGETING *NR2C2* IN PATIENTS WITH BREAST CANCER DURING PREGNANCY

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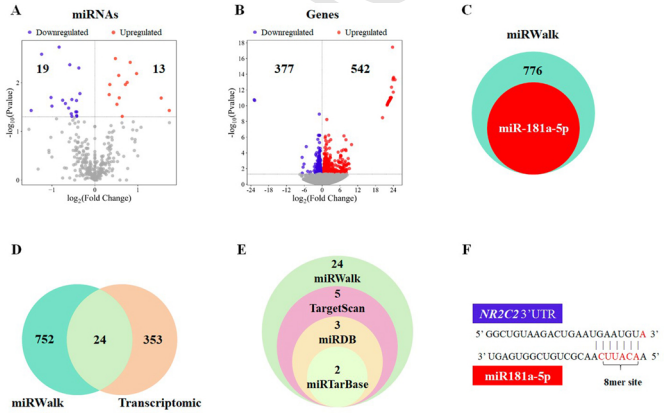
Objectives: Evaluate the influence of placental miRNAs and potential targets in patients with breast cancer during pregnancy (PrBC).
Methods: Placentas were obtained from patients diagnosed with PrBC who received chemotherapy (case group, n=7) and from healthy participants (control group, n=8) (Ethics Committee: #65070122.0.0000.5404). miRNA and gene expression were analysed using RT-qPCR and RNA-Seq transcriptomics, respectively, in the placental chorionic villi. The Differentially Expressed miRNAs (DEmiRs) and Genes (DEGs) were calculated by *t*-test and DESeq2, respectively. The respective potential targets were identified by bioinformatics tools. *P*-value<0.05 was considered significant for all analyses.
Results: The gestational age (35.4±4.4 *vs* 38.9±1.1), placental weight (450.6±153.4 *vs* 682.9±138.6), and neonatal birth weight (2262.5±874.1 *vs* 3363.6±342.3) were lower in the case group compared to the control group. A total of 32 DEmiRs and 919 DEGs were identified in the case group compared to the control group. Among them, 19 miRNAs and 377 genes were downregulated, while 13 miRNAs and 542 genes were upregulated (Figure 1A and 1B). Following validation analyses, the upregulated miR-181a-5p was selected for the identification of potential target genes. Initially, 776 targets were predicted using the miRWalk database (Figure 1C). These predicted targets were then compared with the 377 downregulated genes, revealing 24 overlapping genes (Figure 1D). Of the 24 genes, *MIDEAS*, *NR2C2*, and *RIMKLB* were identified as potential targets based on their presence in at least three of four databases: miRWalk, TargetScan, miRDB, and miRTarBase (Figure 1E). Among these, the 3'UTR of *NR2C2* contains a predicted 8mer binding site for miR-181a-5p (Figure 1F).



Conclusion: Our findings suggest that miR-181a-5p may negatively regulate *NR2C2* gene expression, potentially affecting key placental biological processes such as development, cellular differentiation, and homeostasis in patients with PrBC. Consequently, the regulatory effects of miR-181a-5p may contribute to adverse outcomes, including intrauterine growth restriction and preterm birth.

P2.68.
CIRCULATING BIOMARKER TAGLN AS A REGULATOR OF AUTOPHAGY INDUCED VASCULAR REMODELING IN PLACENTA ACCRETA SPECTRUM

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Objectives: As a critical obstetric condition, placenta accreta spectrum (PAS) disorders are increasingly prevalent and strongly associated with iatrogenic uterine injury.
Methods: Our research group has closely monitored the evolving landscape of PAS diagnosis, treatment, and mechanistic investigation, emphasizing key elements in the design of translational and basic science studies. We employed diverse sample types, enrichment strategies, and multi-omics modalities to identify differential patterns linked to disease severity.
Results: Notably, transgelin (TAGLN) emerged as a potential biomarker for predicting adverse outcomes. To further explore targeted intervention mechanisms, we utilized single-cell omics and spatial transcriptomics at the maternal-fetal interface, examining microenvironmental variations during trophoblast migration and vascular remodeling. Our findings suggest that specific decidual and smooth muscle cell subtypes influence extracellular matrix remodeling, modulate immune tolerance, and affect vascular restructuring. These insights were validated through co-culture systems demonstrating underlying mechanisms. Furthermore, leveraging phenotypic differences in animal models of uterine injury and initial autophagy-targeted interventions,
Conclusion: The findings in circulation could be used for tracing the maternal fetal interface and provide foundational evidence for precision preconception and perinatal management strategies for PAS.

P2.69.
MELATONIN, A MULTI-WEAPONED SOLDIER: EFFECT OF OXIDATIVE STRESS IN TROPHOBLAST CELLS.

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Objectives: Melatonin is a powerful antioxidant that is produced by and protects healthy trophoblastic cells from oxidative stress. On the other hand, in choriocarcinoma cells, this indolamine promotes cell death, but the mechanism behind this effect remains to be studied. **The main objective** was to determine the effect of melatonin on human BeWo choriocarcinoma cell line by evaluating its effect on oxidative stress markers and its antioxidant defence enzymes.
Methods: BeWo cells were exposed under normoxia (8% O_2) or hypoxia-reoxygenation (H/R : 0.5% O_2) to melatonin (1 nM, 1 uM and 1 mM). Reactive oxygen species (ROS) levels were measured by the Carboxy-DCFDA assay and enzyme antioxidant expression and activity was analyzed by immunoblot and by colorimetry assays respectively. Lipid peroxidation was analyzed by the thiobarbituric acid test (TBARS) as well as by ELISA and protein carbonylation by fluorimetry. The apoptotic marker caspase 8 was assessed by western blot.
Results: Melatonin increases ROS levels ($P \leq 0.0001$) and Xantine Oxidase (XO) expression ($P<0,05$), but decreases the activity of superoxide dismutases (SODs) (1µM; $P<0,05$), glutathione peroxidases (GPx), (1 µM and 1nM; $P \leq 0,05$) and catalase (CAT) (1µM $P<0,01$) compared with vehicle control (dimethylsufoxide, DMSO) in BeWo cells. Melatonin also increased lipid peroxidation and protein carbonylation (1µM and 1 nM ; $P < 0,05$) in BeWo cells as well as the cleavage of caspase 8 compared with vehicle control (DMSO) in BeWo cells.

Conclusion: Our results show that melatonin increases oxidative stress in placental choriocarcinoma cells, suggesting an anti-tumour effect for this indolamine. These findings could help generate a more complete and effective approach to cancer treatments.

P2.70.
EFFECTS OF CHEMOTHERAPEUTIC AGENTS ON THE TRANSCRIPTOMIC AND SECRETORY PROFILES OF TROPHOBLAST ORGANOIDS

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Objectives: Integrate transcriptomic and secretome analyses of trophoblast organoids exposed to different chemotherapeutics treatment, including epirubicin, cyclophosphamide, paclitaxel, and carboplatin.
Methods: Trophoblast organoids (TOs) were established from CT29 cell line. Once established, the TOs were validated by immunohistochemistry to confirm the presence of an inner syncytiotrophoblast (STB) layer and an outer cytotrophoblast (CT) layer. RT-PCR was performed to verify the expression of key markers, including *CGB*, *SCD1*, *GCM*, and *ERVW1*. After validation, TOs were treated for 72 hours with epirubicin and cyclophosphamide, both separately and in combination. Following treatment, TOs were collected for transcriptome sequencing, and the culture medium was collected for secretome analysis. An integrative analysis of the transcriptomic and secretomic data will be performed.
Results: Trophoblast organoids (TOs) were established from bTS5 cells using the medium described by Sheridan et al. (2020) (Figure 1). Expression of sdc1, gcm1, ervw1, and cgb3 confirms syncytiotrophoblast differentiation (Figure 2). cgb3 expression is exclusive to syncytiotrophoblast, may serve as a functional marker to assess responses to chemotherapeutics
Conclusion: TOs were validated as syncytiotrophoblast models to be exposed to chemotherapeutics.

P2.71.
VISUALIZATION OF THE DOWNSTREAM BEHAVIOR OF TWIN-TWIN ANASTOMOSES IN DYE INJECTED PLACENTAE WITH 3D VIRTUAL HISTOLOGY USING X-RAYS

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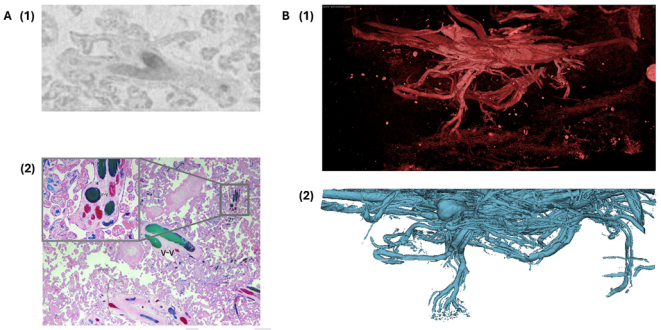
Objectives: Twin to twin transfusion syndrome (TTTS) is a complication occurring in 10-15% of monochorionic twin pregnancies and in case of unbalanced blood flow often resulting in fetal loss without treatment. It is characterized by a disproportionate blood flow between twins due to vascular anastomoses leading to complications in both donor and recipient twin. To validate ultrasound findings and identify missed or newly developed anastomoses after laser coagulation in TTTS, dye can be injected into the umbilical cord vessels. However, deep anastomoses can be missed in gross pathology identification. Through 3D virtual histology using X-rays and following segmentation work we are able to visualize vessel trees of dye injected TTTS placentae in 3D showcasing anastomoses with actual intersections which are important for determining a change in behavior of said vessels.
Methods: Placentae were prepared, injected with dye, and fixed with formalin according to Turowski. The paraffin-embedded blocks were scanned by Histomography GmbH using a non-destructive, tissue-retaining 3D X-ray imaging technique. Those blocks were cut into histological slides and could be compared to the segmentation of the vessel trees. The segmentation of the 3D vessel trees was performed with the open source "3D Slicer" platform. The measured intensities of the dyes ensured the visibility of the vessels in the 3D scan.



Results: Downstream anastomoses can be identified in HE slides as well as in the X-ray tomography data (Fig.A). The visualization of grey levels shows the long range connections of the vascular system (Fig.B.1). The resulting 3D segmentations of the vascular tree based on the tomography scans are shown in Fig.1 B.2.
Conclusion: Combining histology with 3D virtual X-ray histology allows to localize anastomoses and investigate the downstream behavior. Being able to scan placental vessel trees in continuity may contribute to understanding pathophysiology, support diagnostics, staging, management, and identifying associated TTTS findings.

P2.72.
ALTERED UTERO-PLACENTAL BLOOD FLOW FOLLOWING CANAL OCCLUSION SHOWN BY CONTRAST-ENHANCED ULTRASOUND

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Objectives: Impaired utero-placental blood flow underpins the most common cause of placental dysfunction (maternal vascular malperfusion, MVM). Previously, we showed selective occlusion of one or more intraplacental canal vessels in the mouse placenta resulted in asymmetric fetal growth restriction and reduced placental glycogen stores. Redistribution of uterine blood flow through remaining unoccluded canal(s) is therefore insufficient to maintain fetal growth. Despite the occlusion, the placental labyrinth showed no evidence of infarction or other pathologies. To understand the impact of canal occlusion on murine placental exchange, we monitored this acute hemodynamic insult using contrast-enhanced ultrasound (CEUS).
Methods: At embryonic day (E)15.5, pregnant CD-1 mice (N=8) were anesthetized, catheterized via the tail vein, and had their uterus exteriorized. Canal vessels were identified using 40MHz B-mode ultrasound. Baseline placental perfusion was assessed by CEUS following a 50μL bolus of Vevo MicroMarker microbubbles (21MHz, nonlinear contrast mode). The identified canal was then occluded, and a second 50μL bolus was administered to assess post-occlusion perfusion.
Results: CEUS revealed altered enhancement patterns post-occlusion, especially in placental regions distal to the occlusion site. Placental response varied, with two patterns identified in the affected region: slow-enhancement (SE) of the tissue or minimal to no enhancement (MNE). This was associated with significantly longer rise times in the post (SE $\bar{x}$=90.92 ±89.63s; MNE $\bar{x}$=212.73±23.20s) compared to the pre (SE $\bar{x}$=59.69±76.34s; MNE $\bar{x}$=83.49 ±43.40s) scan; SE t(3)=-3.510, p-value=0.0392, d=0.37; MNE t(3)=-7.268, p-value=0.005, d=3.71. Parametric modelling will further characterize perfusion changes and fetal impact.
Conclusion: The hemodynamic response to placental canal occlusion may vary, reflecting differences in vascular organization that influence placental flow. This study will deepen understanding of murine placental perfusion and may inform the underlying basis of human MVM disease and development of fetal growth restriction.

P2.73.
IMPACT OF GESTATIONAL STRESS AND IRON TRANSPORTER MUTATIONS ON PLACENTAL IRON HOMEOSTASIS

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Objectives: The placenta is a transient organ vital for materno-fetal nutrient transfer, known to be highly susceptible to gestational stress (GS). Considering that both environmental stress and iron deficiency are highly prevalent in pregnant women, we studied the effects of GS and selected iron transporters on placental iron homeostasis. Thus, we investigated: 1) the effects of GS on placental iron transport in humans and mice, and 2) the specific role of the iron transporters DMT-1/Slc11a2 and ZIP-8/Slc39a8 in the mouse placenta during late gestation.
Methods: We applied an established protocol of GS in mice and analyzed in parallel placental samples from women who experienced mild stress during pregnancy. To explore transporter-specific effects, we generated conditional transgene mice, where we selectively knocked-down (KD)/knocked-out (KO) DMT-1 or ZIP-8 in the placenta.
Results: GS significantly increased iron uptake, causing iron accumulation in the placenta via upregulation of transferrin receptor 1 (TfR1) and low-density lipoprotein receptor-related protein 1 (LRP-1) in mice and humans. Reduced iron levels were found in human female umbilical cord serum. Homozygous DMT-1 mutation led to high embryonic lethality, while heterozygous DMT-1 mutation induced a significant reduction in DMT-1 that did not affect placental/fetal weight or iron content. In the second mouse line, both heterozygous and homozygous mutations of ZIP-8 led to reduced ZIP-8 expression in both sexes. In ZIP-8 KD/KO mice placental/fetal weight were normal, and placental iron content was reduced only in males.
Conclusion: Placental iron homeostasis is affected by GS, especially in females, leading to reduced iron levels despite normal maternal serum concentrations and increased placental uptake. DMT-1 appears to play a critical role in fetal development, causing embryonic lethality when knocked-out. ZIP-8 appears to play a different role, inducing sex-dependent compensatory mechanisms. The implications for fetal programming will be further investigated.

P2.74.
TREATMENT WITH INTEGRASE-INHIBITOR BASED HIV ANTIRETROVIRALS ALTERS EXPRESSION OF FATTY ACID TRANSPORTERS AND ITS REGULATORS IN THE PLACENTA OF HEALTHY MICE

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Objectives: Integrase strand transfer inhibitors (INSTIs) are the recommended antiretrovirals (ARVs) for people with HIV, including pregnant persons. However, little safety data are available for INSTI use in pregnancy. *In utero* exposure to HIV/ARVs has been associated with higher risk for neurodevelopmental deficits in HIV-exposed but uninfected children. Placental fatty acid (FA) transport, which is vital for fetal brain development, relies on several transport proteins to ensure fetal FA demands are met. We explored the effects of INSTI exposure on placental expression of FA transporter genes in a mouse pregnancy model.
Methods: Healthy C57BL/6 mice (N=10-20 dams/arm) were treated with INSTI-based ARVs (bictegravir, cabotegravir, dolutegravir, raltegravir) with or without a nucleoside reverse transcriptase inhibitor backbone of TDF/FTC, or water as a control, at therapeutic concentrations via oral gavage from conception until gestational day 15.5. Placental gene expression was assessed by qPCR. Generalized linear models assessed differences versus controls. Gene expression was correlated with fetal and placental weight by Spearman's rank correlation.
Results: Treatment with INSTIs + TDF/FTC altered placental gene expression of FA transport proteins (*Fatps*), FA binding proteins (*Fabps*), lipases (*Lpl*, *Lipg*), and FA translocase (*Fat/Cd36*) when compared to controls, across treatment regimens. Significant reductions in expression of nuclear transcription factors *Pparg* and *Rxra* were observed across INSTI + TDF/FTC regimens. Expression of *Pparg* was strongly associated with expression of several FA transporters ($R > 0.9$, $p < 0.0001$). INSTIs alone induced more modest changes in gene expression. Expression of lipases was strongly associated with placental weight but not fetal weight in the cabotegravir treatment arm.
Conclusion: Our findings demonstrate that INSTI-based regimens alter expression of mouse placental FA transporters. Altered *Pparg* expression, which regulates expression of *Fatps, Fabps*, and lipases, suggests *Pparg* drives corresponding changes in transporter expression. Further research is required to understand how INSTIs impact placental transfer of FAs.

P2.75.
SINGLE-NUCLEUS TRANSCRIPTOMICS REVEALS ENDOTHELIAL EXPRESSION OF KEY EFFLUX TRANSPORTERS IN THE TERM PLACENTA

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Objectives: The syncytiotrophoblast, cytotrophoblast and fetal endothelium form key components of the placental barrier. Transcellular transfer of substrates across these layers requires transporter mediated uptake on one side and transporter mediated efflux on the other. Efflux transporters have often been considered to be localised to the basal membrane of the syncytiotrophoblast with transfer across the endothelium mediated by paracellular routes. However, the cellular expression of these transporters in the placental barrier is not well described. This work aims to further our understanding of membrane transporter localisation in the placental barrier using single nuclear transcriptomic data.
Methods: Membrane transporter data was extracted from a publicly available term placental single nucleus transcriptomic dataset[1]. Transporters mediating efflux to the fetus were identified including for amino acids (SLC43A1, SLC43A2 and SLC16A10), iron (SLC40A1) and calcium (ATP2B1 and ATP2B4). Normalised gene expression was determined in syncytiotrophoblast, cytotrophoblast and endothelial nuclei using the AverageExpression function in the Seurat package in RStudio.
Results: Syncytiotrophoblast nuclei had high expression of SLC43A2 (2.49) and SLC40A1 (0.41) but not SLC43A1 (0.002) or SLC16A10 (0.06). However, there was lower calcium transporter expression ATP2B1 (0.08) and ATP2B4 (0.38). Conversely, cytotrophoblast nuclei showed high expression for SLC43A2 (2.31), SLC40A1 (1.67), ATP2B1 (1.00) and ATP2B4 (0.99) but low expression of SLC16A10 (0.09). Endothelial nuclei showed high expression for SLC43A2 (1.53), SLC40A1 (0.40) and ATP2B4 (0.82) with lower ATP2B1 (0.32) and SLC16A10 (0.1) expression.
Conclusion: This study demonstrates that the endothelium has high expression of the transporters necessary for directional transport across this barrier. This suggests that the endothelium is actively mediating transfer of these solutes and that any paracellular transfer across the endothelium is not sufficient to meet fetal demand.
1Wang, M., Liu, Y., Sun, R. *et al.* Single-nucleus multi-omic profiling of human placental syncytiotrophoblasts identifies cellular trajectories during pregnancy. *Nat Genet* 56, 294–305 (2024).

P2.76.
PREDICTING PLACENTAL DRUG PERMEABILITY BY INTEGRATING IN SILICO OPEN-SOURCE PHYSIOLOGICALLY-BASED KINETIC (PBK) MODELLING WITH IN VITRO BEWO B30 TRANSFER DATA AND EX VIVO HUMAN PLACENTAL PERFUSION STUDIES.

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Objectives: *In vitro* and *ex vivo* model systems such as the BeWo b30 cells and the human placenta perfusion technique can be used to study

placental transfer of chemicals. However, the data often cannot be directly incorporated in pregnancy-specific physiologically-based kinetic (p-PBK) models to parameterize placental transfer. *In silico* modelling tools can be employed to estimate clearance and/or permeability values, which serve as input for p-PBK modelling. This study aimed to predict placental permeability using *in silico* modelling software, informed by *in vitro* BeWo b30 transfer and *ex vivo* placenta perfusion data.
Methods: The Open Systems Pharmacology software was used to derive placental permeability values for four compounds: thalidomide, valproic acid, amoxicillin, and antipyrine. In short, the software was used to slightly modify an *in silico* cotyledon model previously published to analyse placental perfusion data. Next, an *in silico* model representing an insert culture system was developed to analyse drug transfer across the BeWo b30 cell layer. Various models incorporating either a single bidirectional permeability parameter or two unidirectional parameters, along with a partition coefficient for the placenta perfusion data, were evaluated to optimize these parameters.
Results: The placenta permeability values of valproic acid, thalidomide, and antipyrine, as derived from the perfusion studies, were found to be $7.52*10^{-3}$ and $3.39*10^{-3}$, $3.30*10^{-3}$ cm/min, respectively. The permeability for amoxicillin was estimated to be an order of magnitude lower, at $1.43*10^{-4}$ cm/min. In the BeWo b30 experiments, the permeability values for these compounds were estimated to be $1.05*10^{-3}$, $8.27*10^{-4}$, $1.65*10^{-3}$, $1.27*10^{-4}$ cm/min, respectively.
Conclusion: Both models were able to accurately capture the experimental data using the model with one bidirectional permeability value. Future steps would be to study how the different permeability values estimated here would impact parameterization of placental transfer in a p-PBK model and how this would impact fetal exposure predictions.

P2.77.
THE PARALLEL RELATIONSHIP BETWEEN FOLIC ACID DEFICIENCY AND EXCESS IN HUMAN PLACENTAL ENDOCRINE FUNCTION: UNDERSTANDING THE ROLE OF FOLATE METABOLISM

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Objectives: Folic acid (FA) food fortification and maternal FA supplementation elevate maternal folate status. Excess FA intake is a risk factor for gestational diabetes mellitus (GDM), but the mechanisms remain unknown. We hypothesize that excess FA dysregulates placental function via one of its receptors/transporters.
Methods: Early and mid-gestation placental explants (6-16 weeks' gestation) were treated with FA at 0 nM (deficiency), 40 nM (adequate), or 2000 nM (excess). Tissue and culture media were harvested 72h post-treatment for downstream analyses that included proliferation (Ki67 and PCNA), apoptosis (cleaved caspase 3) and hormone secretion (human chorionic gonadotropin, hCG; pregnancy-associated plasma protein A, PAPP-A; progesterone, P4; human placental lactogen, hPL; placental growth hormone, GH2)). mRNA expression of genes encoding for folate transporters (FOLR1, SLC19A1, SLC46A1) and folate metabolism (DHFR, MTHFR, MTHFD1/2, MTR, MTRR) were quantified using qPCR.
Results: Compared to 40 nM (physiological norm equivalent), 0 nM and 2000 nM increased hPL secretion by 24% ($p=0.03$) and 29% ($p=0.02$), respectively. GH2 secretion was 25% ($p=0.03$) higher in response to 2000 nM FA. hCG, PAPP-A and progesterone secretion, as well as proliferation and apoptosis, were unchanged. Whilst FA did not alter the expression of any folate receptor or metabolism genes, FOLR1 protein expression was 19% ($p=0.05$) and 45% ($p=0.005$) higher with 0 nM and 2000 nM FA, respectively, relative to 40 nM.
Conclusion: Maternal folate deficiency is a well-established risk factor for GDM. We show that FA doses that represent physiological folate deficiency and excess, both dysregulate secretion of placental hormones (which maintain glucose homeostasis) and the expression of FOLR1 protein (a transcriptional factor). This provides a novel link between excess FA and GDM risk. Given nearly 20% of Australian pregnancies are affected by GDM and that many women are consuming excess FA, it is imperative to understand the mechanisms of excess FA regulation of placental function.

P2.78.
ENHANCED TRANSPLACENTAL ANTIBODY TRANSFER IN EARLY PRETERM PLACENTAL INSUFFICIENCY

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Objectives: To characterize transplacental antibody transfer patterns in pregnancies complicated by early preterm placental insufficiency (e.g., fetal growth restriction and preeclampsia) compared to gestational age-matched controls, and to investigate mechanisms underlying these transfer patterns.
Methods: In this multicenter prospective cohort study (2021-2024), we collected samples from 200 mother-infant dyads across three gestational age groups. Early preterm pregnancies with placental insufficiency ($n=30$) were compared to gestational age-matched controls ($n=31$). Using multiplex Luminex assays, we quantified IgG antibodies against nine pathogens (HBV, pertussis, diphtheria, tetanus, mumps, influenza, COVID-19, RSV, and CMV) in paired maternal and cord blood samples. Transfer ratios (TR; cord blood antibody concentration/maternal concentration) were calculated to assess placental transfer efficiency. Simultaneous assessment of these pathogens provides a comprehensive evaluation of placental functionality in antibody transfer. Further analyses will include immunohistochemistry to assess IgG receptor spatial distribution and expression (FcRn, FcγR2, FcγR3), single-cell mRNA sequencing to explore transcriptional differences, and RSV-specific neutralization assays to evaluate antibody functionality.
Results: In normal placental function, antibody transfer ratios increase with gestational age. In early preterm placental insufficiency cases, the transfer ratio was remarkably higher (mean 0.9) compared to controls (mean 0.6) ($p<0.05$), with this enhanced transfer consistently observed across all 9 pathogens studied.
Conclusion: This study reveals surprisingly efficient transplacental antibody transfer in early preterm placental insufficiency, despite other functional impairments. Further planned analyses are expected to reveal the molecular drivers of this enhanced transfer in placental insufficiency. Together, these findings highlight the potential benefits of maternal vaccination strategies for these vulnerable pregnancies.

P2.79.
MRI-BASED MONITORING OF IRON DEFICIENCY IN THE MATERNAL-FETAL UNIT

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Objectives: To develop a non-invasive MRI approach for studying iron transport during pregnancy, enabling us to uncover the maternal-fetal unit adaptations to iron deficiency.
Methods: Using 15.2T MRI, we investigated iron homeostasis in pregnant mice divided into four experimental groups: (1) control mice on standard diet, (2) iron-deficient mice generated through iron-deficient diet, (3) iron-deficient mice treated with Venofer (iron sucrose), and (4) control mice treated with Venofer serving as an iron overload model. We measured $R2^*$ values as indicators of tissue iron content in maternal, placental, and fetal tissues, complemented by protein analysis to examine tissue-specific iron regulation mechanisms.

Results: MRI successfully detected iron content variations across maternal, placental, and fetal tissues (Figure 1). Iron deficiency resulted in compromised embryonic development and cases of Hydrops Fetalis. The placenta demonstrated remarkable buffering capacity, maintaining stable ferritin levels while modulating iron transport proteins (Figure 2). Venofer administration showed immediate effects on maternal liver R2* values, with sustained elevation for weeks. In iron overload conditions, both placental and fetal tissues exhibited decreased R2* levels, suggesting a protective mechanism against excess iron transfer (Figure 1).

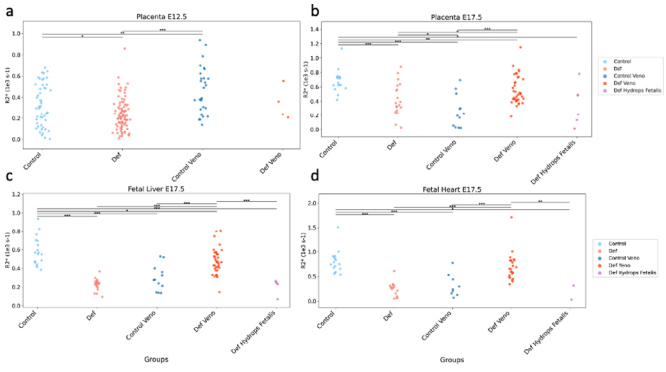

Figure 1Effects of Venofer Treatment on median R2* Values in Placenta and Fetal Organs During Pregnancy.

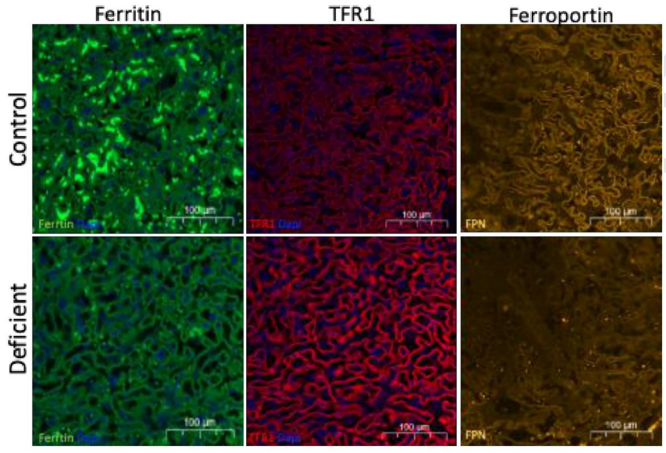

Figure 2: Immunofluorescence imaging of placental iron transport proteins in control vs. iron-deficient conditions.

Conclusion: This study establishes ultra-high field MRI as non-invasive method for comprehensive iron status assessment during pregnancy. The findings reveal complex tissue-specific adaptations to iron status variations and suggest protective mechanisms against iron overload during pregnancy. This approach opens new possibilities for early detection of iron deficiency and provides a valuable tool for understanding iron transport dynamics, potentially leading to targeted therapeutic strategies.

P2.80.
MFSD2A-MEDIATED TRANSPLACENTAL TRANSFER OF LYSOPHOSPHATIDYLCHOLINE-DOCOSAHEXAENOIC ACID IN MICE

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Objectives: Docosahexaenoic acid (DHA) is essential for brain development and therefore needs to be provided to the developing fetus by the mother. There is a need for clarification of the mechanism responsible for the transplacental transfer of DHA. Major facilitator superfamily domain containing 2A (MFSD2A), which transports DHA in the form of lysophosphatidylcholine (LPC), is known to be expressed in the human placenta. In this study, we aimed to clarify the localization and function of MFSD2A in the murine placenta.

Methods: The polyclonal antibody was raised against residues at the C-terminus of mouse MFSD2A. MFSD2A protein expression in the plasma membrane fraction of placental decidua, junctional zone, and labyrinth of wild-type and *Mfsd2a*$^{-/-}$ mice was analyzed by Western blot. LPC-[^{14}C]DHA was administered intravenously to pregnant mice, and the fetal-to-maternal plasma concentration ratio (F/M ratio) was measured by liquid scintillation counter.

Results: The expression of MFSD2A protein at 75 kDa in the plasma membrane fraction of the placenta of wild-type mice disappeared in that of *Mfsd2a*$^{-/-}$ mice. Compared to the junctional zone and the decidua, the expression level of MFSD2A protein was higher in the labyrinth, which constitutes the placental barrier. MFSD2A protein expression varied with gestation, peaking at embryonic day 15.5 (E15.5). At 18 hours after administration of LPC-[^{14}C]DHA to E15.5 pregnant *Mfsd2a*$^{+/-}$ mice, the F/M ratio of LPC-[^{14}C]DHA in *Mfsd2a*$^{-/-}$ fetuses was significantly lower than that in wild-type fetuses of littermates. These results suggest that placental MFSD2A plays a role in the transport of LPC-DHA from mother to fetus.

Conclusion: MFSD2A localizes to the plasma membrane of the placental labyrinth, peaking at E15.5, and is involved in the fetal supply of maternal LPC-DHA.

P2.81.
INCREASED EXPRESSION OF AMINO ACID TRANSPORTERS SNAT1, HCAT2 AND NTRK2 IN PLACENTAE FROM PREGNANCIES WITH GESTATIONAL DIABETES MELLITUS.

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Objectives: Pre-existing maternal obesity and Gestational diabetes mellitus (GDM) are two significant pregnancy complications. They lead to increased fetal adiposity which is associated with many adverse metabolic disturbances in the offspring. It is postulated that increased placental transport of nutrients such as glucose, fatty acids and amino acids is one of the mechanisms responsible for metabolic disorders. While previous studies show increased placental expression of glucose and fatty acid transporters complicated by maternal obesity and/or GDM, there is a paucity of data on amino acid transporters. Thus, the aim of this study was to determine the mRNA expression of amino acid transporters in placentae from obese/non-obese women with GDM compared with BMI matched uncomplicated pregnancies.

Methods: Amino acid transporters was investigated in human placentae obtained from women with diet-managed GDM (n=22 non obese and 12 obese), insulin-controlled GDM (n=19 non obese and 16 obese) and BMI matched normal glucose tolerance (NGT) (n=18 non obese and 15 obese). The mRNA expression of aminoacid transporters was determined using a Fluidigm Biomark™ HD system. Data was analysed using the average of three housekeeping genes (18S rRNA, YWHAZ and TBP) and relative quantification was performed according to the $2^{-\Delta\Delta CT}$ method. Statistical difference was performed using Mann-Whitney test and $p < 0.05$ was considered significant.

Results: SNAT1, hCAT2 and NTRK2 showed significantly increased mRNA relative to the three housekeeping genes. Specifically, placental SNAT1 and hCAT2 mRNA was significantly increased in diet-managed GDM non-obese and obese compared with BMI matched NGT women. Placental NTRK2 mRNA was also significantly increased in obese women with insulin-controlled GDM when compared with BMI-matched NGT women.

Conclusion: This study reports that GDM-affected pregnancies (non-obese/obese) are associated with an increase in placental mRNA expression of SNAT1, hCAT2 and NTRK2 amino acid transporters, which may lead to fetal overgrowth possibly via neoglucogenesis pathway.

P2.82.
PLACENTAL PROTEIN EXPRESSION OF IDO2 AND TPH CORRELATE WITH CORD TRYPTOPHAN METABOLITES INDEPENDENTLY OF MATERNAL MENTAL STRESS STATUS

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Objectives: Intrauterine signals of maternal mental stress received by the fetus via the placenta are thought to program the fetal brain and influence subsequent neurodevelopment. Tryptophan, an essential amino acid, is vital for healthy fetal growth and development. Placental tryptophan is catabolised via the serotonin and kynurenine pathways, producing metabolites with neuroactive and other functions. This study aimed to characterise placental expression of tryptophan catabolic enzymes and their association with cord tryptophan metabolites stratified by maternal mental stress status.
Methods: Maternal mental stress at 26-28 weeks' gestation was determined by a combined stress score incorporating the Beck Depression Inventory-II, State-Trait Anxiety Inventory and Edinburgh Postnatal Depression Scale. Placental protein expression of the upstream tryptophan catabolic enzymes in the serotonin/kynurenine pathway (IDO1, IDO2 and TPH1) was examined by immunoblotting in a pilot study of 7 high maternal mental stress cases and 5 low maternal mental stress controls obtained from the GUSTO mother-offspring cohort. Cord blood tryptophan and metabolites: kynurenine, 3-hydroxykynurenine, kynurenic acid, xanthurenic acid, anthranilic acid, 3-hydroxyanthranilic acid, picolinic acid and quinolinic acid were measured by liquid chromatography-mass spectrometry. Statistical analyses utilised Student's t-tests and Pearson correlation as appropriate.
Results: Neither placental protein expression of IDO1, IDO2 and TPH, nor cord tryptophan metabolites levels differed between high maternal mental stress cases and controls. While placental IDO1 showed no association with any cord metabolites, IDO2 positively correlated with 3-hydroxykynurenine ($r=0.77$, $p=0.04$) and TPH positively correlated with kynurenine ($r=0.89$, $p=0.008$) and 3-hydroxykynurenine ($r=0.87$, $p=0.01$).
Conclusion: Placental protein expression of IDO1, IDO2 and TPH did not differ by maternal mental stress status. Although IDO2 and TPH expression correlated with cord tryptophan metabolites, these were not direct metabolites of these enzymes, suggesting modulation of other tryptophan metabolic pathways. Ongoing work is examining placental expression of downstream tryptophan catabolic enzymes and placental tryptophan metabolites.

P2.83.
HIGHER FETAL MEMBRANE MYO-INOSITOL CONTENT ASSOCIATES WITH INCREASED TENSILE STRENGTH LINKED WITH ALTERATIONS IN ACYLCARNITINE LIPIDS AND HYDROXYPROLINE CONTENT

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Objectives: Previous studies implicate myo-inositol supplementation with lowering preterm birth (PTB) risk. Furthermore, the randomised controlled trial of the NiPPeR intervention supplement containing myo-inositol reduced PTB associated with preterm prelabour rupture of membranes (PPROM). We hypothesised that myo-inositol strengthens fetal membranes by influencing changes in membrane lipids and extracellular matrix (ECM) content.
Methods: Fetal membranes (n=23) were collected at term elective caesarean sections. Membrane tensile strength (biaxial testing), inositol content (Megazyme®), lipid profiles (LCMS), and ECM remodelling markers [hydroxyproline (collagen component), matrix-metalloproteinase-9 (MMP-9)] were quantified in different membrane zones: cervical (CZ), mid (MZ) and placental (PZ) zones. Separately, membranes were treated *in-vitro* with 0, 10, 30, 60 and 100 μM of myo-inositol for 48 hours and assessed as above.
Results: From the CZ to the PZ, fetal membrane tensile strength increased by 28% ($P_{trend} = 0.017$), with correspondingly increased inositol content (mean ± SD, CZ: 660.4 ± 425.2 μg/g; MZ: 1396 ± 758.4 μg/g, PZ: 1432 ± 752.5 μg/g; ANOVA-P<0.05). Total acylcarnitine lipids (mean z-score change relative to CZ: MZ 0.634 ± 0.458, $p=0.0058$; PZ: 1.349 ± 1.002, $p = 0.0066$; ANOVA-P = 0.0082) and hydroxyproline content (fold-change relative to CZ ± SD, PZ: 1.167 ± 0.210, Dunnett's post-hoc-P<0.05) were higher in the MZ and PZ compared with CZ, but there was no change in MMP-9 activity. However, fetal membranes treated with increasing doses of myo-inositol *in-vitro* did not exhibit any differences in these parameters.
Conclusion: Chronic exposure to higher myo-inositol levels *in-utero* may alter fetal membrane lipid and collagen content, strengthening fetal membranes and reducing PPROM risk. However, an acute increase in myo-inositol exposure cannot alter membrane characteristics and tensile strength. If so, myo-inositol supplementation may need to be commenced early in pregnancy to potentially affect PPROM risk. Further studies are required to confirm this.

P2.84.
SYSTEMIC INFLAMMATION INDICES GENERATED FROM FIRST-TRIMESTER BLOOD TESTS CAN BE POTENTIAL BIOMARKERS FOR PREDICTING PREECLAMPSIA

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Objectives: Preeclampsia is a leading cause of maternal and neonatal morbidity and mortality, affecting 3% to 8% of pregnancies. Currently, no clinically useful predictive biomarker exists. Preeclampsia is characterized by systemic inflammatory responses that may be triggered by placental materials released into the maternal circulation, leading to an overly active maternal immune system. Recently, systemic inflammation indices have been used in various diseases as indicators of inflammation. Here, we investigated whether systemic immune inflammation indices generated from peripheral blood tests in the first trimester could be potential predictors for preeclampsia.
Methods: Blood samples, ranging from 6 to 12 weeks, were collected from 109 women who later developed preeclampsia and 177 women who did not develop any complications until delivery. The counts of white blood cells, neutrophils, lymphocytes, monocytes, and platelets were obtained, and systemic inflammation indices, including the systemic immune inflammation index (SII), the systemic inflammation response index (SIRI), the neutrophil-to-lymphocyte ratio (NLR), and the pan-immune inflammation value (PIV), were calculated.
Results: Significantly higher counts of white blood cells, neutrophils, lymphocytes, and monocytes were observed in women who later

developed preeclampsia (p<0.0001). Additionally, all four systemic inflammation indices were significantly elevated in women who later developed preeclampsia (p<0.001). The increased indices were primarily associated with late-onset preeclampsia. No significant differences in these indices were observed between women who developed severe or mild preeclampsia and those who did not later.
Conclusion: Our study demonstrates that systemic inflammation indices derived from first-trimester blood tests may serve as additional predictors for preeclampsia.

P2.85.
ESTIMATED PLACENTAL VOLUME : NOVEL TOOL FOR PREDICTION OF PREGNANCY (MATERNAL & FETAL) COMPLICATIONS.

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Objectives: The main objective of study is to know how estimated placental volume (EVP) which is measured by the ultrasound helpful for the prediction of pre eclampsia, maternal diabetes, Pregnancy induced hypertension, Placental insufficiency, LGA and SGA.
Methods: Prospective study of population of 108 pregnant women attending antenatal checkups during second trimester from 17 weeks to 24 weeks gestation at Tulasi Multi speciality hospital, Guntur.
By using ultrasound, we measure placental volume by taking placental width, height and thickness of placenta.
EPV calculated by merwins calculator and percentile charts are prepared. By this method, all pregnancy complications are identified and by taking earlier interventions.
Results: Women estimated with placental volume more than 75th percentile has increased odds of delivering LGA infants and EPV less than 25th percentile had an increased odds of delivering SGA infants.
Above 108 women, 88 women are have EPV of normal range, 20 women less than 25 th percentile and 2 women are more than 75 th percentile.
Among 20 women who has abnormal EPV, 12 babies had LBW of range 1.4 - 2.2 kgs and 8 babies had weight more than 4.2 kgs.
Larger placentas may be associated with GDM and smaller placental volumes are seen in SGA fetuses & pre eclampsia.
Conclusion: Simple and rapid placental volume estimation is feasible in routine ultrasound screening in second trimester which is very helpful for identification of placental insuffiency, LGA & SGA.
Measuring EPV is best tool for the patients from 15 wks to 40 wks of gestation and screening for closer surveillance.
EPV is a new tool for detecting placental insufficiency by ultrasound with two dimensional method by using three parameters in second trimester. There by we will start early intervention to get better neonatal outcome and prevent maternal complications.

P2.86.
EARLY PREGNANCY CIRCULATING BIOMARKERS OF VASCULAR DYSFUNCTION AND LNCRNA EXPRESSION IN MATERNAL HYPERCHOLESTEROLEMIA

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Objectives: Maternal supraphysiological hypercholesterolemia (MSPH), present in ~25% of pregnancies, is associated with oxidative stress, endothelial dysfunction, and increased long-term cardiovascular risk in neonates from MSPH women. These early maternal alterations may impair vascular adaptation and placental development, contributing to adverse maternal-fetal outcomes. However, the circulating markers underlying MSPH remain poorly characterized. Long non-coding RNAs (lncRNAs) have recently emerged as promising biomarkers in pregnancy complications. However, their role in MSPH remains unexplored.
This study aims to investigate early changes in circulating vascular dysfunction biomarkers during the first trimester in pregnancies later classified as MSPH, and to explore the expression of lncRNAs in maternal plasma from the first and third trimesters.
Methods: A retrospective study was conducted using first-trimester serum samples (11–14 weeks) from pregnant women later classified as normocholesterolemic (MPH) or MSPH based on third-trimester lipid levels. Samples were analyzed for lipid profile, apolipoproteins (ApoB, ApoAI), lipid peroxidation (MDA and oxLDL), total antioxidant capacity (TAC), cytokines (IL-10, IL-12, IL-6), and adhesion molecules (sICAM, sVCAM). In a subgroup, exploratory lncRNA profiling was performed on maternal plasma collected at the first and third trimesters.
Results: Compared to MPH, MSPH women showed significantly elevated total cholesterol, LDL-C, ApoB, ApoAI, and MDA levels, along with reduced TAC, indicating early oxidative stress. IL-10 and IL-12 were decreased, suggesting altered inflammatory regulation, while IL-6, oxLDL, sICAM, and sVCAM showed no significant differences. Preliminary transcriptomic analysis revealed differential lncRNA expression between MSPH and MPH groups and across gestation.
Conclusion: This study identifies early changes in circulating vascular dysfunction biomarkers in MSPH, detectable prior to clinical hypercholesterolemia. These findings offer the first indication that circulating lncRNAs could be explored as early biomarkers in maternal hypercholesterolemia. Together, the results support further investigation into early risk stratification and regulatory pathways in MSPH, with potential implications for pregnancy adaptation and maternal-fetal health.

P2.87.
EVALUATING THE CLINICAL UTILITY OF PLACENTAL SPECIFIC BETA-1 GLYCOPROTEINS(PSG) AS A NOVEL BIOMARKER FOR PRE-ECLAMPSIA

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Objectives:
Aim: To evaluate the clinical utility of serum placenta-derived pregnancy-specific beta-1 glycoproteins as an early biomarker for pre-eclampsia.
Objectives: To investigate the expression patterns of serum PSG in normal pregnant women and pre-eclampsia cases and to analyze the performance of PSGs as biomarkers in PE with respect to established biomarkers (sFlt-1 and PlGF ratio).
Methods: 40 patients (Confirmed cases of Pre-eclampsia (hypertension with/without Proteinuria). 20 patients with PIH and 20 health controls PSG expression in Placental tissue samples was done by PCR while ELISA was used for estimating plasma PSG levels long with sft-1 and PlGF.
Results: a.The sFlt-1/PlGF ratio was significantly elevated in the severe PE cohort and PIH subjects relative to normotensive controls, indicating its association with hypertensive disorders of pregnancy.
b. ROC curve analysis of downregulated PSGs demonstrated that PSG4 exhibited the strongest performance in differentiating PE from PIH, with an area under the curve (AUC) of 0.65 in PE, which declined to 0.438 in PIH. In contrast, PlGF displayed similar AUC values for PE and PIH limiting its utility in distinguishing between the two conditions
Conclusion: The outcomes of this study indicate that PSG9 and PSG4, as well as PSG9/PSG4 ratio offer greater diagnostic precision in differentiating PE from PIH compared to the widely used sFlt-1/PlGF ratio

P2.88.
MULTIOMIC APPROACH TO STUDY THE IMMUNOLOGIC PROFILE PRECEDING PRETERM BIRTH

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Objectives: Preterm birth (PTB) occurs in 10% of pregnancies worldwide, with approximately 60-70% of these cases arising spontaneously (sPTB) and without prior clinical warning. Currently, there is a significant gap in reliable tools for early prediction of sPTB and in effective preventive treatments. Emerging evidence suggests that a dysregulation in the maternal immune response to pregnancy may play a critical role in the development of sPTB. However, our understanding of the specific immunological changes that precede sPTB remains limited.
Methods: In our study, we collected serial peripheral blood samples before the onset of sPTB from 24 participants (n = 58) across all trimesters. We employed advanced techniques such as single-cell mass cytometry and plasma proteomics to profile over 5,000 cell-specific functional phenotypes and circulating proteins. To provide a robust comparison, we used demographically and gestational age-matched samples from term birth (TB) pregnancies (N = 46 samples, n = 118) as controls. Notably, our multivariable model, which integrates longitudinal single-cell and plasma proteomic data, achieved high accuracy in differentiating sPTB from TB, with an AUC of 0.81 (p = 9.51e-06, cross-validation).
Results: Our findings underscore the intricate nature of immune maladaptation that distinguishes term pregnancies from those at risk of preterm birth, even before clinical pathology is evident. Early indicators of sPTB included heightened neuroimmune responses to adrenergic stimulation in myeloid cells, detectable as early as the first trimester. Conversely, late pregnancy was characterized by increased proportions of granulocytes and elevated pro-inflammatory cytokine production, revealing notable immune dysfunctions. Additionally, our single-cell transcriptomic analysis confirmed maladaptive T cell cytokine responses, pointing to a Th17-skewed, pro-inflammatory CD4+ T cell signature in sPTB pregnancies during the second trimester.
Conclusion: In summary, we were able to successfully identify biomarkers in early pregnancy for predicting sPTB, paving the way for the development of predictive tools and preventive treatments for sPTB.

P2.89.
EARLY PREGNANCY METABOLIC SYNDROME, FOLATE AND VITAMIN D STATUS, PLACENTAL HORMONES AND GESTATIONAL DIABETES.

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Objectives: We have previously shown in the international SCOPE cohort that metabolic syndrome (MetS) identified in early pregnancy is associated with a nearly 4-fold increased risk for gestational diabetes (GDM), independent of maternal BMI, age and socioeconomic status. Furthermore, high maternal Vitamin D status in early pregnancy was protective against GDM. In the STOP cohort, recruited 10 years after Adelaide SCOPE at the same hospital GDM was 3 times more common than in SCOPE (15.2% vs 5%). We have recently shown that excess maternal folate status in STOP increases risk for GDM in STOP. Here we assessed whether maternal MetS increased risk for GDM in the STOP Study and explored interactions between MetS, folate and vitamin D status and key placental hormones that govern maternal glucose homeostasis.
Methods: Maternal assessments were made in early pregnancy (9-16 weeks) in n=1300 participants in the STOP Study, in Adelaide Australia. MetS was diagnosed using international guidelines. Vitamin D and red cell folate (RCF) were quantified by a pathology service. Serum prolactin (PRL), human placental lactogen (hPL) and growth hormone variant (GH2) were measured by ELISA. To assess risk for GDM data were compared between MetS and No MetS by univariable and multivariable tests.
Results: MetS status was available for n=1208 participants. 112 had MetS and 42 (38.2%) of these developed GDM compared to 142 (13.5%) of those without MetS. Serum Vitamin D and RCF levels, as well as the ratio of RCF: Vitamin D, were significantly higher in women who had MetS versus those who did not. Serum PRL and hPL were both significantly lower in women with MetS and associated with GDM.
Conclusion: Maternal MetS in early pregnancy substantially increases risk for GDM and alters placental and maternal secretion of hormones that govern maternal glucose homeostasis. Causal mediation analyses are ongoing.

P2.90.
MATERNAL OVERWEIGHT AND EXCESS WEIGHT GAIN DURING PREGNANCY IMPACT THE MATERNAL-PLACENTAL-FETAL HPA AXIS

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Objectives: Maternal overweight and excess gestational weight gain (GWG) have been linked to adverse perinatal outcomes, potentially mediated by alterations in the maternal–fetal stress axis. Placental 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2), a key enzyme protecting the fetus from excessive glucocorticoid exposure, may play a pivotal role in fetal programming under conditions of maternal metabolic stress.
Methods: In this prospective longitudinal study, 176 healthy singleton pregnancies were categorized according to pre-pregnancy body mass index (BMI) as adequate (control; 18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2). Maternal anthropometrics and weight trajectories were assessed throughout gestation. Glucocorticoid indices were measured using maternal hair and serum cortisol assays. Placental samples were analyzed for 11βHSD2 mRNA and protein expression and promoter methylation patterns at defined CpG sites. Correlation and regression analyses evaluated the impact of BMI and GWG on markers of HPA axis activity and placental adaptations, with additional exploration of sex-specific differences in neonatal outcomes.
Results: Obese women demonstrated a higher prevalence of excess GWG compared to adequate BMI women. Hair cortisol levels, reflective of chronic stress, were significantly higher in overweight and obese women compared to adequate BMI women. Although absolute serum cortisol levels were similar among groups, obese women exhibited a markedly greater increase from second trimester to third trimester. 11βHSD2 protein levels were significantly higher in placentas from excessive GWG women compared to below GWG women. In females, promoter methylation at specific CpG sites was significantly positive correlated with neonatal anthropometrics.
Conclusion: Maternal overweight and excess GWG are associated with markers of chronic HPA axis activation and increased placental 11βHSD2 protein levels. Possible sex-specific placental adaptations may underpin altered fetal glucocorticoid exposure and may contribute to the developmental programming of offspring health risks, underscoring the need for integrated perinatal strategies targeting both nutritional and stress-related pathways.

P2.91.

EFFECT OF MATERNAL IMMUNE ACTIVATION ON THE KYNURENINE PATHWAY OF THE TRYPTOPHAN METABOLISM IN THE PLACENTA-FETAL BRAIN AXIS IN RATS

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Objectives: Maternal immune activation during pregnancy is a well-established risk factor for neuropsychiatric disorders in offspring, including schizophrenia, autism spectrum disorders, and cognitive impairments. Although epidemiological evidence strongly supports the link between maternal inflammation and altered fetal neurodevelopment, the underlying mechanisms remain poorly understood. Given that neurodevelopmental disorders often originate from early placental dysfunction, we propose that altered placental tryptophan (TRP) metabolism may play a key role, due to the neuroactive and immunomodulatory properties of TRP-derived metabolites. This study investigates how maternal inflammation alters TRP metabolism in the placenta and fetal brain, focusing on the KYN pathway and its potential role in fetal neurodevelopmental programming.
Methods: Pregnant Wistar rats received intraperitoneal injections of Poly I:C (5 or 15 mg/kg) or LPS (1 mg/kg) on gestational days 17 and 18. Dams were sacrificed at 4, 24, or 48 hours after the second injection. Inflammatory markers were analyzed in plasma and amniotic fluid. Gene and protein expression of TRP-metabolizing enzymes were assessed in the placenta and fetal brain, and placental KYN metabolites were quantified by HPLC.
Results: Inflammatory markers were elevated in both maternal plasma and amniotic fluid. Inflammation also altered the expression of key enzymes in the KYN pathway in the placental and fetal brain. Treatment with LPS and/or Poly I:C significantly increased the expression of *Kmo*, *Kynu*, *Haao*, and *Qprt*. These changes were associated with elevated placental levels of KYN and its downstream metabolites, with sex-specific differences: quinolinic acid was increased in male fetuses, while kynurenic acid was elevated in females.
Conclusion: Our findings suggest that systemic maternal inflammation disrupts tryptophan metabolism in the placenta and fetal brain, potentially affecting fetal neurodevelopmental programming.
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P2.92.

IMPACT OF DEPRESSION AND SELECTIVE SEROTONIN REUPTAKE INHIBITORS (SSRIS) TREATMENT DURING PREGNANCY ON IRON HOMEOSTASIS-RELATED PROTEIN EXPRESSION IN PLACENTAL TISSUE

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Objectives: Antenatal depression affects up to 20% of pregnancies and is frequently treated with selective serotonin reuptake inhibitors (SSRIs). Several studies have suggested an association between abnormal iron status and depression. Pregnancy involves significant changes in iron homeostasis, and disruptions in placental iron regulation—potentially linked to SSRI treatment—may increase vulnerability in pregnant individuals with depressive symptoms. Despite the widespread use of SSRIs during pregnancy, their impact on placental iron regulation remains unexplored. In this study, we investigate the potential relationship between antenatal depression, SSRI treatment during pregnancy, and alterations in the placental iron system.
Methods: Full-term placental tissues were obtained from the healthy (n=18), depressed/SSRIs treated (n=18), and depressed/no SSRI (n=34) pregnant individuals. Snap-frozen tissues were powdered, extracted, and then digested with trypsin. Bottom-up proteomics analysis was conducted with data-independent LC-MS/MS and TripleTOF 5600 (Sciex). Differential expression analysis was performed using the DEP package for R, followed by the identification of iron-related proteins based on Reactome. The effect of depression and SSRIs were separately assessed by comparing the expression between healthy vs. depressed/ no-SSRI and depressed/no-SSRI vs. depressed/SSRI treated groups, respectively.
Results: In placentas from individuals with depression not treated with SSRIs, compared to healthy controls, the expression of iron storage proteins (transferrin and lactotransferrin) and heme-regulating proteins (hemoglobin subunits γ1, γ2, α, ϕ; α-hemoglobin stabilizing protein; hemopexin; and biliverdin reductase B) was significantly increased (*adj. p* < 0.05). In contrast, the expression of iron transporters, including the transferrin receptor, ferroportin, and LDL receptor-related protein 1, was reduced. In placentas from depressed individuals treated with SSRIs, compared to depressed/no-SSRI group, both iron storage and transport proteins expression showed a reversed pattern.
Conclusion: Our findings suggest that maternal depression and SSRI treatment during pregnancy may alter the placental iron transport and homeostasis.

P2.93.

ESTROGEN ALPHA RECEPTOR DEFICIENCY IN INNATE LYMPHOID CELLS IMPAIRS PROPER FETAL DEVELOPMENT IN MURINE PREGNANCY

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Objectives: Innate lymphoid cells (ILCs), including NKp46+ ILCs, represent the largest proportion of immune cells at the fetal-maternal interface in the early stages of pregnancy. They support trophoblast invasion, spiral artery remodeling and promote fetal tolerance. Steroid hormones have been shown to regulate the distribution and activity of ILCs. However, the exact mechanisms of this hormonal regulation are still unclear. Therefore, we investigated whether the absence of the estrogen receptor alpha (ERα) in maternal NKp46+ ILCs affects the properties of these cells and thus pregnancy.
Methods: Female heterozygous ($NKp46^{ERα+/-}$) and homozygous ($NKp46^{ERα-/-}$) C57BL/6 mice were mated with BALB/c males. $NKp46^{ERα+/+}$ females served as controls. Dams were sacrificed at gestational day 12. Pregnancy-associated weight gain of the dams, implantation numbers and abortion rates as well as fetal and placental weights were recorded in all three groups. Moreover, frequencies of NKp46+ cells were determined in the uterus by flow cytometry and spiral artery remodeling was assessed by histology.
Results: Dams of all genotypes showed comparable weight gains, implantation numbers, abortion rates, and placental weights until mid-gestation. However, fetuses of $NKp46^{ERα-/-}$ dams presented with a decrease in weights, placing 43% of them below the 10th percentile (small for gestational age; SGA), while 26% of fetuses from $NKp46^{ERα+/-}$ dams were SGA fetuses compared to fetuses from $NKp46^{ERα+/+}$ control dams. Notably, impairment in fetal weight gain was neither associated with altered uterine NKp46+ cell frequencies nor with interferences in spiral artery remodeling.
Conclusion: Our results suggest that the lack of ERα in NKp46+ ILCs leads to SGA fetuses, supporting the importance of ILC and their hormonal regulation for fetal development. However, our findings can neither be explained by reduced intrauterine NKp46+ cell numbers nor by disturbances in spiral artery remodeling. Thus, further investigations are needed to unravel the underlying causes of this fetal impairment.

P2.94.
IMPAIRED DECIDUAL MACROPHAGE EFFEROCYTOSIS OF TROPHOBLASTS IN PREECLAMPSIA

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Objectives: Preeclampsia (PET) is associated with defective trophoblast invasion, with elevated numbers of apoptotic trophoblasts observed. Decidual macrophages (DMφ) are thought to be key in removal of these cells via efferocytosis, which drives macrophages into an anti-inflammatory, tolerogenic phenotype. However, in PET DMφ appear more pro-inflammatory which may affect their ability to efferocytose. This study investigated whether *ex vivo* DMφ efferocytosis of trophoblasts is altered in preeclampsia.
Methods: Placentas were collected from healthy pregnancies (healthy controls HC, >37 weeks gestation, n=7-11), and pregnancies diagnosed with PET (30-37 weeks gestation, n=6-7). Decidua basalis macrophages (DBMφ) and decidua paritelais macrophages (DPMφ) were isolated and underwent phenotyping by flow cytometry. For efferocytosis, DMφ were co-cultured with apoptotic BeWo trophoblasts labelled with cell tracker green, for 90 minutes, and uptake measured by flow cytometry. Co-currently, phagocytosis of fluorescently labelled *Streptococcus agalactiae* for 4 hours was measured by flow cytometry
Results: PET DBMφ efferocytose significantly less apoptotic trophoblasts compared to HC (29±9% vs 61±9%, $p<0.05$). PET DBMφ also phagocytose significantly less *S. agalactiae* compared to HC DBMφ (10±3% vs 24±4%, $p<0.05$). Similarly, efferocytosis by PET DPMφ was significantly reduced compared to HC (39±10% vs 60±10%, $p<0.05$), and phagocytosis by PET DPMφ was significantly reduced compared to HC (10±2% vs 29±8%, $p<0.05$). There was no significant difference in phenotypic receptor expression between patient groups for either DM φtype.
Conclusion: Our study is the first to demonstrate efferocytic function of *ex vivo* DMφ, and demonstrates that in PET, DMφ have impaired ability to both efferocytose apoptotic trophoblasts and phagocytose bacteria. Impaired DMφ function in PET may lead to elevated trophoblast necrosis in the placenta, driving inflammation. Further understanding the effect of pregnancy complications on DMφ function is vital to increase our understanding of the pathogensis of these conditions and identify novel immunomodulatory treatments.

P2.95.
ANGIOTENSIN II-MEDIATED MODULATION OF MACROPHAGE PHENOTYPE AND FUNCTION BY DECIDUALIZED ENDOMETRIAL STROMAL CELLS

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Objectives: This study investigated how decidualized human endometrial stromal cells (DC-T-HESC) and Angiotensin II (Ang II) influence macrophage differentiation, phenotype and function, critical processes at the maternal-foetal interface for successful placental development and pregnancy. The study characterized the effects of conditioned media (dCM) from DC-T-HESC and Ang II-treated DC-T-HESC (Ang-dCM) on macrophage activity.
Methods: T-HESC cells were decidualized for 6 days, treated or not with Ang II during the last 48 h, and conditioned media (dCM and Ang-dCM) were prepared. PBMC-derived macrophages were differentiated and treated with dCM or Ang-dCM for 6 days. Cytokine/chemokine levels were measured by ELISA. Mitochondrial content was assessed using MitoTracker green (MTK), flow cytometry, and imaging. Macrophage phenotype was analysed by image cytometry. Statistical analysis included ANOVA and Dunnet's *post hoc* test.
Results: Ang-dCM decreased IL-6 but increased IL-10 and VEGF production in macrophages, compared to dCM, indicating a shift towards an anti-inflammatory, pro-angiogenic state. These effects were reversed by Ang II receptor blockade. Ang-dCM contained more mitochondria-derived content, including extracellular particles positive for MTK, MDC and Annexin V.
dCM-treated macrophages showed increased cell size and modified mitochondrial networks. Ang-dCM-treated macrophages showed a distinct phenotype, with further increases in mitochondrial membrane potential (MMP), lysosomal volume and cell morphology modifications, and a decrease in MTK staining, compatible with a shift towards oxidative metabolism. Both dCM and Ang-dCM decreased macrophage apoptosis. Ang-dCM-treated macrophages exhibited enhanced efferocytosis and mitochondrial content transfer from DC-T-HESC.
Conclusion: In conclusion, decidualized T-HESC cells, particularly treated with Ang II, significantly alter macrophage differentiation and function, promoting an anti-inflammatory, pro-angiogenic and metabolically active macrophage phenotype. These findings highlight the critical role of Ang II signalling in regulating macrophage function at the maternal-foetal interface during pregnancy.

P2.96.
GENERATION OF A HIGH-SPECTRAL FLOW CYTOMETRY PANEL TO CHARACTERISE PLACENTAL HOFBAUER CELL PHENOTYPE.

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Objectives: Within the placenta a network of blood vessels enable efficient oxygen/nutrient delivery to the fetus. Impaired placental vascular development is seen in fetal growth restriction (FGR). Hofbauer cells (fetal-derived macrophages) support placental vascular development. However, limited/conflicting data describes Hofbauer cell abundance in FGR, Hofbauer cell phenotype has not been examined, and many studies plagued by maternal macrophage contamination. This work utilised advances in understanding of Hofbauer markers to develop a novel high-spectrum flow cytometry panel to improve Hofbauer cell characterisation at term.
Methods: A pre-existing villous core panel was modified to include a broader range of macrophage (CD36, CD39, ICAM1, CD206, CCR2, CD11b, CD80, CD86, CD163, CD209) and serotype (HLA-A2, A3, B7) markers. Fluorophore titrations and fluorescence-minus-one controls were used to optimize gating accuracy. SpectroFlo's *Autofluorescence Wizard* tool was employed to compensate for the impact of autofluorescence. Normal placentae were processed for panel testing by a) washing maternal blood from tissue (to obtain maternal immune cells for serotype matching) and b) enzymatic digestion of fetal villous cores.
Results: Our 20-colour flow panel successfully resolved Hofbauer cells ($CD45^+FOLR2^+CD36^+Lin^-$) from term placental digests. Whilst CD14 is a common macrophage marker, it exhibited 'smearing' characteristic of its downregulation in tissue macrophages. Rather, a combination of CD36 (expressed primarily by monocyte/macrophages) and CD19 (to exclude B-cells that also express CD36) better identified Hofbauer cells. Serotype markers allowed identification of HLA mismatches between maternal and fetal cells, providing reliable discrimination of contaminating maternal macrophages (~13% of cells) from fetal-derived Hofbauer cells in 80% (8/10) of placental digests.
Conclusion: The ability to clearly distinguish contaminating maternal macrophages from Hofbauer cells will improve accurate Hofbauer cell phenotyping. Future work will deploy this validated panel to understand how Hofbauer cell phenotype is altered in FGR, and how this may contribute to impaired vascular development in this disorder.

P2.97.
THE IMPACT OF COVID-19'S CYTOKINE STORM ON THE PLACENTA

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Objectives: The increased risk of maternal inflammation due to COVID-19 infection raised significant concerns for expectant mothers during the pandemic. In particular, COVID-19's link to the cytokine storm, as inflammation is considered to be the principal cause of adverse pregnancy and neonatal outcomes. Therefore, this could have a significant impact on placental development and functioning. Hence, this study aims to elucidate if the COVID-19 cytokine storm impacts placental functioning in the South African cohort.
Methods: Plasma and placental samples were obtained from pregnant women in the third trimester from the Inkosi Albert Luthuli Central Hospital in South Africa. MIP-1 alpha, IFN gamma, TNF alpha, and IL-6 plasma levels were analyzed utilizing the ProcartaPlex 4 Plex assay. Thereafter, placental samples were examined histologically through hematoxylin and eosin (H&E) and Masson's trichrome stains to assess for any morphological alterations.
Results: A significant increase in MIP-1 alpha, IFN gamma, TNF alpha, and IL-6 plasma levels was observed in COVID-19-positive pregnancies as compared to COVID-19-negative pregnancies. This indicates that the pro-inflammatory cytokine profile is impacted as a result of COVID-19 infection. These findings were accompanied by a significantly increased prevalence of inflammation, maternal and foetal malperfusion in the placentae from COVID-19-positive pregnancies compared to COVID-19-negative pregnancies.
Conclusion: These findings suggest that the pro-inflammatory cytokine profile observed as a result of COVID-19 infection during pregnancy can be linked to altered placental functioning in the South African cohort.

P2.98.
DEFINING FUNCTIONS OF MATERNAL MACROPHAGE SUBPOPULATIONS DURING PLACENTAL DEVELOPMENT

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Objectives: About a fifth of human pregnancies terminate in miscarriage. Immune system deficiencies occurring during implantation, decidualization and placentation are closely linked to Recurrent Spontaneous Abortions (RSA). Immune cells constitute 40% of the cells within the uterus during decidualization, and macrophages are one of the most abundant populations. Macrophages were shown to have pivotal roles in the processes of implantation and vascular remodeling during placenta development. Tissue macrophages in the developing decidua can arise from resident macrophages in the myometrium, or from circulating monocytes that differentiate into macrophages in the decidua.
Methods: Our study focuses on mouse models which allow fate mapping of different types of macrophages and probing their functional importance during the early stages of implantation and placental development. As iron plays a pivotal role in supporting processes during development, it is essential to investigate macrophages, which function as mediators of iron storage and distribution. Specifically, we utilize a binary transgenic mouse model designed to track Slc40a1^{+} (ferroprotein positive) macrophages that can store iron and control its availability. We further will study perivascular, hyaluronan receptor expressing macrophages (1).
Results: Furthermore, we seek to characterize monocyte-derived macrophages as recent evidence identified two origins for classical monocytes that give rise to macrophage populations in mice – GMP-Mo and MDP-Mo (2). We aim to elucidate whether these distinct monocyte subtypes contribute to the composition of the macrophage pool during placental development. Utilizing the Ms4a3^{cre}:dtTomato^{homo} Cx3cr1^{GFP} mouse model. Preliminary data indicate a shift in the relative proportions of monocyte subtypes in the maternal blood during pregnancy, suggesting their role in the early stages of pregnancy.
Conclusion:
1. Kim J-S et al. *Immunity* 54(1):176-190. PMID: 33333014
2. Trzebanski S et al. *Immunity*, 57(7):1710-1712. PMID: 38986443

P2.99.
THE PLACENTAL IMMUNE PROFILE IN TYPE 1 DIABETES PREGNANCIES AND ITS ASSOCIATION WITH PREGNANCY OUTCOMES

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Objectives: Pregnancy and fetal growth pose significant challenges for the maternal immune system. In women with Type 1 diabetes (T1D), these challenges are further compounded by an autoimmune state sustained by chronic inflammation, increasing the risk of complications such as preterm birth and macrosomia. However, the mechanisms underlying pregnancy and neonatal complications in T1D remain poorly understood. We hypothesized that maternal-fetal immune interactions play a key role in these outcomes.
To explore this, we analysed placental immune profiles and investigated links between immune markers and maternal-child health outcomes in a longitudinal cohort.
Methods: Pregnant women with T1D, gestational diabetes mellitus (GDM), and healthy controls were enrolled into INSIGHT-2 pregnancy-to-early-life cohort (IRAS 326577, REC 23/WS/011). Placentas were collected post-delivery, and the decidua basalis was dissected to isolate immune cells using Histopaque gradient centrifugation. Cells were analysed by flow cytometry to profile 76 immune cell populations.
Results: Our results offer valuable insights into T1D-affected pregnancies. While more mothers are still being recruited and analyses are ongoing, preliminary findings indicate that γδ T cell populations were present in the decidua basalis across all sample groups. Interestingly, there was a trend toward lower Vδ1 subset frequencies in placentas from diabetic mothers compared to the control group. NK cell subset frequencies also varied between diabetic and non-diabetic pregnancies. Access to detailed clinical data enabled us to explore associations between immune profiles and factors such as maternal infection and fetal sex.
Conclusion: This study enhances our understanding of the placental immune landscape in both normal and diabetic pregnancies. Ongoing analyses continue to reveal novel insights into maternal-fetal immune interactions, which may support improved management of T1D and other high-risk pregnancies.

P2.100.
EXPLORING THE IMMUNOLOGICAL CROSSTALK OF TROPHOBLAST ORGANOIDS AND DECIDUAL NATURAL KILLER CELLS IN CO-CULTURE

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Objectives: The goals of the present study were to determine which cytokines and chemokines were secreted in co-cultures of trophoblast organoids and decidual natural killer cells (dNKs), and to determine the impacts of the immunological crosstalk that occurred in co-cultures on the growth and development of culture constituents.
Methods: This study was performed using our co-culture system of human trophoblast organoids with allogeneic dNKs, involving both villous trophoblast organoids (vTO) and those induced to produce extravillous trophoblast (EVTs) with media modifications. Primary cytotrophoblast from first trimester placental samples was used to derive trophoblast organoids using the protocols described by Turco *et al*, 2018. First trimester decidual samples were subjected to magnetic bead-based negative selection to obtain dNK-enriched populations. Cultures were analyzed through multiplex bead-based measurements of 48 cytokines and chemokines, ELISAs, flow cytometry, and whole mount microscopy.
Results: Negative selection to enrich for NK cells yielded an 88% CD45+/CD56+ population. There were significantly ($p<0.01$) higher levels of IL-15 measured in dNK-containing cultures performed in vTO media, but not EVT media. Cultures containing dNKs that were performed in vTO media showed significantly ($p<0.01$) higher levels of G-CSF, IL-6, IL-8, and MCP-1.

Cultures containing dNKs that were performed in EVT media contained significantly ($p<0.01$) higher levels of IL-8 and IL-27. We previously observed that vTO co-culture led to significant ($p<0.05$) increases in organoid cross-sectional area, and upregulation of EVT-associated gene signatures. However, exogenously added IL-8 was not found to impact vTO cross-sectional area ($p>0.05$) in the absence of dNKs.
Conclusion: Cultures performed in vTO media contain more immunomodulatory factors that are present at the maternal-fetal interface *in vivo* than those performed in EVT media. IL-8 does not impact vTO growth with respect to cross-sectional area, but whether it promotes EVT development will be investigated with upcoming work.

P2.101.
MODULATION OF PRIMARY CILIA BY BIOACTIVE FACTORS IN ADULT MESENCHYMAL STROMAL/STEM CELLS FROM THE HUMAN PLACENTA

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Objectives: Primary cilia are essential organelles for signal transduction and cellular homeostasis. We previously demonstrated that mesenchymal stromal/stem cells derived from the chorionic villi of preeclamptic placentas (hCV-MSCs) exhibit shortened primary cilia, accompanied by impaired signaling, differentiation, and motility. This highlights the importance of ciliary integrity for MSC functionality. In this project, we aimed to investigate whether maternal metabolic conditions, specifically obesity and gestational diabetes mellitus (GDM), alter primary cilia in hCV-MSCs. GDM is characterized by insulin resistance, β-cell dysfunction, and altered adipokine profiles, particularly elevated leptin levels. Given that leptin impacts on neuronal primary cilia, we hypothesized that similar effects may occur in placental MSCs.
Methods: hCV-MSCs were isolated from term placentas from healthy donors, and from individuals with obesity or GDM. Cells were characterized via FACS, immunofluorescence, and confocal microscopy, and cultured in 2D monolayers and 3D spheroid models. Ciliogenesis, cell cycle, and gene expression of cilia-related intraflagellar transport markers (e.g., *IFT88*, *IFT172*) and leptin signaling proteins (e.g., *SOCS3*, suppressor of cytokine signaling 3) were analyzed by immunostaining or qPCR. Findings were also corroborated with the HTR-8/SVneo trophoblast cell line as a model control.
Results: Leptin treatment elongated primary cilia of hCV-MSCs from healthy pregnancies without significantly altering cell cycle progression. This was accompanied by increased expression of *SOCS3* and IFT genes. Similar effects were also observed in HTR-8/SVneo cells under leptin as well as insulin treatment. In contrast, hCV-MSCs from donors with obesity or GDM exhibited shortened cilia upon leptin exposure, indicating a differential cellular response to metabolic cues.
Conclusion: Our data suggest that leptin and other bioactive factors modulate ciliogenesis of hCV-MSCs in a pregnancy context-dependent manner. The opposing effects observed in healthy vs. obese/GDM conditions underscore a potential link between maternal metabolism, ciliary signaling, and placental mesenchymal stem cell function.

P2.102.
UPREGULATION OF CREBBP CAN OFFSET THE EFFECTS OF EP300 DEFICIENCY IN THE DIFFERENTIATION OF TROPHOBLAST CELLS

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Objectives: Previous data showed that inhibition of EP300 prevents human trophoblast stem cell (TSC) differentiation into extravillous trophoblasts (EVTs) and syncytiotrophoblasts. This effect was not observed with inhibition of the highly homologous protein CREBBP. However, since EP300 mRNA levels are more than three times higher than those of CREBBP in trophoblasts, it remains unclear whether the effect is due to EP300 alone or the combined deficiency of CREBBP and EP300. This study aims to clarify whether EP300 alone or the combined levels of CREBBP and EP300 are involved in TSC differentiation.
Methods: CREBBP and EP300 knock-down/knock-out TSCs were generated by siRNA and CRISPR/Cas9 gene editing. The sensitivity of these cells to the CREBBP/EP300 inhibitor A-485, concerning EVT differentiation, was determined through analysis of cell morphology and marker gene expression. Rescue experiments were performed on EP300 knock-down cells by overexpressing CREBBP to evaluate its ability to restore EVT differentiation.
Results: EP300 knock-down TSCs were more sensitive to the inhibitory effect of A-485 on EVT differentiation than CREBBP knock-down TSCs, which in turn were more sensitive than control TSCs. Moreover, TSCs with lower CREBBP levels were more sensitive to A-485, as CREBBP homozygous knock-out TSCs showed increased sensitivity compared with heterozygous knock-out. Furthermore, Overexpression of CREBBP was able to restore the inhibitory effect of EP300 knock-down on EVT differentiation.
Conclusion: Our experiments indicate that the combined levels of CREBBP and EP300 are crucial for TSC differentiation into EVTs, rather than EP300 exclusively. However, given the significantly higher expression levels of EP300 compared to CREBBP in trophoblasts, EP300 may still be the most essential denominator for proper EVT differentiation and hence early placenta development.

P2.103.
DECODING EXTRAVILLOUS TROPHOBLAST CELL DIFFERENTIATION: A PROTEOMIC PERSPECTIVE

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Objectives: Extravillous trophoblast (**EVT**) cells invade the uterus and contribute to the vascular remodeling required for proper blood flow and nutrient delivery to the developing fetus. Impaired EVT cell invasion is a key factor in several obstetrical complications. Human trophoblast stem (**TS**) cells can be captured, maintained in vitro, and differentiated into EVT cells. However, the regulatory mechanisms governing TS cell self-renewal and their differentiation into EVT cells remain poorly understood. This study aims to identify and characterize key regulators of EVT cell differentiation using a proteomic approach
Methods: Quantitative mass spectrometry was performed to capture proteome and phospho-proteome on human TS cells maintained in the stem state and on cells progressing from the stem state to EVT cells (differentiation days 3, 6, and 8). Tandem Mass Tag labeling of the individual samples was done after trypsin digestion. All spectra were acquired using an Orbitrap Fusion Lumos mass spectrometer controlled by Xcalibur 2.0 software. For phosphopetides, sample were enriched using a titanium dioxide affinity chromatography and ferric nitroacetate affinity chromatography. Protein identification and quantification was performed using Protein Discoverer v.2.4. We validated our findings using western blotting, and *in-situ* hybridization on human TS cells undergoing EVT differentiation and with first trimester human placenta tissue. Involvement of specific proteins and kinases in the EVT cell differentiation were tested using loss-of-function approaches
Results: Distinct protein profiles were identified for each day of analysis. Prominent transitional cell populations were uniquely present on day 3 of EVT cell differentiation, which were readily distinguishable from other time points. Proteomic analysis led to the identification of candidate kinases contributing to EVT cell differentiation
Conclusion: Through a proteomic approach, we identified key developmental targets and characterized transitional cell populations during EVT cell differentiation, highlighting dynamic changes in kinase expression and activity.
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P2.104.

INVESTIGATING STEM CELL ANTIGEN-1 EXPRESSION AND EFFECTS IN MOUSE TROPHOBLAST STEM CELLS

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Objectives: Trophoblast stem (TS) cells give rise to the differentiated trophoblast of the placenta, with small populations maintained beyond mid-gestation. Stem cell antigen-1 (Sca-1) is a murine cell surface marker expressed by many stem and progenitor populations and is heterogeneously expressed in mouse (m)TS cell populations. Sca-1^{NEG} mTS cells maintain the same differentiation potential as the Sca-1^{HIGH} cells. However, undifferentiated Sca-1^{NEG} cells form colonies more slowly. Importantly, Sca-1^{NEG} mTS cells become Sca-1^{POS} as the colonies expand, suggesting that the loss of Sca-1 does not indicate a path to differentiation. Based on an observation that over-confluent mTS cells have a larger Sca-1^{NEG} subpopulation, we speculated that there may be a relationship between Sca-1 expression and colony expansion.
Methods: To assess whether Sca-1 regulates cell number, proliferation and expression of mTS cell markers, anti-Sca-1 antibody treatment was performed. To investigate the relationship between cell density and Sca-1 expression, mTS cells were plated at different seeding densities for different durations to assess Sca-1 subpopulation sizes by fluorescence-activated cell sorting (FACS), with immunocytochemistry performed to assess spatiotemporal expression.
Results: Sca-1 Ab-treated mTS cells had increased expression of *Eomes* and *Cdx2*, reduced cell numbers, and no change in proliferation, suggesting that Sca-1 may accelerate the cell cycle. Supporting this finding, there was an inverse relationship between cell density and the size of the Sca-1^{HIGH} population and a positive relationship with the Sca-1^{NEG} population. Staining revealed more Sca-1^{NEG} cells as colonies expanded with Sca-1^{POS} cells on the periphery.
Conclusion: These findings support the possibility that Sca-1 may accelerate the cell cycle and regulate the expansion of Sca-1-expressing populations, potentially explaining why the Sca-1 knockout is not embryonic lethal but reduces the capacity to respond to stress. Whether there is a similar regulatory mechanism in human TS cells is unknown.

P2.105.

ELEVATED ENDOTHELIN-1 PRECEDES PREECLAMPSIA: DOES BLOCKADE OF ITS RECEPTORS HAVE EXCITING THERAPEUTIC POTENTIAL?

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Objectives: The potent vasoconstrictor, endothelin (ET)-1, is implicated in the pathogenesis of preeclampsia. We aimed to evaluate circulating ET-1 concentrations both before and after the clinical onset of preeclampsia and investigate whether selective inhibition of ET-1 receptors, ET_A and ET_B, could modulate molecular and functional features associated with disease.
Methods: Circulating ET-1 levels were quantified in (1) an established cohort of pregnant people with early onset preeclampsia (<34 weeks' gestation), and (2) a prospective cohort sampled at 28 and 36 weeks' gestation prior to diagnosis of term preeclampsia, compared to gestation-matched normotensive controls. In vitro, primary human umbilical vein endothelial cells (HUVECs) and placental tissue explants were treated with selective ET_A and ET_B inhibitors. Expression and secretion of key anti-angiogenic (sFLT1), antioxidant (*HMOX1*), pro-inflammatory (*IL-1b*, *IL-6*), and endothelial dysfunction (*VCAM*) markers were assessed. Vascular reactivity to ET-1 was measured in ex vivo omental arteries following selective or combined ET_A/ET_B inhibition.
Results: Circulating ET-1 was significantly elevated in established early onset preeclampsia (2.46 $\pm$ 0.18 pg/mL) compared with controls (0.87 $\pm$ 0.06 pg/mL; $p<0.0001$), and also increased at 28 weeks' ($p<0.001$) and 36 weeks' ($p<0.05$) gestation, prior to diagnosis of term disease. In vitro, neither ET_A nor ET_B receptor inhibition altered gene expression or protein secretion in primary HUVECs and placental explant tissue. However, selective ET_A inhibition significantly attenuated ET-1-induced vasoconstriction in omental arteries, while ET_B inhibition had no effect.
Conclusion: Circulating ET-1 is elevated prior to, and following onset of preeclampsia, supporting its potential as an early biomarker. Although ET_A blockade reduced ET-1-mediated vasoconstriction, inhibition of ET_A or ET_B did not modulate key molecular features in placental or endothelial cell models of preeclampsia.

P2.106.

INGENOL MEBUTATE AS A NOVEL THERAPEUTIC CANDIDATE FOR PREECLAMPSIA.

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Objectives: Elevated circulating levels of placental-derived sFlt-1 is a key driver of preeclampsia. It acts as a vascular endothelial growth factor (VEGF) trap, preventing VEGF from activating its key receptor, VEGF receptor 2 (VEGFR2), in endothelial cells. This study aimed to identify novel small molecules that activate VEGFR2, even in the presence of high sFlt-1 to mitigate endothelial dysfunction.
Methods: Using a VEGFR2-responsive HEK293 cell line, 6,132 compounds were screened to identify VEGFR2 activators. Top 10 drugs were selected for further investigation, including Ingenol Mebutate (IM), a FDA-approved drug used in dermatology. IM's ability to activate VEGFR2 was tested in the presence/absence of sFlt-1. To evaluate functional effects, primary human umbilical vein endothelial cells (HUVECs) were treated with IM and assessed for viability (MTS) and proliferation (IncuCyte). To mimic endothelial dysfunction, HUVECs were pre-treated with TNFα, followed by IM, and the effect on adhesion molecules and Endothelin-1 was assessed. IM's angiogenic potential was assessed using mouse aortic rings. IM's effect on sFlt-1 secretion was measured in HUVECs, primary trophoblasts, and human trophoblast stem cells (hTSCs).
Results: We identified 134 compounds activating VEGFR2 (z-score $\geq$ 4, fold change $\geq$ 2). IM was selected for further study as the most potent candidate. Increasing doses of IM activated VEGFR2, even in the presence of high sFlt-1 levels ($p=0.0121$). A low dose of IM (1.25μM) doubled HUVEC proliferation, relative to recombinant VEGF ($p<0.0001$). Furthermore, in the presence of TNFα, IM significantly reduced mRNA expression of endothelial dysfunction marker, *VCAM1*, ($p=0.0051$) and potent vasoconstrictor *ET-1* ($p=0.0007$), while upregulating expression of cytoprotective molecule *HO-1* ($p=0.0006$). IM did not enhance aortic ring sprouting relative to VEGF. No changes were observed in sFlt-1 secretion from HUVECs, trophoblasts, and hTSCs.
Conclusion: Our findings suggest that IM restores VEGFR2 signalling and improves endothelial cell health, supporting its potential as a therapeutic candidate for preeclampsia.

P2.107.
SOLUBLE ENDOGLIN (SENG) IMPAIRS FETOPLACENTAL ENDOTHELIAL BARRIER INTEGRITY IN PREGNANCIES COMPLICATED BY PRE-GESTATIONAL DIABETES.

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Objectives: sEng has been shown to increase permeability in human brain endothelial cells concomitant with decreased junctional VE-cadherin expression. Elevated maternal sEng is also a feature of gestational diabetes, type1 (T1DM), and insulin- treated type2 (T2DM) but the effect of this on the fetal endothelial barrier is not known and was the aim of this study.
Methods: Primary human umbilical vein endothelial cells (HUVEC) were isolated from normal (N, 6), T1DM (insulin treated, 6), m+iT2DM (metformin+ insulin, 6), and mT2DM (metformin, 3) term pregnancies. Upon confluence the barrier integrity was assessed by trans-endothelial electrical resistance (ECIS). VE-cadherin junctional occupancy was analyzed by immunocytochemistry. ELISA was used to measure sEng levels in culture supernatants from the above and cord serum from N (5), T1DM (3), and m+iT2DM (2).
Results: nHUVEC monolayer impedance (706± 145.2Ω), was higher than T1DM (394.9± 61.49Ω; ***P< 0.001). Paracellular resistance in mT2DM and m+iT2DM were not significantly different from nHUVEC (822.2± 91.09Ω; P>0.05 and 626.5± 135.3Ω; P>0.05, respectively). 70% of paracellular clefts in nHUVEC showed full VE-cadherin junctional occupancy visualized as continuous staining. T1DM HUVEC showed a decrease in the percentage of junctional VE-cadherin compared to nHUVEC (25%; ****P<0.0001). mT2DM and m+iT2DM HUVEC showed lesser disruption than TIDM (50%; **P<0.01 and ***P<0.001 respectively). T1DM HUVEC secreted more sEng (2.561± 0.5836ng/ml) than normal (1.183± 0.5009ng/ml; ***P< 0.001) but not m+iT2DM (1.576± 0.4441ng/ml; P>0.05) or mT2DM (0.7037± 0.2332ng/ml; P>0.05). sEng levels in T1DM cord serum (8.843± 2.551ng/ml) was higher than normal (4.677± 0.8063ng/ml; *P< 0.05), but not m+iT2DM (5.079± 0.4776ng/ml; P>0.05).
Conclusion: These findings suggests that sEng plays a role in the impairment of fetal endothelial barrier function seen in the insulin-treated T1DM, but not in mT2DM or m+iT2DM. Here, metformin may be protecting against sEng increase and junctional VE-cadherin disruption similar to our finding of metformin treatment protecting junctional occludin in m-GDM

P2.108.
PHARMACOLOGICAL MODULATION OF ACETYLSALICYLIC ACID ON THE INFLAMMATION AND TISSUE DAMAGE CAUSED BY *TRYPANOSOMA CRUZI* IN THE PLACENTAL BARRIER.

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Objectives: *Trypanosoma cruzi (T. cruzi),* a protozoan parasite, is the etiological agent of Chagas disease (CD) that can infect the fetus and cause adverse pregnancy outcomes. The trophoblast recognizes the parasite through *Toll*-like receptors 2 and 4 (TLRs), leading to a pro-inflammatory response that increases the parasite-induced tissue damage. However, no studies have been conducted to modulate this placental inflammation and tissue damage. Acetylsalicylic acid (ASA) is a non-steroidal anti-inflammatory drug used in conditions related to non-infectious pregnancy. One of its mechanisms of action involves triggering the generation of specialized pro-resolving mediators (AT-SPMs). Two of the most studied AT-SPMs act through the formylated peptide receptor 2 (FPR2), a G protein-coupled receptor. This study aims to evaluate the effect of ASA on FPR2 protein expression, placental inflammation, and tissue damage caused by *T. cruzi* infection.
Methods: Human placental explants (HPEs) were co-incubated with LPS 100 ng/ml as a positive control, *T. cruzi* 10^5 parasite/ml and ASA 100 μM *ex vivo* for 2 and 24 hours, and after processing the samples, we evaluated i) the protein expression of FPR2 by Western blot, ii) inflammatory response by cytokines expression and secretion levels through RT-qPCR and flow cytometry, and iii) tissue damage through hematoxylin-eosin staining.
Results: Our results show that *T. cruzi* increases the protein expression of FPR2, the expression and secretion levels of TNF-α, IL-1β, IL-6, and IL-10, and the histological damage. At the same time, ASA reduces TNF-α and IL-1β expression and secretion levels and the histological damage during *T. cruzi* infection.
Conclusion: These results suggest a protective effect of ASA in HPEs co-incubated with *T. cruzi* and a possible role of FPR2 in the host-pathogen modulation at the maternal-fetal interface. These findings support ASA as a potential therapeutic approach for CD during pregnancy.
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P2.109.
PLACENTAL COMPLEMENT COMPONENT C5 LEVELS ARE ELEVATED IN TERM PREECLAMPSIA - ITS INHIBITION DOES NOT MITIGATE PLACENTAL DYSFUNCTION

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Objectives: The immune complement system is essential for maintaining immunotolerance in pregnancy. However, complement overactivation is implicated in the serious pregnancy complication, preeclampsia - via elevated circulating complement protein levels. Though complement activation is canonically driven by circulating effector proteins, intracellular complement can regulate inflammation independently. As placental dysfunction is central to preeclampsia pathogenesis, here we determine placental levels of key complement effectors C3, C4 and C5, and assess whether inhibiting complement could mitigate placental dysfunction.
Methods: *C3*, *C4* and *C5* expression was measured in placental tissue collected from first trimester surgical terminations (7-11 weeks; n=11), early preterm (24-30 weeks; n=15), and term caesarean deliveries (38-39 weeks; n=10) (qPCR). Expression was assessed in placentas from preterm (<34 weeks) and term (>37 weeks) cases of preeclampsia, fetal growth restriction (FGR), and gestation-matched controls (n=10-25/group). Term preeclamptic placentas were treated with 125-1000nM eculizumab (biologic C5 inhibitor) for 48h (n=5). Expression and secretion (Luminex/ELISA) of inflammatory and anti-angiogenic markers elevated in preeclampsia were assessed.
Results: Placental *C3* and *C4* expression were higher in first trimester compared to later gestation. C5 expression was lower in term compared to preterm placentas. Neither *C3* nor *C4* expression was altered in preterm or term pathological samples compared to gestation-matched controls. *C5* expression was decreased in both preterm preeclampsia and FGR placentas, compared to preterm controls. In contrast, *C5* expression was elevated in term preeclamptic placenta compared to term controls and FGR placenta. Eculizumab treatment (all doses) did not alter levels of inflammatory interleukin-1β, interleukin-6 or tumor necrosis factor, nor antiangiogenic factor soluble fms-like tyrosine kinase.
Conclusion: These data demonstrate the distinct placental regulation of complement effectors throughout gestation, and in early and late-onset disease. Inhibiting C5 did not reduce inflammatory or anti-angiogenic factors in the preeclamptic placenta, suggesting that targeting C5 is unlikely to mitigate placental dysfunction in preeclampsia.

P2.110.

PETN ATTENUATES ANGIOGENIC MARKER PROFILES AND HYPERTENSION IN PREGNANCIES COMPLICATED BY IMPAIRED UTERINE PERFUSION– A SECONDARY ANALYSIS OF THE RANDOMIZED CONTROLLED PETN-TRIAL

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Objectives: The PETN trial investigated the efficacy of Pentaerytrithyltetranitrate (PETN) in improving pregnancy outcomes for women with impaired uterine perfusion, a significant risk factor for hypertensive disorders and fetal growth restriction (FGR). This secondary analysis focuses on maternal outcomes, adverse events, and angiogenic marker profiles associated with PETN treatment.
Methods: In a multicenter, randomized, double-blind, placebo-controlled trial, pregnant women with a mean uterine artery Pulsatility Index above the 95th percentile between 19+0 and 22+6 weeks gestation were randomized to receive Pentalong® 50 mg or placebo twice daily. Maternal data collected included blood pressure, adverse events, and serum samples for sFlt-1/PlGF ratios and preeclampsia-related parameters. Residual samples from 100 participants (46 PETN, 54 placebo) were used to measure soluble endoglin and PETN metabolite serum concentrations. Outcomes were analyzed using the intent-to-treat principle, with continuous variables compared via Mann-Whitney U test and categorical variables via Fisher's exact test.
Results: The incidence of pregnancy-induced hypertension (PIH) was significantly lower in the PETN group (23.9%) compared to placebo (36.6%). Preeclampsia occurred in 20% of PETN participants versus 29.2% in the placebo group (p=0.07). The maximum systolic blood pressure was lower in the PETN group (130 mmHg vs. 135 mmHg). Hospitalization rates were also significantly reduced (hazard ratio 0.51). Serum analysis showed fewer PETN participants exceeded critical sFlt-1/PlGF ratio cut-offs. Additionally, soluble endoglin levels were significantly lower in the PETN group. Drug adherence was confirmed through serum metabolite levels.
Conclusion: The secondary analysis suggests that PETN may significantly improve hypertensive disorders and angiogenic balance in high-risk pregnancies with impaired uterine perfusion. The favorable effects on sFlt-1/PlGF ratio and soluble endoglin indicate a positive impact on placental angiogenic regulation. These findings support further research to validate PETN as a potential intervention for preventing pregnancy complications related to placental dysfunction and hypertensive disorders, ultimately improving maternal and fetal outcomes.

P2.111.

THE ATRN-HSPB1-FERROPTOSIS AXIS IN PREECLAMPSIA: MENDELIAN RANDOMIZATION INSIGHTS INTO PATHOGENESIS AND EARLY BIOMARKER POTENTIAL

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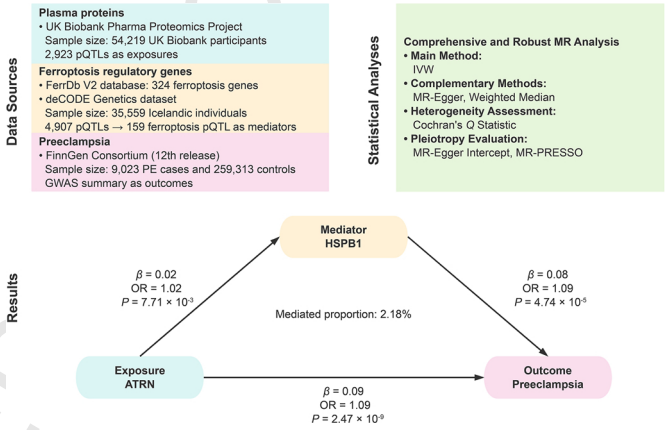
Objectives: Preeclampsia (PE) is a pregnancy-specific hypertensive disorder characterized by systemic inflammation and oxidative stress. Dysregulated ferroptosis, regulated by proteins involved in iron metabolism and antioxidant defense, has been implicated in early placental pathogenesis. We explored the potential causal role of ferroptosis-related proteins in PE using Mendelian randomization (MR) analysis.
Methods: 4,907 protein quantitative trait loci (pQTLs) were obtained from the deCODE Genetics, and 159 were mapped to 324 ferroptosis-regulatory genes from the FerrDb V2 as potential mediators. We incorporated pQTLs for 2,923 proteins from the UK Biobank as exposures. PE outcome data were derived from the FinnGen. A two-step mediation MR analysis was conducted using the inverse variance weighted method as the primary approach, with Bonferroni correction. Sensitivity analyses confirmed the robustness.
Results: Genetically predicted circulating levels of heat shock protein beta-1 (HSPB1) was the only ferroptosis-related protein significantly associated with higher PE risk after Bonferroni correction ($\beta = 0.08$, odds ratio [OR] = 1.09, 95% confidence interval [CI]: 1.04–1.13, $P = 4.74 \times 10^{-5}$). Genetically predicted circulating levels of attractin (ATRN) was also positively associated with higher PE risk ($\beta = 0.09$, OR = 1.09, 95% CI: 1.06–1.12, $P = 2.47 \times 10^{-9}$). ATRN was also associated with increased HSPB1 levels ($\beta = 0.02$, OR = 1.02, 95% CI: 1.01–1.04, $P = 7.71 \times 10^{-3}$), suggesting a potential upstream regulator role. Mediation analysis indicated that approximately 2.18% of ATRN's effect on PE risk was mediated via HSPB1. No pleiotropy or heterogeneity was detected.



Conclusion: Consistent with previous clinical findings of elevated serum HSPB1 levels in early pregnancy among women with PE, our results support HSPB1 as a potential early biomarker and therapeutic target. Furthermore, we identified a causal link wherein ATRN may promote PE development partly through upregulation of HSPB1, suggesting a pathogenic role for the ATRN–HSPB1 axis in placental dysfunction.

P2.112.

NOVEL REDOX 2D NANOZYMES AS PROMISING CANDIDATES FOR THE SAFE TREATMENT OF INFLAMMATORY DISEASES DURING PREGNANCY

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Objectives: Gestational inflammation is pivotal for an uncomplicated pregnancy, however persistent and excessive inflammation can underline various pregnancy complications, primarily linked to placental dysfunction, posing risks to both maternal and fetal well-being. Currently, gynecologists rely on conventional anti-inflammatory and immunosuppressive therapies, which have not been clinically tested for safety and efficacy during pregnancy. On this ground, we aim to develop safe and effective nanotherapeutics to treat gestational inflammatory diseases using nanomaterials with enzyme-like properties (nanozymes), which have shown great promise for the therapy of numerous oxidative stress- and inflammation-driven conditions.
Methods: We engineer vanadium MXene nanozymes (V-MXenes) with pro- and anti-oxidant activities for bacterial eradication and resolution of oxidative stress and inflammation, respectively. Their enzyme-like activities are investigated *in chemico* as proof-of-concept and will be confirmed in a human placenta explant inflammation model. Given that nanodrug safety is crucial, a comprehensive multi-endpoint toxicological assessment (impact on barrier integrity, placenta functionality and possible direct/indirect embryotoxicity) will be performed using *in vitro* placental co-cultures, placental explant cultures and a microphysiological placenta-embryo chip.
Results: Preliminary results revealed that V-MXenes maintain their stability in complex cell culture media and exert catalase (CAT)-like and peroxidase (POD)-like activities. V-MXenes did not interfere with the

viability of BeWo b30 trophoblasts or placenta barrier integrity (BeWo b30/HPVEC co-cultures), as evidenced by transepithelial electrical resistance (TEER) and Sodium Fluorescein (NaF) measurements. Additionally, no apparent damage in the integrity of *ex vivo* placenta tissue was observed. Efficacy studies demonstrated the V-MXene potential to decrease IL-1b-mediated inflammation (induced by monosodium urate crystals) in *ex vivo* placenta tissue. These preliminary findings show great promise towards nanomedicine-driven gestational inflammation treatment.

Conclusion: Ongoing studies focus on the capacity of V-MXenes to restore the placenta functionality after inflammation resolution and on the establishment of a non-sterile (bacterial-induced) inflammation model to investigate their antimicrobial activity.

P2.113.
IDENTIFYING AND CHARACTERIZING MEMBRANE-BINDING PARTNERS OF THE PLACENTAL HOMING PEPTIDE NKG

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Objectives: Delivery of targeted placental therapies using tissue-specific homing peptides is a promising approach to correcting placental dysfunction more safely and effectively and treating pregnancy complications. Our lab has previously identified the novel peptide sequence CNKGLRNK (NKG), which selectively binds to the endothelium of the uterine spiral arteries and placental labyrinth in mice, and the syncytiotrophoblast layer of human placental explants. NKG peptide decoration of nanoparticles successfully guided their accumulation in these regions; however, the cell surface receptor sequence(s) that the NKG peptide recognizes remain unknown. This study aimed to identify the potential NKG binding target(s) that confer its tissue-specificity. We hypothesized that NKG recognizes one or more placental surface proteins and that the peptide-receptor complex's mode of interaction is essential for the precision of payload delivery.

Methods: Putative NKG receptors were identified by affinity chromatography and mass spectrometry (MS) using human term placental tissue homogenates. Membrane-bound and cytosolic protein fractions were separated and passed through a column containing beads conjugated to NKG or a scrambled peptide. Bound proteins were eluted and subjected to MS. Peptide-receptor interactions were assessed by immunofluorescence. Binding affinities and kinetics were characterized by Surface Plasmon Resonance (SPR).

Results: MS and bioinformatic stratification yielded 23 hits, among which annexin A11 (ANXA11) and hexokinase 1 (Hex1) were the strongest candidates. ANXA11 and Hex1 are highly expressed on the syncytiotrophoblast microvillous membrane, and immunofluorescent staining of placental explants pre-incubated with carboxyfluorescein-labelled NKG indicated colocalization with both proteins. The interacting domains and peptide-receptor binding kinetics were obtained by biophysical characterization studies.

Conclusion: We have identified ANXA11 and Hex1 as placental membrane-bound proteins that bind to the NKG placental homing peptide. Gaining a better understanding of this interaction will improve our understanding of the mechanisms of nanoparticle uptake and aid in the design of more effective placenta-targeting nanomedicines.

P2.114.
SEMAGLUTIDE REWIRES PLACENTAL TROPHOBLAST DYNAMICS: EFFECTS ON SURVIVAL AND SIGNALING

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Objectives: The placenta, a critical regulator of maternal-fetal nutrient exchange, is increasingly recognized as a dynamic target of metabolic therapeutics. Celebrated for its metabolic prowess in diabetes, semaglutide's placental impact remains a mystery. This study unveils its effects on BeWo (syncytiotrophoblast) and SW71 (cytotrophoblast) cells, probing viability, proliferation, and signaling pathways critical to placental function.

Methods: Trophoblast cells were treated with 100nM semaglutide for 24 hours. Cell number, viability, and mitochondrial respiration were determined. Protein lysates were obtained, and western blot mTOR, p70, 4EBP-1, AKT, and AMPK were assessed.

Results: Our experiments reveal striking, cell-specific responses to semaglutide exposure. Semaglutide boosted BeWo cell number and viability, amplifying mitochondrial respiration—a powerhouse boost mirrored by surging pAMPK and pp70 activation. This metabolic ignition suggests enhanced energy and protein synthesis, fortifying the syncytial shield against placental stress.

Meanwhile, SW71 cells danced subtly: viability rose modestly, with mitochondrial respiration ticking upward, yet tempered by nuanced shifts in pAMPK, pp70, and p4EBP1. These hints of restrained vigor point to a cytotrophoblast balancing act—survival without overdrive, perhaps preserving their progenitor role.

Conclusion: These divergent responses spotlight trophoblast-specific roles—syncytiotrophoblasts thriving under metabolic enhancement, while cytotrophoblasts tread a tighter rope of resilience. As GLP-1 agonists gain traction in clinical use, our results underscore the need to decode their placental effects—offering a tantalizing glimpse into how metabolic therapies might shape maternal-fetal health. This work sets the stage for deeper investigations into semaglutide's therapeutic potential and safety in pregnancy, captivating the intersection of metabolism, placental biology, and fetal outcomes.

P2.115.
TGFβ-INDUCED SMAD1/5 PHOSPHORYLATION OCCURS THROUGH A DUAL RECEPTOR ACTIVATION AND MEDIATES TRANSIENT EMT IN TROPHOBLASTS DURING HUMAN PLACENTAL DEVELOPMENT

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Objectives: During human placental development, fetal trophoblasts undergo epithelial-to-mesenchymal transition (EMT) to invade the extracellular matrix towards maternal spiral arteries, followed by mesenchymal-to-endothelial transition (MEndT) to replace the endothelium and induce spiral artery remodeling for adequate placental perfusion. These processes seem to be dysregulated in preeclampsia, a pregnancy disease which affects 2 to 8 % of all pregnancies and is the main cause for fetal and maternal mortality worldwide. However, molecular mechanisms underlying both differentiation processes at the fetal-maternal interface remain elusive. Emerging data indicate that the transforming growth factor-β (TGFβ) is a key factor in human trophoblast invasion, but reports on its role are controversial.

Methods: We investigated the TGFβ-SMAD signaling cascade and its effect on EMT marker expression as well as on cell migration in HTR8/SVneo cells and human trophoblast stem cells (hTSC) using standard biochemical methods and ChIP-Seq and IHC stainings of healthy and preeclamptic placentas.

Results: We discovered that TGFβ uses a novel mode of a dual receptor activation, the TGFβ type I receptor ALK5 and the BMP type I receptor ALK2, to induce a transient SMAD1/5 phosphorylation in the HTR8/SVneo trophoblast cell line as well as in hTSCs. TGFβ-induced pSMAD1/5 contributed to the upregulation of the EMT transcription factor SNAIL which is highly associated with the invasive properties of human trophoblasts. Interestingly, SMAD1/5 phosphorylation and SNAIL expression levels were reduced in placental samples from preeclamptic donors compared to healthy donor samples whereas pSMAD2/3 levels were increased in the preeclamptic condition.

Conclusion: Hence, the transient TGFβ-induced ALK5/ALK2/SMAD1/5 signaling cascade might be a prerequisite for trophoblasts to enter EMT,

facilitating MEndT along the differentiation trajectory towards pseudo-endothelial cells. These studies provide new insights into upstream mechanisms of placental EMT which could be of great importance for the understanding of physiological and pathological process regulation at the fetal-maternal interface.

P2.116.
A WNT-DOW INTO MOUSE PLACENTAL DEVELOPMENT: A TEMPOROSPATIAL ATLAS OF WNT SIGNALLING

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Objectives: Wingless-related integration site (Wnt) signalling is a key driver of placental development, directing processes including trophoblast differentiation, lineage specification and vascular remodelling. Reported dysregulation suggests contributions to placental insufficiency pathogenesis. While gene disruption studies have identified crucial Wnt pathway members, transcriptomic datasets reveal variable expression across cell types and developmental stages. Thus, a comprehensive understanding of compartment-specific Wnt expression remains incomplete. We sought to generate a temporospatial Wnt signalling atlas to capture patterns across placental development.
Methods: We measured temporal expression of Wnt-related members in wild-type C57BL/6 mouse placental mRNA (n=minimum 3 litters/timepoint) at placental development milestones: e8.5, e9.5, e10.5, e12.5, e14.5, e16.5 and e18.5 via qRT-PCR. Spatial expression patterns were evaluated via chromogenic *in situ* hybridisation. To validate and contextualise findings, we integrated publicly available snRNA-seq/scRNA-seq/stereo-seq datasets (e9.5-14.5, Marsh & Blelloch, *eLife* 2020; e7.5-14.5, Jiang et al., *Cell Discovery* 2023; e7.5-14.5, Wu et al., *Cell Discovery* 2024) to extract Wnt pathway dynamics across distinct placental development processes.
Results: Of 59 Wnt-related genes analysed, 36 showed increased expression across gestation, 14 were stably expressed, and 9 genes decreased. *In situ* hybridisation mapped these genes to stage- and placental compartment-specific patterns across mouse placental development. Notably, 4 genes (*Axin1*, *Lgr5*, *Ror2*, *Tcf7l1*) localised to labyrinth trophoblast progenitor populations, supporting existing transcriptomic datasets and reinforcing proposed functional roles. 11 genes demonstrated expression in broader placental cell compartments than previously reported, including compartments critical to labyrinth, junctional zone formation, and stromal support. Furthermore, we describe novel temporospatial patterns of 7 Wnt-related genes in mouse pregnancy, deepening insight into uncharacterised dimensions of Wnt signalling in placental development.
Conclusion: This integrated analysis provides a comprehensive spatio-temporal Wnt signalling atlas in the developing placenta. It offers a more complete framework for understanding Wnt-related mechanisms driving important placental developmental processes and potential for dysregulation in disease.

P2.117.
SUPERCOMPLEX ASSEMBLY SUPPORTS HIGHER MITOCHONDRIAL BIOENERGETIC CAPACITY OF CYTOTROPHOBLASTS, COMPARED TO THE SYNCYTIOTROPHOBLAST.

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Objectives: Placental mitochondria display remarkable adaptability, meeting the bioenergetic demands of cytotrophoblasts, and lower energy requirements of the syncytiotrophoblast. Mitochondria produce energy in the form of ATP, via the electron transport chain (ETC), a series of multi-protein complexes (I-IV) that couple electron and proton flux, with energy production by ATP synthase. Emerging evidence in other mammalian species, suggests the existence of higher-order structures known as supercomplexes, whereby complexes I, III, and IV, assemble together to minimise electron leakage and increase the efficiency of ATP synthesis. The presence of supercomplexes, however, has not yet been investigated in the placenta. In this study, we investigated the composition of the ETC, and compared the abundance of supercomplexes between mitochondria from the cytotrophoblast (Cyto-Mitos) and syncytiotrophoblast (Syncytio-Mitos).
Methods: Cyto- and Syncytio-Mitos were isolated from healthy full-term human placental villous tissue. LC-MS was performed (n=6), to quantify the log2 fold change of subunit proteins from complexes I-IV and ATP synthase. Clear-native PAGE was used to compare the abundance of supercomplexes between Cyto- and Syncytio-Mitos (n=14 placentae).
Results: Our proteomic analysis identified 21 differentially expressed subunit proteins from complex I, six proteins from complex II, three proteins from complex IV, and eight proteins from ATP synthase, several of which are suggested to coordinate supercomplex assembly. Additionally, using clear-native PAGE, we found that Cyto-Mitos had a higher abundance of supercomplexes I+III+IV ($p<0.005$) and III+IV ($p<0.005$), compared with Syncytio-Mitos.
Conclusion: These findings suggest that subunit-specific variations of complexes in the ETC promote greater supercomplex assembly in Cyto-Mitos, compared with Syncytio-Mitos. The formation of supercomplexes in Cyto-Mitos may underlie the increased bioenergetic capacity and energy demands of the cytotrophoblast. This understanding is a critical first step toward revealing the mechanisms that underpin mitochondrial transformations in the placenta.

P2.118.
NEUREGULIN-1 AND EGFR/ERBB SIGNALING REGULATE CELL COLUMN FORMATION AND EVT DIFFERENTIATION IN THE FIRST-TRIMESTER HUMAN PLACENTA

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Objectives: Our group has previously demonstrated that neuregulin-1 (NRG1) promotes extravillous trophoblast (EVT) survival through ERBB2/ERBB3 signaling and is routinely used to initiate EVT differentiation in trophoblast organoid models. Conversely, epidermal growth factor (EGF) is used to maintain proliferation and stemness; nonetheless, like NRG1, it can also induce EVT differentiation in trophoblast organoids. The mechanisms of action and the downstream targets of these ligands in different trophoblast subtypes, particularly during cell column formation and EVT maturation, remain unclear. This study investigates the specific effects of NRG1 on trophoblast populations and compares them to other EGFR-family ligands.
Methods: Trophoblast organoids undergoing a two-step EVT differentiation protocol were stimulated with NRG1 during the second phase, with or without TGF-β inhibition. EVTs were isolated via HLA-G immunopurification and compared to placental and interstitial EVTs from patient-matched samples. Bulk RNA-sequencing results were validated by qPCR, Western blotting, and immunofluorescence. Additional stimulation experiments were performed on primary trophoblasts and placental explants using NRG1 and other EGFR-family ligands.
Results: Bulk RNA-seq analysis showed that NRG1 stimulation of organoid-derived HLA-G–positive EVTs upregulated markers of both mature

EVTs and early cell column trophoblasts (e.g., *ITGA2*, *NOTCH1*), suggesting a dual role in EVT differentiation and cell column development. Western blot and immunofluorescence analyses confirmed that NRG1 stimulation upregulates *ITGA2* in freshly isolated EVTs, and increases *ITGA2*, *ERBB3*, and *NOTCH1* expression in cytotrophoblasts, suggesting a role in promoting EVT differentiation. Similar results were observed with EGF and other EGFR ligands, indicating shared downstream pathways.
Conclusion: Activation of EGFR-family receptors by NRG1 and related ligands supports EVT survival, promotes cell column formation, and facilitates EVT differentiation. EGF-family ligands are essential for maintaining the EVT progenitor niche and for mature EVT functions.

P2.119.
SEROTONIN IMPACT IN HUMAN TROPHOBLAST CELLS: SEROTONYLATION, GENE REGULATION, PHENOTYPE, AND POTENTIAL PHYSIOPATHOLOGY

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Objectives: Serotonin (5-hydroxytryptamine) plays a critical role in human development in utero. Recent evidence suggests that it is taken up from the maternal circulation and gets concentrated in trophoblasts (Kliman et al., 2018). A novel mechanism involving serotonin has been described: serotonylation, a covalent linkage of 5-HT with protein glutamines mediated by a tissue transglutaminase (Farelly et al., 2019).
Our objective was to understand the role of serotonin, through serotonylation, in protein, gene and phenotype expression in human placenta.
Methods: Trophoblast purification and culture, western blot (WB), immunochemistry (IHC), and RNAseq (with IPA) were performed. Experiments were repeated at least 3 times, and corresponding statistical analysis was done.
Results: Through WB and IHC, we found that serotonylation seems to be present in human placenta and cytotrophoblasts.
Moreover, through IHC and RNAseq/IPA, serotonylation seems to have consequences on gene and phenotype expression; especially on fusion, proliferation and invasion, which leads to implantation and placental structure, initial steps of pregnancy.
Through IPA analysis, those phenotype and serotonylation impacted-genes were also found to be involved in embryonic, organismal, tissue and organ development, and especially the brain and behavior, foreshadowing central role in human body physiological development.
Lastly, IPA results also showed that those same genes were also involved in many different pathologies, especially ones like Alzheimer, Parkinson, ALS, depression, ASD or cancer (EMT).
Conclusion: To conclude, these findings seem to show that the human placenta, through the cytotrophoblast, is an essential organ to understand the origin of human life and pathophysiological development.
Serotonin, through serotonylation, seems to be central by regulating *original embryonic* genes involved in different processes during life, especially in initiation of pregnancy (invasion and placentation), development of the human body, behavior, and different pathologies, leading to potential diagnostic or therapeutic options for patients.

P2.120.
EXTRACELLULAR ATP REGULATES HUMAN SYNCYTIOTROPHOBLAST FORMATION BY ACTIVATING THE P2X7 CHANNEL.

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Objectives: Trophoblast fusion is a crucial stage in the development and maintenance of multinucleated syncytiotrophoblast (STB) that form placenta. Reports suggest that adenosine and ATP plasma levels are elevated in preeclampsia, as compared with normal pregnant women. Since extracellular ATP (eATP) is known to be the ligand for P2X7 receptor, we hereby propose to explore the role of eATP and P2X7 channel during trophoblast fusion.
Methods: We used the invitro trophoblast fusion model where BeWo cells were treated with forskolin (FSK), resulting in STB formation. eATP was measured during trophoblast fusion and the cell surface expression of the P2X7 receptor was checked by immunofluorescence. To investigate the role of the P2X7 receptor during trophoblast fusion, we used three different approaches: first, a biochemical approach by desensitizing P2X7 channels in the presence of eATP; second, pharmacological modulators targeting P2X7 channels; and finally, genetic modulation of P2X7 channels through overexpression and knockdown in BeWo cells.
Results: We found a significant increase in the release of eATP during trophoblast fusion suggesting its role in STB formation. The FSK treated BeWo cells shows increased P2X7 expression by immunofluorescence, compared to proliferating cells. Desensitized BeWo ATP-R cells lacking the P2X7 channel were unable to fuse and form STB however, when ATP was removed, the cells became sensitized and restored back the trophoblast fusion**.** Furthermore, we observed that agonist targeting P2X7 channel and its overexpression resulted to greater STB formation however, in the presence of antagonist, or genetic knockdown approach targeting P2X7 channel decreased STB formation, suggesting the involvement of P2X7 channel during trophoblast fusion.
Conclusion: Overall, we conclude that purinergic signaling pathway regulates the trophoblast fusion by maintaining delicate balance of eATP, activating P2X7 channel.

P2.121.
BCL6-MEDIATED REGULATION OF PLACENTAL REACTIVE OXYGEN SPECIES: UNRAVELING NOVEL MECHANISMS IN PREECLAMPSIA

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Objectives: Placental development relies on tightly regulated processes, including the controlled production of reactive oxygen species (ROS), which support key functions like cell proliferation and angiogenesis. Excessive ROS can lead to oxidative stress, impairing placental function. In preeclampsia (PE), marked by hypertension and organ dysfunction, elevated ROS levels contribute to abnormal placental development, inflammation, and vascular complications, adversely affecting maternal and fetal outcomes. Interestingly, it has been reported that the transcriptional repressor B-cell lymphoma 6 (BCL6), which is deregulated in PE, modulates ROS. This study aims to reveal the potential role of BCL6 in regulating ROS in placenta.
Methods: To address this issue, 3D-placental organoids will be generated from TS^{CT} cells, and term trophoblasts, including those from preeclamptic placentas. BCL6 expression will be selectively modulated using shRNA-mediated knockdown and specific pharmacological inhibitors. Basal and stress-induced ROS levels will be quantified to assess redox homeostasis and resilience. Comprehensive transcriptomic and proteomic analyses will be performed to identify BCL6-regulated genes and pathways, with integrated bioinformatic approaches employed to define signaling networks and potential direct BCL6 targets relevant to oxidative stress and placental dysfunction.
Results: Initial data show that BCL6 inhibition, particularly with FX1, significantly upregulates ROS-associated genes, such as *CYP1A1*, *CYP1B1*, and *OSGIN1*, while this inhibition downregulates *CYP2C18*, indicating altered redox regulation. RNA-seq of FX1-treated TS^{CT}-derived placental organoids ($p\text{-}Org^{ins.out}$) revealed 72 differentially expressed genes involved in ROS production, detoxification, and signaling compared to DMSO controls. Similar but milder effects were observed with two other BCL6 inhibitors BI3812, BI3802, and in FX1-treated 2D TS^{CT} cells. Further functional studies are underway to validate and extend these observations.

Conclusion: These findings suggest that BCL6 may be a key regulator of oxidative stress in the human placenta. Ongoing studies aim to further define its molecular targets and functional impact, offering potential insights into placental dysfunction in disorders, such as preeclampsia.

P2.122.
DESCRIPTIVE ANALYSIS OF ENDOCANNABINOID SYSTEM GENE AND PROTEIN EXPRESSION IN HUMAN PLACENTAL MODELS

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Objectives: The endocannabinoid system (ECS) plays a critical role in placental function, yet its expression across human placental models remains poorly defined. This study aims to comprehensively characterize ECS gene and protein expression in commonly used human placental models—including cell lines, primary cells, and villous explants—to establish a reference data supporting model selection and advancing ECS research in placental biology.
Methods: ECS expression was analyzed in term-placental villous explants, primary trophoblast cultures, and trophoblast-derived cell lines. Villous explants were cultured for seven days to assess ECS expression dynamics during syncytiotrophoblast regeneration. Primary trophoblasts isolated from the term placenta were analyzed at the cytotrophoblast (CTB) and syncytiotrophoblast (STB) stages. Cell lines included BeWo (cytotrophoblast-like), forskolin-treated BeWo (syncytialized), HTR-8/SVneo (extravillous trophoblast-like), and ACH-3P (combined villous/extravillous features). Initially, 16 ECS-related genes were screened by quantitative RT-PCR. Eight key components—CB1, CB2, MAGL, DAGL, FAAH, NAPE-PLD, TRPV1, and COX-2—were further quantified by droplet digital PCR (ddPCR). Protein expression and localization were assessed by Western blot and immunofluorescence (IF).
Results: Quantitative RT-PCR screening revealed differential ECS gene expression across models. Subsequent ddPCR confirmed distinct expression patterns dependent on trophoblast subtype and differentiation state. Western blot and IF analyses showed clear, model-specific differences in ECS protein expression and localization, validating transcript-level findings and highlighting potential functional distinctions between trophoblast subpopulations.
Conclusion: This comparative study provides novel evidence of significant variability in ECS gene and protein expression among widely used human placental models. Our findings emphasize the importance of model selection for ECS-focused placental research and contribute a foundational reference for investigating ECS roles in placental physiology and trophoblast differentiation.

P2.123.
EXTRACELLULAR VESICLES DERIVED FROM HUMAN PLACENTAL TROPHOBLAST INHIBITED ENDOMETRIOTIC CELL MIGRATION: A POTENTIAL THERAPEUTIC FOR ENDOMETRIOSIS.

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Objectives: Endometriosis is generally thought to be caused by the migration of endometrial cells or tissue similar to the lining of the endometrium outside the uterus, resulting in an often-painful clinical condition and an increased risk of infertility. The human placenta is a tumour-like organ in its growth and invasion, but its invasion is well-regulated during pregnancy, partially contributed to by factors released from the placenta. Previous studies reported that the phagocytosis of EVs derived from the placenta inhibited ovarian cancer cell growth by delaying the cell cycle. Given the similarities between the nature of endometriosis and cancer cells, particularly in cell migration, we investigated whether placental trophoblast EVs can inhibit the growth and migration of endometriotic cells.
Methods: 1st trimester placental tissues were collected after surgical suctions, explants were cultured overnight, and trophoblast EVs were collected from conditioned media. 12Z cells, an endometriotic cell line, were seeded until confluent and cultured with placental trophoblast EVs for 24 and 48 hours. The cell proliferation was then measured using Alamar Blue and CCK8 assays. Additionally, 12Z cells were treated with placental explant culture-conditioned media for 24 and 48 hours to measure their migration, using scratch wound healing and invasion assays.
Results: Compared to controls, the Alamar Blue and CCK8 assays showed a significant reduction of 12Z cell proliferation following 24 or 48 hours of treatment. Additionally, scratch wound healing assay showed a significantly smaller area of 12Z cell migration after the treatment with placental explant culture conditioned media for 24 hours, compared to controls. After treatment, the transwell migration assay confirmed a significant reduction of 12Z cell migration/invasion. Proliferation and invasion-associated miRNAs from C19MC, unique to the human placenta, were significantly increased in 12Z cells after treatment.
Conclusion: Placental trophoblast EVs inhibited endometriotic cell proliferation and migration, suggesting a potential therapeutic for endometriosis.

P2.124.
THE ROLE OF STAT3 SIGNALING IN HUMAN PLACENTAL EXTRAVILLOUS TROPHOBLAST DIFFERENTIATION

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Objectives: Extravillous trophoblasts (EVTs) play a central role in placental development and maternal-fetal interface remodeling. These cells differentiate from cell column trophoblasts at the distal tips of anchoring villi, but the molecular cues driving this process remain incompletely defined. While our lab and others have characterized the roles of WNT, NOTCH, and TGF-β signaling in EVT biology, key differences between in vitro and in vivo EVT differentiation persist. One pathway that remains understudied in this context is the Leukemia Inhibitory Factor-Signal Transducer and Activator of Transcription 3 (LIF-STAT3) signaling axis. Given LIF's abundant expression during pregnancy, we aimed to investigate its role and the downstream activation of STAT3 in EVT differentiation.
Methods: We employed 3D trophoblast organoids, first-trimester placental explants, and freshly isolated EVTs derived from human placental tissue. These models were analyzed using brightfield imaging, immunofluorescence microscopy, qPCR, Western blotting, and zymography to assess morphological, phenotypic, and molecular changes.
Results: Immunofluorescence of first-trimester placental sections revealed strong STAT3 phosphorylation (Tyr705) in distal EVTs and increased LIF receptor expression. In contrast, STAT3 phosphorylation declined over time in cultured primary EVTs and was absent in trophoblast organoids under EVT differentiation conditions. However, LIF supplementation restored STAT3 phosphorylation, confirming ligand-dependent activation. LIF-treated organoids and primary EVTs upregulated late EVT markers (HPGD, DAO, PAPPA2) at both RNA and protein levels. Interestingly, LIF exposure reduced explant invasiveness and suppressed active MMP2, suggesting a shift towards a more differentiated, sessile phenotype.
Conclusion: STAT3 activation at the distal ends of anchoring villi may promote EVT maturation toward a late-stage, less migratory phenotype. These findings highlight LIF-STAT3 as a key regulator of EVT differentiation and offer insight into refining in vitro models to better mimic in vivo trophoblast development.

P2.125.
BCL6 REGULATES TROPHOBLAST DIFFERENTIATION AND WNT SIGNALING: IMPLICATIONS FOR PREECLAMPSIA

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Objectives: The placenta is essential for fetal growth and development during pregnancy. Key developmental processes depend on the differentiation of villous cytotrophoblasts (vCTBs) into syncytiotrophoblasts (STB) and extravillous trophoblasts (EVTs), which are governed by multiple signaling pathways, including Wnt. B-cell lymphoma 6 (BCL6), an established oncogene involved in cell differentiation, has recently been identified as a regulator of trophoblast survival, fusion, and invasion. Dysregulated BCL6 expression has been associated with preeclampsia (PE), a leading cause of maternal and neonatal complications. This study aimed to elucidate the role of BCL6 in trophoblast differentiation and function.
Methods: We employed both two-dimensional (2D) trophoblast cell lines (JEG-3, TS^{CT}) and three-dimensional (3D) placental organoid models. Organoids with inverted (p-$Org^{ins.out}$) and physiological (p-$Org^{phys.pol.}$) polarity were generated from TS^{CT} cells, term, and preeclamptic placental trophoblasts. Organoid polarity and cellular composition were assessed by immunostaining. Gene expression was analyzed using RNA-sequencing approaches. BCL6 activity was modulated via siRNA knockdown and pharmacological inhibitors (BI3812, FX1, BI3802). Transcriptomic comparisons were performed between 2D and 3D cultures, as well as following BCL6 inhibition. STB differentiation was evaluated by assessing marker gene expression (GCM1, CGB3, SYN2, LEP) and β-HCG secretion in treated organoids.
Results: We successfully established 3D organoids from TS^{CT} and primary trophoblasts, recapitulating villous structure and showing appropriate differentiation. BCL6 inhibition with BI3812, FX1, and BI3802 led to downregulation of known targets (CAV2, MYLK) and transcriptomic changes. Notably, Wnt pathway components (WNT7B, FZD5, TCF7L2, N-MYC) were consistently downregulated, indicating a central role for BCL6 in Wnt-mediated trophoblast differentiation. STB marker expression and β-HCG secretion were reduced upon BCL6 inhibition, underscoring its importance in STB formation and placental development.
Conclusion: BCL6 is a key regulator of trophoblast differentiation and STB formation through modulation of the Wnt pathway. Its dysregulation may contribute to the development of preeclampsia, offering potential markers or targets for therapeutic intervention.

P2.126.
EXPRESSION AND REGULATION OF AGBL2 IN HUMAN PLACENTAL TISSUE AND TROPHOBLAST CELL LINES

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Objectives: AGBL2 (ATP/GTP-binding protein-like 2) encodes a carboxypeptidase that catalyzes the detyrosination of alpha-tubulin, influencing microtubule stability. We previously showed that detyrosinated tubulin (detyr-tubulin) was enriched in villous placental endothelial cells, while tyrosinated tubulin (tyr-tubulin) was also expressed in villous cytotrophoblasts (VCT) and extravillous trophoblasts (EVT). Both detyr- and tyr-tubulin were absent in syncytiotrophoblast (SCT). Importantly, detyr-tubulin expression was reduced in preeclamptic (PE) placentas, suggesting impaired microtubule remodeling. We further demonstrated that RARRES1, a proposed inhibitor of AGBL2, is epigenetically regulated and overexpressed by trophoblasts in PE, suggesting a potential upstream influence on the α-tubulin tyrosination cycle in placental dysfunction. Building on these findings, the current study investigates the compartment-specific expression and regulation of AGBL2 as the enzymatic driver of detyrosination in human placenta and model cell lines.
Methods: AGBL2 expression was analyzed in third-trimester human placenta immunohistochemically and via qPCR of primary villous trophoblasts. In vitro, the cell lines Swan71, JEG-3, JAR, and BeWo were analyzed for AGBL2 expression and its modulation by the DNA demethylating agent 5-Aza-2′-deoxycytidine (AZA).
Results: AGBL2 was expressed in third-trimester VCT and EVT, but absent in SCT. Only vascular endothelial cells of the maternal placental bed showed AGBL2-positive staining. Among the cell lines, Swan71 exhibited the highest AGBL2 expression, while JEG-3 showed only basal levels that were significantly upregulated upon AZA-treatment, suggesting epigenetic repression. No significant AZA effect was observed in the other cell lines.
Conclusion: AGBL2 is compartment-specifically expressed in the third-trimester placenta and appears to be epigenetically regulated in selected trophoblast cell lines. These findings support a functional role for AGBL2 in cytoskeletal regulation and placental development. Further studies are needed to elucidate how AGBL2-mediated detyrosination influences placental function, how upstream regulators such as RARRES1 modulate this process, and whether these pathways may be therapeutically targeted in gestational diseases such as PE.

P2.127.
THE ROLE OF METALLOPROTEASES IN TROPHOBLAST PROGENITOR CELL MAINTENANCE

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Objectives: Progenitor cells of the developing placenta, called cytotrophoblasts (CTB), are capable of long-term self-renewal and differentiation into specialized extravillous trophoblasts and syncytiotrophoblasts (SCT) that are necessary for placental function. Remarkably, there is limited understanding of processes controlling the maintenance of progenitor trophoblasts.
A specialized tissue microenvironment, or niche, is important in progenitor cell identity and function. Tissue niches that support progenitor maintenance and growth have unique compositions of extracellular matrix (ECM). ECM components are a rich source of growth factors that can be liberated by matrix metalloproteases. Protease-directed ECM turnover is important in controlling progenitor self-renewal, senescence, and differentiation.
Single-cell transcriptomic profiling of human placentas identified 11 metalloproteases specifically expressed in progenitor CTB. My *objective* is to investigate the role of CTB-specific metalloproteases in human trophoblast progenitor growth and maintenance.
Methods: Human trophoblast stem cells were cultured as three-dimensional organoids and treated with 1μM batimastat, a potent and broad-spectrum metalloprotease inhibitor. Trophoblast organoids cultured with equal volumes of DMSO served as controls. The effect of batimastat was measured on organoid establishment and growth (live imaging microscopy), proliferation (EdU immunofluorescence), apoptosis (Annexin V flow cytometry), human chorionic gonadotropin (hCG) production (ELISA), and gene expression (Bulk RNA-sequencing; $n=3$).
Results: Inhibiting metalloproteases significantly reduced the size of trophoblast organoids; however, the frequency of organoid establishment did not differ from controls. Treatment with batimastat did not affect organoid apoptosis or proliferation, however there was a significant reduction in hCG secreted by the SCT core of trophoblast organoids. RNA-sequencing identified 3,445 differentially expressed genes (DEGs) in response to batimastat, where top DEGs suggest impaired SCT development. Together, these results suggest that inhibiting metalloproteases impairs SCT development, yielding reduced trophoblast organoid growth.

Conclusion: This work provides insight into the role of metalloproteases in the differentiation of human trophoblast progenitor cells, and advances knowledge around processes regulating early placental development.

P2.128.
IMPACT OF ARTERIAL AND VENOUS ENDOTHELIAL CELL ORIGIN ON TROPHOBLAST INVASION USING A 3D BIOENGINEERED PCL/PLA SCAFFOLD SYSTEM

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Objectives: Proper invasion of trophoblasts into maternal vessels during the first trimester of pregnancy is crucial for successful placentation. Although differences in the invasion patterns between maternal arteries and veins are recognized, the cellular mechanisms underlying these processes are not yet fully characterized. Here, we investigate whether the type of endothelial cells influences trophoblast invasion behavior using a bioengineered 3D cell culture system.
Methods: A modular 3D cell culture system based on electrospun PCL/PLA scaffolds was established, enabling endothelialization on one side and trophoblast seeding on the other side of the scaffold. Human iliac vein endothelial cells (HIVEC) and human iliac artery endothelial cells (HIAEC) were used to represent venous and arterial endothelium, respectively. Initially, ACH-3P trophoblast cells were applied, followed by the use of first trimester villous explants containing native trophoblasts. Cultures were maintained under controlled oxygen conditions (21% or 8% O_2). Invasion and attachment were assessed using histological and immunofluorescent staining (H&E, CK7, HLA-G, CD31) for qualitative analysis combined with automated quantitative image analysis. Additionally, biochemical methods including qPCR and cytokine expression analysis via ELISA were applied.
Results: Preliminary results demonstrated successful endothelialization with both primary HIVEC and HIAEC, as well as attachment and initial invasion of ACH-3P cells and first trimester villous trophoblasts into the PCL/PLA scaffolds. Initial observations suggest that the endothelial cell type, reflecting their vascular origin (arterial vs. venous), may influence the extent of trophoblast invasion, although oxygen conditions must also be considered in data interpretation.
Conclusion: The established bioengineered 3D system enables controlled investigation of first trimester trophoblast invasion towards different types of endothelial cells. Preliminary data suggest that endothelial origin may influence invasion behavior, with oxygen conditions acting as an additional modulatory factor. Future studies will incorporate dynamic flow to further enhance the physiological conditions of the system.

P2.129.
SPATIAL PROFILING OF THE EARLY HUMAN FETAL-MATERNAL INTERFACE REVEALS A UNIQUE TISSUE RESTRUCTURING PROCESS AND A DISTINCT SPATIAL DISTRIBUTION OF IMMUNE CELLS LINKED TO THE DEGREE OF EXTRAVILLOUS TROPHOBLAST INVASION

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Objectives: Extravillous trophoblasts (EVTs) are specialized fetal cells that invade the maternal decidua during pregnancy. The decidua features a complex immune cell composition, including macrophages and decidual natural killer (dNK) cells. The decidual microenvironment interacts with but generally tolerates the EVTs. However, the impact of EVT invasion on the decidual tissue architecture and immune landscape is still poorly understood.
Methods: A first trimester decidual tissue cohort (gestational age 5-12 weeks, matched *decidua basalis* and *parietalis* tissue and unique archival specimens, n=49) was used to systematically assess the early decidua, with particular emphasis on structural and cellular heterogeneity of the tissue. We applied integrative spatial and single-cell transcriptomics analysis in combination with classical histological approaches that were quantitatively analyzed with semi-automated image analysis (HLA-G, CD3, CD8, CD56, CD14, CD163), observer-based systematic assessment (H&E, for debris, fibrinoid, epithelial integrity, extravasal erythrocytes, neutrophils) and staining series with an extensive marker panel (CD66b, fibrin, CD235a, CD31, HLA-G, CD3, CD8, CD14, CD163, CD56, CD11c).
Results: When defining the tissue areas for this study (*decidua parietalis*: (i) *zona compacta*, (ii) *zona spongiosa*; *decidua basalis*: (iii) weak invasion, (iv) strong invasion), we identified areas of obvious morphological change within the strongly invaded *decidua basalis*. We defined these areas as (v) remodeling lesions. These remodeling lesion areas exhibited unique properties, characterized by fibrin deposits, altered tissue integrity, extravasal erythrocytes, eroded blood vessels, frequent interstitial gaps and the presence of neutrophils. Additionally, we observed a decline in the number of local immune cells (T cells, macrophages, dNK cells).
Conclusion: Taken together, our study reveals morphologically distinct areas, defined as remodeling lesions, within the early *decidua basalis*. Furthermore, the degree of invading EVTs is closely intertwined with changes in the decidual immune landscape. We propose that remodeling lesions are crucial for the appropriate restructuring of the decidual tissue architecture.

P2.130.
EFFECTS OF MATERNAL THC ADMINISTRATION ON TRYPTOPHAN METABOLISM IN THE PLACENTA AND FETUS OF WISTAR RATS

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Objectives: This study aimed to investigate the effects of prenatal tetrahydrocannabinol (THC) exposure on tryptophan metabolism-related gene expression in the placenta and fetal organs. Tryptophan metabolism plays a crucial role in serotonin biosynthesis, immune regulation, and the production of neuroactive kynurenine metabolites, making it essential for fetal development. Understanding these molecular changes may provide insight into the potential risks and outcomes associated with THC use during pregnancy.
Methods: Pregnant Wistar rats were randomly assigned to treatment and control groups. The treatment group received 3 mg/kg THC daily via oral administration in sesame oil from gestational day (GD) 6 to GD 21, while the control group received sesame oil alone as the vehicle. A total of 16 rats were used in this study. At GD 21, placental tissues and fetal organs (brain, lung, liver, heart, kidney and intestine) were collected from the control group and THC-treated pregnant dams. RNA was isolated using TRIzol®. cDNA was synthesized using iScript™ cDNA Synthesis Kit, and qPCR was performed to analyze the expression of tryptophan metabolism-related genes, including *IDO2, KMO, KAT1, QPRT, HAAO, KYNU, TPH1, MAO-A, MAO-B, SERT, OCT3, LAT1, LAT2, SLC3A2*.
Results: Fetuses from THC-exposed pregnancies exhibited lower fetal and placental weights compared to controls, suggesting impaired placental function and fetal growth restriction. In addition, analysis of gene expression revealed changes in genes associated with tryptophan metabolism in both the placenta and fetal organs.
Conclusion: Prenatal THC exposure led to impairment in tryptophan metabolism in the placenta and fetal organs, suggesting potential dysregulation in tryptophan metabolites that could affect fetal growth and programming. The observed reduction in fetal and placental weights further points to possible placental insufficiency and growth restriction, highlighting the impact of maternal cannabis use on fetal development.

P2.131.
MATERNAL PARITY AFFECTS PLACENTAL GENE EXPRESSION AT TERM IN AN EQUINE MODEL

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Objectives: Primiparity is a natural model of intrauterine growth restriction in horses. Foals born to primiparous mares are typically smaller and lighter, with lower placental weights, compared to those from multiparous mares. These differences persist postnatally, as primiparous offspring show delayed testicular and metabolic development. Despite these long-term effects, placental function in primiparous mares remains poorly understood. Previous studies report reduced feto-maternal exchange surfaces and decreased uterine artery blood flow in primiparous placentas, but gene expression data are lacking. This study aimed to compare term placental gene expression between primiparous and multiparous mares.
Methods: Six mares were artificially inseminated for two consecutive years. In Year 1, all mares were primiparous (P, n=6); in Year 2, the same mares were multiparous (M, n=6). Placentas were sampled immediately after birth near the umbilical cord, snap-frozen, and stored at −80°C until analysis. Total mRNA was extracted using the TRIzol method. Paired-end directional sequencing was performed on a NextSeq500 Illumina platform. Differential gene expression was analyzed using the DESeq2 package in R software. Gene network analysis was conducted using STRING (Gene Ontology, KEGG, Reactome), with redundancy reduction via Revigo. Significance was set at adjusted $p < 0.05$.
Results: No differences in gestation length, placental weight nor foal weight at birth were observed between P and M groups. In P vs. M placentas, 2,110 genes were upregulated and 3,551 downregulated (fold change >2). Upregulated genes were associated with RNA translation, protein synthesis, metabolism, vesicle transport, inflammation, catabolism, migration and cell motility, stress response, blood vessel contraction, and apoptosis. Downregulated genes were related to cell division, cytoskeleton and microtubule organization, RNA transcription, primary cilia synthesis and function, and DNA repair.
Conclusion: These findings suggest that pathways involved in parturition, such as inflammation, vasoconstriction, apoptosis, and extracellular matrix remodelling, are more active in primiparous placentas compared to multiparous ones.

P2.132.
EFFECTS OF HEAT STRESS DURING LATE GESTATION ON PLACENTAL MORPHOMETRY AT TERM IN PRIMIPAROUS AND MULTIPAROUS DAIRY COWS

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Objectives: The aim of this study was to evaluate the impact of voluntary access to natural shade (NS) at term placental morphometry in Primiparous (P) and Multiparous (M) dairy cows exposed to heat stress (HS) during final third gestation (FTG).
Methods: The experiment was carried out at Faculty of Agronomy (Udelar, Latitude: 31° 23'S and Longitude: 57° 57'W) in summer 2024 with an average ITH index 80 (critical ITH ≥ 72). Approved ethical protocol No. 1389. Twenty-two pregnant cows (P; n=10 and M; n=12), BW=566±55 kg and BCS=2,9±0,27, managed in a grazing system were dried off 72±17.1 days before calving and randomly assigned to two treatments: with (ANS) and without (NNS) voluntary access to NS (Eucalyptus stand). Placentas were collected at birth (4.6±2.4 h) and analyzed for placental weight (PW), intercotyledonary membrane weight (ICW), and cotyledon number (CN), weight (CW), and surface area (CSA). Cotyledons were grouped into six size-based categories (1=smallest to 6=largest) and the means values were analyzed.
Results: P showed higher PW (6.0 vs 5.0 kg; p=0.044), ICW (4.3 vs 3.1 kg; p=0.007), CN (94 vs 72; p=0.025), MCW (19 vs 28 g; p=0.008), and MCSA (0.040 vs 0.059 m^2). Additionally, higher CW3 and CN1, and larger SA1, SA2, and SA3 were observed compared to M, with tendencies for higher CW2 and CN2, and lower CW5.
Conclusion: P exhibited a compensatory placental development, probably due to the overlapping growth and gestation requirements.

P2.133.
PLACENTAL INTERFACE IN POLISH DRAFT MARES WITH RETAINED FETAL MEMBRANES - HISTOLOGICAL OBSERVATIONS.

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Objectives: Fetal membranes retention (FMR) of unknown etiology is a life-threatening condition in horses. In draft breeds, its occurrence can reach up to 50%. The aim of this study was to analyse biological factors associated with FMR.
Methods: In the 2024-2025 breeding seasons, 17 parturitions were monitored in draft mares bred in similar conditions. Mares' age, weight, gestation length and placental volume were compared between affected (FMR) or healthy (noFMR) mares. Median difference between groups were analyzed, using a percentile bootstrap. Both allantochorion and endometrium were biopsied after foal delivery. Endometrial crypt depth and villi length were measured on HE stained slides. Two-way mixed ANOVA with Bayes factor (BF) was performed.
Results: Altogether, FMR occurred in 10 mares (frequency=58,8%). Weight and age distribution were fairly similar in both groups (median differences: 20.5kg, 0 years; 95%CIs: -88 to +122 kg, -2 to +3 years; P=0.62 and P=0.83; respectively). Gestation length did not clearly differ between groups (median difference: 3.5days shorter with FMR; 95%CI: -20 to +12 days; P=0.69). Placental volumes were also similar in both groups (median difference: 0 liters; 95%CI: -1.0 to +0.8 liters, P=0.75). For technical reasons, histological analyses could only be performed in 10 mares (5FMR and 5noFMR). FMR was associated with longer villi and deeper crypts than noFMR. The interaction between villi and crypts was 2.7-times stronger in FMR vs. noFMR (BF=2.7, P=0.05 is equivalent to BF≤2.46).
Conclusion: These data indicate that deeper endometrial crypts and longer villi are associated with higher risk of FMR. More data are being collected to increase animal numbers. Stereological and gene expression analyses are pending.
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P2.134.
EXPOSURE TO BISPHENOL A JEOPARDIZES DECIDUALIZATION AND CONSEQUENTLY TRIGGERS PREECLAMPSIA BY UP-REGULATING CYP1B1

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Objectives: Preeclampsia (PE) is a pregnancy-related disease that poses a significant threat to the health of both the mother and the fetus. Previous studies have primarily focused on the role of the placenta in PE pathogenesis; however, normal decidualization is crucial for the subsequent development of the placenta and pregnancy. Bisphenol A (BPA) is an environmental endocrine disruptor commonly used in the synthesis of

polycarbonate and epoxy resins. Overexposure to BPA can result in severe reproductive issues.
Methods: To further investigate the effects of BPA exposure on pregnancy, C57BL/6 mice were continuously exposed to either 0 or 100 mg/kg of BPA in this study.
Results: As a result, these mice developed symptoms of hypertension and proteinuria, indicative of PE. Additionally, their decidualization process was impaired. Transcriptome sequencing of artificially induced decidua revealed a significant upregulation in the expression of CYP1B1 within the BPA-treated group. This upregulation accelerated the metabolism of estrogen and progesterone, leading to significant decreases in their levels. Furthermore, the expression levels of estrogen and progesterone receptors and their responding genes were significantly reduced.
Conclusion: These findings suggest that BPA exposure can negatively impact decidualization and placental development, potentially contributing to the development of PE.

P2.135.
STUDY OF THE MOLECULAR BASIS OF HUMAN TROPHOBLAST FUSION

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Objectives: This study aims to investigate the molecular pathways that regulate the fusion of human trophoblasts into syncytiotrophoblasts, a key process for proper placental development. Special emphasis is placed on exploring how defects in trophoblast fusion contribute to the onset and progression of preeclampsia, a major hypertensive disorder of pregnancy
Methods: BeWo cells, a human choriocarcinoma-derived trophoblastic cell line, are used as an in vitro model in this study due to their capacity to undergo syncytial fusion upon treatment with forskolin, a known activator of the cyclic AMP (cAMP) signaling pathway. The *CLCN6* gene is knocked out in these cells using the CRISPR-Cas9 genome editing system. The resulting cellular phenotype is characterized using multiple molecular and cellular biology techniques, including RT-qPCR, Western blotting, immunofluorescence, ELISA, and the Incucyte live-cell imaging system
Results: The initial key finding of this study is the successful establishment of *CLCN6* knockout (KO) BeWo cell lines using CRISPR-Cas9-mediated gene editing. Subsequent phenotypic analyses indicate that *CLCN6*-deficient cells exhibit a significantly higher rate of syncytial fusion compared to wild-type (WT) controls. This enhanced fusogenic potential is correlated with an increased intracellular level of cyclic adenosine monophosphate (cAMP), suggesting that the absence of *CLCN6* may modulate signaling pathways involved in trophoblast differentiation and fusion. These results point toward a potential regulatory role of *CLCN6* in cAMP-mediated trophoblast syncytialization, a process that is crucial for normal placental development and often impaired in pregnancy-related disorders such as preeclampsia
Conclusion: Preliminary analyses provide support for the hypothesis that CLCN6 plays a role in the pathophysiology of preeclampsia. Alterations in its expression may disrupt the trophoblastic fusion process, leading to placental dysfunctions that could contribute to the pathogenic mechanisms underlying the disease