promotes the derivation of fetal MSCs from term placentae above that of more commonly employed DMEM/F12 media. However, the extent of contamination in first-trimester MSC preparations, and the effects of these media on MSC phenotype remain unclear. Therefore, we aimed to determine the effects of culture media on maternal cell contamination and fetal MSC phenotype across gestation.

Methods: MSCs were isolated from first-trimester (n=10) and term (n=18) placentae explants in advanced-DMEM/F12 or EGM-2 media. Male placentae were identified using PCR for SRY. Fluorescence *in-situ* hybridisation was used to evaluate the proportion of maternal (XX) and fetal (XY) cells within male MSC isolates. MSC phenotype was analysed by flow cytometry, immunohistochemistry and Alamar blue proliferation assays.

Results: All first-trimester MSCs isolated in advanced-DMEM/F12 exhibited maternal contamination (x= 84±22% XX cells, n=5). In contrast, the majority (7/9) term MSC isolates contained >98% fetal cells. When isolated in EGM-2, first-trimester MSCs first-trimester (n=10) and term (n=14) placentae were 95-100% fetal. Advanced-DMEM/F12 halved the rate of fetal MSC proliferation compared to EGM-2 ($p<0.005$, n=3). Fetal MSCs in both media expressed the generic MSC phenotype (CD90+, CD105+, CD73+, CD31-, CD34-, CD45-). However MSCs transferred from EGM-2 to advanced-DMEM/F-12 increased the expression of smooth muscle markers Calponin and α-smooth muscle actin, and decreased the endothelial cell marker VEGFR2 (n=4).

Conclusion: MSCs from first-trimester placentae are highly prone to maternal contamination despite a lack of visible decidual tissue. However, EGM-2 media can be used to isolate pure fetal MSCs. Media affects the phenotype of fetal MSCs, and thus careful consideration should be given to conditions in which MSCs are differentiated.

P1.136.
DERIVATION OF HUMAN TROPHOBLAST STEM CELLS FROM HUMAN PLURIPOTENT STEM CELLS

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Objectives: Trophoblast (TB) derived from human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs), is an attractive model for early human trophoblast development. Here we describe the derivation of human trophoblast stem cells (hTSCs) from hESCs.

Methods: H1 (male) and H9 (female) hESCs cultured in E8 medium were differentiated to the trophoblast lineage by treatment with an activin/nodal signaling antagonist, bone morphogenetic protein (BMP4), and an agonist of the sphingosine 1 phosphate (S1P) receptor in E7 medium (E8 medium without transforming growth factor-beta (TGF-β)). $CDX2^+$ TB obtained following this treatment were further passaged into a chemically defined medium based on E6 medium (E7 without fibroblast growth factor-2 (FGF2)) called TM4, or into trophoblast stem cell medium (TSCM) as previously described by Okae et al. (Cell Stem Cell 2018 22(1):50-63).

Results: HESC-derived TB have been maintained as hTSCs for several passages in both TM4 and TSCM. In TM4, hTSCs expressed CDX2, TFAP2C, YAP, TEAD4, and GATA3, in addition to the pan TB marker, KRT7 and low levels of p63; these cells were denoted $hTSC^{CDX2}$. In contrast, hTSCs in TSCM lost expression of CDX2 and gained high expression of p63; these cells were denoted as $hTSC^{p63}$. Both $hTSC^{CDX2}$ and $hTSC^{p63}$ could be differentiated into mesenchymal extravillous trophoblast (EVT) expressing HLA-G, and multinucleate syncytiotrophoblast (STB) expressing human chorionic gonadotrophin (hCG), using protocols described by Sarkar et al. (J. Biol. Chem. 2015 290(14):8834-48) or Okae et al.

Conclusion: We show that two distinct hTSC states – $hTSC^{CDX2}$ and $hTSC^{p63}$ – can be derived from hESCs. We hypothesize that $hTSC^{CDX2}$ and $hTSC^{p63}$ are representative of the trophectoderm in the blastocyst-stage embryo, and villous cytotrophoblasts of the first trimester placenta, respectively. The ability to routinely derive hTSCs, e.g. from hPSCs generated from tissues associated with placental disorders, can significantly accelerate research on human trophoblast biology.

P1.137.
TROPHOBLAST STEM CELLS ISOLATED USING THE SIDE-POPULATION TECHNIQUE ARE DEPLETED IN PLACENTAE FROM GROWTH RESTRICTED PREGNANCIES

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Objectives: The pathogenesis of FGR develops in early pregnancy, but does not present clinically until later in gestation, making FGR difficult to diagnose and treat. FGR is linked to inadequate trophoblast function, but our understanding of the mechanisms underlying this are poor. The recent isolation of human trophoblast stem cells (TSC) from first-trimester placentae provides a major advance in understanding normal trophoblast differentiation (Okae et al., 2018). However, TSCs could not be isolated from third-trimester placentae, meaning that their role in pregnancy disorders such as FGR remains elusive.

Methods: Our previously published Hoechst side-population technique was used to isolate cells from first-trimester placentae. These cells were maintained in culture and differentiated into mature trophoblast lineages using the conditions defined by Okae et al. The same side-population technique was used to isolate TSCs directly from normal third-trimester and FGR placentae.

Results: First-trimester side-population trophoblasts can be passaged in culture for at least 5 months, and appear morphologically similar to TSCs isolated by Okae et al. Side-population trophoblasts differentiate via a cytotrophoblast-like (β4 integrin positive) phenotype into either 1) multinucleated hCG positive and syncytin-1 positive syncytiotrophoblast-like cells (n=3), or 2) HLA-G positive extravillous trophoblast-like cells that significantly increased their invasion through Matrigel (n=3). Thus, trophoblasts isolated using the side-population technique exhibit the expected potency of a human TSC population. Using the same technique, TSCs could be isolated from third-trimester placentae for the first time, demonstrating that while they were present at consistent levels throughout gestation (~3.5%, n=20 for both first-trimester and third-trimester placentae), TSCs were significantly depleted in FGR placentae (0.32%, n=5, $p<0.001$).

Conclusion: Our novel method of isolating human TSC directly from placental tissue throughout gestation provides the first opportunity to examine their role in pregnancy pathologies. The depletion of TSC in FGR placentae may contribute to their poor trophoblast function.

P1.138.
PLACENTA-ON-A-CHIP MODEL FOR ASSESSING THE TRANSPORT AND TOXICITY OF XENOBIOTICS *IN VITRO*

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Objectives: The study of the transport and toxicity of xenobiotics in women is limited for ethical reasons. *Ex vivo* placenta models have high variability and low success rates. Animal models *in vivo* differ from a human in anatomy, genotype, and proteome. The placenta-on-a-chip model is a compromise. We studied the components of the FAC chemotherapy regimen for breast cancer in this model.

Methods: BeWo b30 cell line was grown in the DMEM with L-glutamine, 4.5 g glucose/l and Earle's salts containing 10% FBS, 1x MEM NEAA, 100 U/

ml penicillin and 100 μg/ml streptomycin in inserts cut from 96-well Transwell plate and placed in a microfluidic chip. Cells were seeded with a density of 10,000 cells per insert. After 7 days, 5-fluorouracil (25 μg/ml), doxorubicin (50 μg/ml), cyclophosphamide (150 μg/ml), or all three drugs were added to the cells for 1 hour. Control cells were cultured in the presence of 0.05% DMSO. The impedance spectrum was measured before and 1 and 24 hours after the addition of the drug. The concentration of the drug was determined by HPLC-MS/MS. Cell viability was assessed using the CellTiter-Blue Assay.

Results: After 1 h incubation with drugs, TEER decreased in experiment and control groups from an average of 90 to 25 $\Omega \cdot cm^2$, and after 24 h TEER was 67.3±17.9 $\Omega \cdot cm^2$ for control, 67.8±16.4 $\Omega \cdot cm^2$ for cyclophosphamide, 90.0±20.1 $\Omega \cdot cm^2$ for 5-fluorouracil, and decreased to the background for doxorubicin and drug mixture. Cell viability did not differ significantly between the control, 5-fluorouracil, and cyclophosphamide groups, but decreased to 40±9% of the control when exposed to doxorubicin and drug mixture. The placenta-on-a-chip model transported the drugs from the apical to the basolateral side.

Conclusion: The developed placenta-on-a-chip model is suitable for assessing the transport and toxicity of xenobiotics *in vitro*.

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P1.139.
CHOLESTEROL UPTAKE AND EFFLUX IS IMPAIRED IN HUMAN TROPHOBLAST CELLS FROM PREGNANCIES WITH MATERNAL SUPRAPHYSIOLOGICAL HYPERCHOLESTEROLEMIA

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Objectives: Placental trophoblasts modulate maternal cholesterol uptake (via lipoprotein receptors as SR-BI and LDLR) and efflux (via cholesterol transporters as ABCA1, ABCG1 and SR-BI). Maternal physiological hypercholesterolemia (MPH) occurs in pregnancy assuring fetal development, but maternal supraphysiological hypercholesterolemia (MSPH) leads to endothelial dysfunction and atherosclerosis in fetal vasculature

The aim of this work is to determine the effect of MSPH on the traffic of cholesterol in human trophoblast.

Methods: pregnant women with total cholesterol (TC) ≤280mg/dL or >280mg/dL were considered as MPH (n=19) or MSPH (n=24), respectively. Trophoblasts were isolated by trypsin/DNAse digestions, from MPH and MSPH placentas. Cholesterol uptake was estimated in cells incubated with HDL or LDL labeled with DiI by fluorescence quantification. Cholesterol efflux to HDL was determined in cells pre-incubated with [^{3}H] cholesterol in absence or presence of inhibitors for SR-BI (BLT-1) and ABCA1 (glyburide). Expression of LDLR, SR-BI, ABCG1, ABCA1 and HMGCR were determined by immunofluorescence or western blot. Cellular content of cholesterol was determined enzymatically and by Filipin staining.

Results: Protein abundance of SR-BI and ABCG1 was lower (~35% and 33%) in trophoblast from MSPH without changes in LDLR or ABCA1. Compared to MPH, LDLR was localized almost entirely in the cellular surface of trophoblast from MSPH; result confirmed in whole placenta. The uptake of HDL-DiI and LDL-DiI as well the efflux of cholesterol to HDL was lower (~72%, 81% and 82%, respectively) in trophoblast from MSPH. Efflux was partially dependent of ABCA1 (~40%) and independent of SR-BI. TC content was comparable in trophoblast from MPH and MSPH; however, free cholesterol was higher (~1,8 folds) in MSPH and was not associated with HMGCR upregulation.

Conclusion: In trophoblasts from MSPH placentas, the expression and function of proteins related with cholesterol uptake, efflux and synthesis is downregulated possibly to avoid the exacerbated entry of this lipid into the fetal circulation.

P1.140.
MONOACYLGLYCEROL LIPASE MOBILISES INTRACELLULAR LIPID STORES IN THE HUMAN TERM PLACENTA

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Objectives: The placenta fulfils transport functions as nutrient supply to the fetus, gas exchange, and returns waste products to the mother. In particular, supply of maternally derived long-chain polyunsaturated fatty acids (PUFAs) is crucial for fetal development due to insufficient endogen synthesis. Considerable amounts of PUFAs are deposited transiently in placental tissue. However, the purpose of intermediate lipid storage, the mechanisms how these lipid stores are mobilised and the transfer of remobilised PUFAs to the fetus are not well understood. Monoacylglycerol lipase (MAGL) catalyses the hydrolysis of monoacylglycerols (MGs) to glycerol and fatty acids (FA). Moreover, MAGL acts as key enzyme in brain endocannabinoid system by degrading the endocannabinoid 2-arachidonoylglycerol. The placental endocannabinoid signalling system regulates implantation, embryo development, maintenance of pregnancy and labour. We aimed to determine the role of MAGL in FA related metabolic processes in the human placenta.

Methods: *Ex-vivo* perfusion, tissue explants, western blot, immunohistochemistry, in-situ hybridization, MAGL activity assay, MG and FA analysis by UPLC-MS/MS.

Results: In placental tissue, MAGL is localized predominantly at the apical membrane of the syncytium, facing maternal circulation, and in the feto-placental endothelium. MAGL enzyme activity was reduced by 74% (p=0.001) in placental explants (n=3) treated with MAGL inhibitor (50nM MJN110, 2h), subsequently an increase in total MG by 50% (±38) was observed. *Ex-vivo* perfusion of placental lobule with MAGL inhibitor (4h, n=4) led to elevated total MG (47% ±35, p=0.029). This was corroborated by attenuated total FA release to maternal and fetal circulation. Inhibition of MAGL resulted in significant reduction of arachidonic acid (53%, p<0.05) and docosahexaenoic acid (56%, p<0.05) in fetal circulation.

Conclusion: Together, these results suggest that MAGL substantially contributes to MG hydrolysis in human placental tissue, providing FA for placental/fetal supply. We conclude that placental MAGL is involved in the regulation of both endocannabinoid and FA metabolism.

P1.141.
VASOACTIVE INTESTINAL PEPTIDE INDUCES GLUCOSE AND NEUTRAL AMINO ACID UPTAKE THROUGH mTOR PATHWAYS IN HUMAN TROPHOBLAST CELLS

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Objectives: Nutrient transport across the placenta and sensing of nutrient levels through mTOR-mediated pathways is crucial for fetal growth. We have shown that vasoactive intestinal peptide (VIP), synthesized by human trophoblast (Tb) cells, increases human Tb migration and invasion. Also, VIP has embryo trophic effect in mice. Our aim was to evaluate the effect of VIP on glucose and neutral amino acid uptake in Tb cells and the interplay between VIP and mTOR.

Methods: Human trophoblast-derived cell lines Swan-71 (SW) and BeWo (BW) treated with VIP or silenced in VIP expression were used. Glucose and