on cultured primary trophoblasts, or bidirectionally on the Transwell® system using BeWo cells treated with forskolin. As forskolin can induce various gene alterations (e.g. cAMP response element genes), we aimed to establish a physiological primary trophoblast model for materno-fetal nutrient exchange studies without forskolin application.

Methods: Human term cytotrophoblasts were isolated by enzymatic digestion and Percoll® gradient separation. The purity of the primary cells was assessed by flow cytometry using the trophoblast-specific marker cytokeratin-7. After screening different coating matrices, we optimized the growth conditions for the primary cytotrophoblasts on Transwell→ inserts. The morphology of 5 days cultured trophoblasts was determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Membrane makers were visualized using confocal microscopy. Additionally transport studies were performed on the polarized trophoblasts in the Transwell® system.
Results: During 5 days culture, the trophoblasts (>90% purity) developed a modest trans-epithelial electrical resistance (TEER) and a size-dependent apparent permeability coefficient (Papp) to fluorescently labeled compounds (MW ~400-70'000D). SEM analyses confirmed a confluent trophoblast layer with numerous microvilli at day six, and TEM revealed a monolayer with tight junctions. Immunocytochemistry on the confluent trophoblasts showed positivity for the cell-cell adhesion molecule E-cadherin, the tight junction protein ZO-1, and the membrane proteins ABCA1 and Na^+/K^+-ATPase. Vectorial glucose and cholesterol transport studies confirmed functionality of the cultured trophoblast barrier.
Conclusion: Evidence from cell morphology, biophysical parameters and cell marker expressions indicate the successful and reproducible establishment of a primary trophoblast monolayer model suitable for transport studies. Application of this model to pathological trophoblasts will help to better understand the mechanism underlying gestational diseases, and to define the consequences of placental pathology on materno-fetal nutrient transport.

P2.119.
TRANSPLACENTAL TRANSFER OF 2- NAPHTHOL ACROSS THE HUMAN PLACENTA

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Objective: To determine the transfer of 2-Naphthol (NPH) in full-term human placental tissues using the ex vivo human perfusion model.

Methods: A total of six placentas were obtained from women undergoing elective Caesarean section or vaginal delivery at term. Schneider's ex vivo human placental perfusion model was adopted. The perfusate at fetal side was circulated at a flow rate of 4 ml/min. The flow at maternal intervillous space was at a rate of 12 ml/min. After equilibration, the perfusate in the maternal compartment was replaced with fresh perfusate containing 2-NPH at 100ug/mL, antipyrine at 50 mg/mL. Samples were obtained from the maternal and fetal circulations at 15, 30, 60, 120, 240, and 360 minutes.
Results: A total of 6 term placentas were perfused. The mean weight of the perfused cotyledon was 26.3 (±5.5) grams. The fluid shift between the fetal and maternal compartments was <3ml/h.
All pre-perfused placental samples contained 2-Naphthol (NPH) in their tissues with a mean NPH level of 7.98 (± 1.73) ug\g compared to a mean of 15.58 (± 4.53) ug\g after 360 minutes perfusion. The initial drop in the concentration of NPH in the maternal compartment was rapid. It dropped 5.54 ug\g in the first 15 minutes and 13.8 ug\g in 360 minutes. However, the increase of NPH concentration on the fetal side was much slower. The transfer rate of NPH was much lower than that of antipyrine. The highest transfer index was at 120 minutes.
In conclusion, pregnant women seem to be exposed to high levels of environmental NAP. Its metabolites, NPH, and accumulates in term placentas. NPH has the ability to rapidly cross the placenta from the maternal to the fetal compartment within 15 minutes. The placenta seems to play a role in limiting the passage of NPH in the fetal compartment.

P2.120.
PLACENTAL NUTRIENT TRANSPORTER EXPRESSION IS DIFFERENTIALLY REGULATED FOLLOWING NANOPARTICLE MEDIATED GENE DELIVERY OF INSULIN-LIKE GROWTH FACTOR-1 AT GESTATIONAL DAY 15.5 IN A MURINE MODEL OF INTRA-UTERINE GROWTH RESTRICTION

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Objectives: Placental insufficiency accounts for 75% of Intra-Uterine Growth Restriction cases and there is no current treatment. We previously demonstrated nanoparticle mediated intra-placental human IGF-1 gene therapy maintains normal fetal growth in a murine model of placental insufficiency. However, in order for translational gene therapy application in the human placenta, the underlying mechanisms associated with placental gene therapy need to be understood. This study investigated the regulation of placental nutrient transport mechanisms by nanoparticle mediated Hu-IGF-1 under the control of the trophoblast-specific promoter PLAC1 in a mouse model of IUGR.

Methods: At day 15.5 of gestation, through laparotomy, uterine artery branch ligation (UABL) was performed and a sub-set of placentas underwent intra-placental injection with HPMA-DMAEMA nanoparticles (NP) containing HuIGF-1, a control group underwent sham surgery. Pups and placentas were delivered at term (GD19.5) by Caesarean section. Pups were weighed and placentas snap frozen or formalin-fixed for expression analysis of Glucose transporters (SLC2, GLUTs), and System A (SLC38, SNATs), and System L (SLC7, LATs) amino acid transporters. Data were analyzed by ANOVA and a P value less than 0.05 was considered significant.
Results: No differences were seen in SLC38A2 or SLC2A3 mRNA expression; however SLC2A1 and SLC2A8 mRNA expression levels were significantly increased in the UABL group but remained at sham levels with NP+HuIGF-1 treatment ($p=0.047$ and $p<0.001$ respectively, $n>3$ dams per group). Interestingly, SLC7A5 mRNA expression was significantly increased in UABL and showed a further increase with NP-HuIGF-1 treatment ($p=0.017$, $n>3$ dams per group).
Conclusion: Following surgical induction of restriction at gestational day 15.5, placental GLUT1, GLUT8, and LAT1 appear to attempt compensatory expression. Treatment of the UABL placentas with the NP+HuIGF-1 maintains glucose transporter expression at sham levels. The further increase in LAT1 expression with the NP-HuIGF-1 treatment indicates possible direct regulation of this transporter by IGF-1.

P2.121-N.
FETAL GROWTH RESTRICTION AND GENDER-SPECIFIC EFFECTS ON PLACENTAL FUNCTION IN A RAT MODEL OF RAMADAN FASTING DURING PREGNANCY

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Introduction: During Ramadan, pregnant Muslim women observe the religious requirement that no food or liquid is ingested during daylight hours over a lunar month. The impact of this pattern of maternal intermittent fasting (IF) on fetal growth and development is not well defined, but reduced placental weight and birthweight have been reported, associated with greater incidence of metabolic disease in adults exposed to Ramadan fasting *in utero*.

Methods: A rat model of repeated IF during pregnancy (mimicking Ramadan fasting) was employed to investigate gender-specific effects on placental function and fetal development. Food was withdrawn daily from pregnant dams between 5 pm to 9 am from day 1 (plug found) to day 21 of gestation. Control pregnant dams received food *ad libitum*. Both groups ($n=30$/each) had free access to water.

Results: IF dams consumed 29±1% less food and gained less weight (P<0.0001) than controls, accompanied by 33±3% and 40±5% reductions in maternal plasma glucose and branched amino acid concentrations, respectively. Maternal insulin concentration was unaffected. IF fetuses of both genders were significantly lighter and shorter, with smaller head circumferences (P<0.001). Placental weights and efficiency (fetal/placental weight ratio) were also reduced (P<0.05) compared to controls. Placental glycogen content and fetal glucose concentration were unaffected by IF; however fetal insulin concentration was significantly lower (P<0.05). *In vivo* transplacental flux of ^{14}C-methylaminoisobutyric acid (^{14}C-MeAIB), a substrate for system A amino acid transporter, was reduced in IF males and females by ~24% and 41% respectively compared to controls (P<0.05). Sodium-dependent ^{14}C-MeAIB uptake (measure of system A activity) into isolated placental plasma membrane vesicles at 60s was only significantly reduced in IF female fetuses (P<0.05).
Conclusion: IF adversely affected maternal physiology and fetal development. Reduced placental system A activity may contribute to fetal growth restriction in both genders but this appeared more pronounced in female fetuses.

P2.122.
ACTIVATION BY INTRAVESICULAR AMINO ACIDS OF SYSTEM L-MEDIATED SERINE UPTAKE INTO HUMAN PLACENTAL MICROVILLOUS PLASMA MEMBRANE VESICLES

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Objective: The system L family of amino acid exchange transporters (LATs) mediate Na^+-independent transport of neutral amino acids across human placenta. By combining mathematical modelling with vesicle experiments, we recently demonstrated that serine transport (LAT2 substrate) across the maternal-facing microvillous plasma membrane (MVM) cannot be fully explained by obligate exchange, with evidence for a non-obligate exchange component (facilitated transport). To explore these mechanisms further, we investigated the influence of an outwardly-directed trans-membrane concentration gradient, imposed by inclusion of various intravesicular amino acids/analogues, on ^{14}C-serine uptake into MVM vesicles.

Methods: ^{14}C-serine uptake (7.5μM) into MVM vesicles isolated from normal term placentas was measured under Na^+-free conditions over 10min. Uptake by MVM vesicles preloaded with unlabelled amino acid/analogue substrates (1mM) was compared to that by non-preloaded vesicles (control). Extravesicular concentration was constant at 50μM; a steep outwardly-directed substrate gradient was imposed.
Results: ^{14}C-serine accumulated in control vesicles in a time-dependent manner, approaching equilibrium by 10min. In contrast, the addition of intravesicular LAT substrates serine, alanine, glycine, methionine and 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH) led to trans-stimulation of ^{14}C-serine uptake of varying magnitude above equilibrium concentration (overshoot at 60s) followed by subsequent reduction with time; consistent with both obligate exchange and facilitated transport mechanisms. Interestingly, intravesicular α-methyl-aminoisobutyric acid (MeAIB) and proline, substrates of the Na^+-dependent accumulative transporter system A, were also effective in eliciting a similar response: relative overshoot maximum was Ser>Ala>BCH>Pro>Gly>MeAIB>Met.
Conclusion: The identity of amino acids present on the internal face of MVM dictates serine uptake capacity across MVM. The broad internal amino acid selectivity by system L in MVM indicates that both the composition and concentration of freely-exchangeable amino acids within syncytiotrophoblast is likely to regulate system L exchange capacity in MVM. Collectively these observations provide further experimental support for previous model predictions regarding serine uptake by system L exchange transporters.

P2.123-N.
STATUS ANALYSIS OF TUMOUR ASSOCIATED FACTORS IN HUMAN PLACENTAE AND STEM-LIKE CELLS DERIVED FROM PLACENTAL CELL LINES

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Tumour and early trophoblast cells have a sub-population of "stem like cells" (SLC's) which can proliferate to form heterogeneous cell groups. However, tumour SLCs proliferates during invasion; while trophoblast cells proliferate and then invade. To date, the factors that are responsible for this uncontrolled versus controlled invasions are not fully understood. Potential candidates/factors include gene families such as BIRC-6 [Baculoviral IAP repeat-containing protein-6]; transcription factors like TWIST [a basic helix-loop-helix transcription factor]; to enzymes such as ALDHs [aldehyde dehydrogenases] and AURK [aurora kinases] A, B & C.

This study aims to (a) compare the status of these factors in human placentae developed from normal trophoblast invasion (normotensive; NT) and pre-eclamptic (PE) pregnancies, where the invasion is low; and (b) investigate the expression patterns of these factors in SLCs derived from transformed placental (TEV-1 and HTR8/sv-neo) and choriocarcinoma (placental tumour; JEG-3 and BeWo) cell lines.
The mRNA expressions of these factors were compared in 13 NT and 12 PE placentae by quantitative real-time RT-PCR. The results have shown that there is no statistical significance in mRNA expressions of ALDH-3, AURK-B and BIRC-6, whilst the mRNA expression of PSP, TWIST and AURK-A were up-regulated in PE ($p< 0.05$). On the other hand, AURK-C was down-regulated in PE ($p<0.05$).
Using cytotoxicity (MTT and LDH) assays, the optimum concentration of (DOX) needed to produce drug resistant SLCs in transformed placental and tumour cell lines was found to be 250 ng/ml.
The data from this study suggests that SLC's isolated from placental cell lines can be used to investigate the status of different factors that are involved in placental invasion. Investigations are currently underway to study the status of above factors in placental cell lines and the SLC's derived from these cell lines.

P2.124-N.
FIRST TRIMESTER TROPHOBLAST CELL LINE ACH-3P AS MODEL TO STUDY INVASION INTO ARTERIES VS. VEINS

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Objectives: Trophoblast invasion during first trimester of pregnancy not only serves to attach the placenta to the uterus. One invasion route of extravillous trophoblast (EVT) goes towards spiral arteries and is responsible for successful establishment of the maternal blood flow into the intervillous space. In the present study we investigated the invasion abilities of the first trimester trophoblast derived cell line ACH-3P towards placental arterial (PLAEC) and venous (PLVEC) endothelial cells including the cytokine expression pattern of the endothelial cells.

Methods: Conditioned medium (Cdm) was prepared by incubating confluent arterial and venous endothelial cells with EGM medium for 48h under standard culture conditions. The impact of Cdm on the invasion ability of ACH-3P was assessed using a colorimetric collagen invasion assay. Non conditioned medium was used as control. Furthermore, paracrine factors in Cdm were analyzed by human angiogenesis array kits. Arrays were evaluated using densitometry and normalized to the internal positive controls.