

**Methods:** The distribution of CB1R and CB2R in amnion tissue was detected by immunohistochemistry (IHC). Human amniotic epithelial cell proliferation and migration in response to THC treatment were measured by MTS and transwell assays, respectively. The PCR array was performed to study the key genes involved in the regulation of cell migration.

**Results:** Our results indicated that both CB1R and CB2R primarily presented in the epithelial layer of term amnion tissue. High-dose of THC (30 $\mu$ M, but not 20 and 10 $\mu$ M) significantly inhibited ( $p < 0.01$ ) human amniotic epithelial cell lines (WISH) proliferation. Meanwhile, THC (10 $\mu$ M and 20 $\mu$ M) ( $p < 0.05$ ) robustly suppressed cells migration in both WISH and primary human amniotic epithelial cells. The PCR array assay indicated that MMP2 and MMP9 expression were greatly reduced and probably correlated with THC-suppressed cell migration.

**Conclusion:** These results suggested THC inhibited the migration of human amniotic epithelial cell, which probably contributed to the THC-downregulated protein expression of MMP2 and MMP9.

### P1.73 EVALUATION OF PLACENTAL FUNCTION USING ULTRASOUND ELASTOGRAPHY

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**Objective:** Recently, it is illustrated that tissue hardness is clinically related to the illness.

The aim of this study is to examine hardness in placental tissue using Ultrasound elastography and to study correlation between placental hardness and obstetrical outcomes.

**Methods:** This study was approved by the ethics committee in our hospital. Forty-two patients with pregnancy was examined using Ultrasound elastography at 160 times. After delivery, 30 of their placenta were pathologically examined.

**Results:** The elastographic findings in placental tissue were divided into 3 categories by Ultrasound elastography HT score: Hardness in placental Tissue score. Estimated fetal body weight (EFBW) was +0.36SD in score1 group, -0.32SD in score2 group and -1.18SD in score3 group. Pathologic abnormality in placenta were observed 40.0%(6/15) in score3 group and 6.7%(1/15) in score1 group

**Conclusions:** Placental hardness in view of elastography correlated lower EFBW and higher risk of pathologic abnormality in placenta. This result shows that perinatal risks might be predicted by HT score.

### P1.74 EARLY FUSION EVENTS AND INVASIVE BEHAVIOUR IN TROPHOBLAST AT SITES OF IMPLANTATION *IN VITRO*

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**Objectives:** In early pregnancy, maternal and embryonic signals prime the receptivity of the uterine luminal epithelium before the trophoblast (TE) of the blastocyst-stage embryo mediates attachment. We hypothesised that a maternal juxtacrine signal initiates trophoblast differentiation, leading to breaching of the epithelium. We tested this proposition using three *in vitro* models.

**Methods:** Mouse or human blastocysts or BeWo cell spheroids were transferred to confluent human endometrial epithelial Ishikawa cells. Attachment sites were studied using high-resolution fluorescence microscopy and RT-PCR; multinucleation was specified by combining E-cadherin with actin and DAPI.

**Results:** Embryos and spheroids attached stably to epithelium, and this was followed by breaching and trophoblast outgrowth. Mouse cells breaching the cell layer developed prominent stress fibres and large nuclei, resembling trophoblast giant cells (TGC). Apposition (E4.5-5.5) led to expression of Hand1, suggesting promotion of TGC differentiation, and induction did not occur in embryos separated from epithelium by porous filters. Day 6 human blastocysts also readily attached to Ishikawa cells. Sites of attachment ( $n=46$ ) at 48h of co-culture revealed onset of expression of GATA3, HLAG and hCG. The majority had breached the cell

layer (37/46, 80.4%). Furthermore, 38/46 (82.6%) contained multinucleated trophoblast at the site of contact, and notably, each embryonic breach point observed consisted of syncytial trophoblast (7/7). Multinucleation was also observed in attached BeWo trophoblast spheroids. Pretreatment of spheroids for 24h with forskolin prior to coculture induced more syncytium, and greatly increased breaching and invasion of the epithelial layer.

**Conclusions:** The results implicate primary syncytium as the pioneer invasive cell type in human embryo implantation, and TGC in mouse implantation. Interaction with receptive epithelium initiates trophoblast differentiation and the onset of invasive behaviour. These findings have clinical implications for assisted reproductive technologies, in which implantation is the rate-limiting step, as well as biological importance in understanding early placentation.

### P1.75 CASPAE 8 INHIBITION INCREASES THE INFECTION WITH *TRYPANOSOMA CRUZI* IN THE HUMAN TROPHOBLAST CELL LINE (BEWO)

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Congenital Chagas' disease is caused by the haemoflagellated protozoan *Trypanosoma cruzi* (*T. cruzi*), which is able to cross the placental barrier and infect both the placenta and fetus. However, congenital transmission rates are low, suggesting the presence of local defense mechanisms. The trophoblast is the first tissue of the placental barrier in contact with the maternal blood; its epithelial turnover is considered part of innate immune system. Previous studies have shown that *T. cruzi* induces proliferation, differentiation and apoptosis in the trophoblast, suggesting an increase in epithelial turnover. Caspase 8 is an essential molecule not only during apoptotic cell death but also during trophoblast differentiation.

**Objective:** To study the possible role of caspase 8 during *T. cruzi* infection since it has not been studied.

**Methods:** BeWo cells (a trophoblast cell line) were incubated in presence and absence of *T. cruzi* trypomastigotes (Ypsilon strain) in a cell:parasite ratio of 1:0.1 and 1:1 and in presence or absence of IETD-CHO (caspase 8 inhibitor) during 48 hours. Caspase-8 and its active (cleaved) form were analyzed by Western Blot and enzymatic assays (Caspase8Glo; Promega®). Parasite infection was assayed by real time PCR as well as by automated cell analysis (with MATLAB® software) in DAPI stained cells. Additionally, DNA synthesis (as proliferation marker),  $\beta$ -human chorionic gonadotropin ( $\beta$ -hCG) (as differentiation marker) and activity of caspase 3 (as apoptotic cell death markers) were determined.

**Results:** *T. cruzi* induces caspase 8 activity but not its protein expression. Interestingly, the inhibition of caspase 8 activity increases parasite infection by increasing the number of intracellular parasites but not the percentage of infected cells. As expected, inhibition of caspase 8 does not affect cell proliferation, but disrupts the cellular differentiation and apoptotic cell death.

**Conclusion:** Our results suggest, that caspase 8 and therefore the trophoblast turnover could be part of the proposed local antiparasitic mechanism of the human placenta.

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### P1.76 BALANCE OF LIN28A AND LIN28B IN BOVINE TROPHOBLAST GIANT CELLS FORMATION

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**Introduction:** Bovine trophoblast giant cells is derived from mononuclear trophoblastic cells by acitokinetic mitosis, but the molecular mechanism is not described. In humans, it is known that TGCs is a differentiation of