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K1

EXPLORATORY PROCESS OF PLACENTATION FROM HUMAN BEINGS TO OCEAN LIVING SPECIES

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1. Placentation process from prehistoric placoderms to sharks and rays.

The placenta with appearance of million years ago has shown a significant evolutionary development that gave use to the vast majority of mammals alive until today from human to Ocean living species. In 2005, Australian scientist Prof. John A. Long has detected fossils of umbilical cord belong to pelvic structures in the ancient fish, Materpiscis in 375-million ago, but no placental fossil was found. It was, therefore, supposed that placoderms had a remarkable advanced reproduction since long time ago. Sharks and Chondrichthyan are generally considered to be primitive fishes. At this chance, astonished placentations of Hammerhead shark and Manta ray are presented.

2. Placentation process from giant sea cow to dugong and manatee.

In 1980, a gigantic skeleton of Takikawa Sea Cow (dugongid sirenia) with 15 millions years ago was detected on riverside of Takikawa, Hokkaido, Japan. This giant sirenia was ancestor of vanished Steller's sea cow in the Behring Sea and dugongs as well as manatee in the warm ocean. They feed upon aquatic vegetation. Their sea cows group was related to Proboscidea (elephant) from 45 millions year, even though their living environments are quite different. They share herbivorous diet and a long gestational period approximately 1.5 to 2 years. In this presentation, their placentation presented zonary endothelial types was compared.

3. A comparative study of placental pathology between great apes (Chimpanzee and Orang-utan), in contrast with placenta of small monkey (Goeldi's monkey, Callimico) in South America.

A purpose of this study was not only considered comparison of placental pathology between human and great apes, but also was expected whether gestational trophoblastic tumor may occur in great apes.

4. Placental pathology in Himalayan mountain people.

The vast majority of Nepal population lives in small rural settlements, where a chronic shortage of essential health care system and low socio-economic status in addition to rough high altitude, result in a lack of prenatal care for pregnant women, thereby causing high perinatal mortality and neonatal mortality rates in Nepal. This study has started with pathological examination of 1,000 Himalayan placentas obtained in Nepal and Tibet since 1977 and the results were compared with Japanese placentas since 1990. As a characteristic of histological findings of the placental villi in Himalayan groups, the incidence of villous chorangiosis and chorangioma was high. Such histogenesis was clarified with ultrastructural observation.

K2

PLACENTAL TROPHOBLAST AS EPITHELIUM: A USEFUL CLASSIFICATION?

Richard Boyd
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It is more than 60 years since electron microscopy revealed the polarized nature of the trophoblast. At that time there was increasing consideration of the placenta as playing a remarkable role acting as the" lungs", the "intestine", the "kidney" and the "liver" of the fetus. Thus functional work in the following decades sought to establish the basis of transplacental transport involving for example primary, secondary and tertiary active transport, transporters (symporters and antiporters) and channels. These studies emphasised the polarized distribution of these membrane proteins. More recently renewed interest in the intracellular cell biology of epithelia, specifically of signalling compartments has begun to be applied to the trophoblast. This work feeds into both the genomic revolution and to epigenetic control, as well as to the immunobiology of materno fetal interaction. For fetal well-being, for successful neonatal outcome and for the long term health of the individual in adult life the placenta is recognized increasingly as having an important role. I will argue that the epithelial properties of the trophoblast are helpful in integrating this. One specific example relates to the role of the placenta in embryonic brain development (McKay 2011 Nature 472 298) where the release into the fetus in a polarized manner of serotonin synthesized from the amino acid tryptophan, taken up from the maternal circulation, has been shown to be critical. Rather remarkably tryptophan has other central roles in placental biology, again involving polarized vectorial transport and these will be reviewed.

NIH

EMBRYONIC AND TROPHOBLAST STEM CELL MODELS OF EMBRYO IMPLANTATION AND PLACENTAL DEVELOPMENT

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The human embryo is not a feasible experimental system for the detailed study of implantation and early placentation, so surrogate systems have been sought for investigating the determination of the trophectoderm lineage, and its differentiation into trophoblasts of the early implantation site and subsequently the definitive placenta. Trophoblast stem cells (TSC) have been identified in several species, and employed most extensively in the mouse in studies of the transcriptional regulation of trophoblast differentiation and placental morphogenesis. TSC have been reported but less extensively studied in porcine and rhesus, and recently in cells from the human chorionic plate. An alternative to the use of embryos for studying early placental development was revealed by work with human embryonic stem cells (hESC), demonstrating either BMP2/4-stimulated trophoblast differentiation, or spontaneous formation from embryoid bodies. These cells display a trophoblastic transcriptome, as well as a placental protein and steroid hormone secretory profile, and invasive and chemotactic behavior resembling human placental trophoblasts. Two-dimensional and three-dimensional paradigms and other modifications of the culture environment, including oxygen tension and extracellular matrix, impact on trophoblast differentiation. Induced pluripotent stem cells (iPSC) also resemble hESC in their capacity to form trophoblasts in BMP-stimulated and embryoid body paradigms. Although the precise placental counterpart of the hESC-derived trophoblast remains unclear, hESC-derived trophoblasts remain an intriguing platform for modeling early implantation. *Supported by NIH grants P01 HD38843 and R01 RR021876.*

SA1

REPRODUCTIVE IMMUNOLOGY IN PERSPECTIVE

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Involvement of the maternal and fetal immune systems in the events of pregnancy was generally overlooked by reproductionists until the mid-twentieth century. At that time, Billingham and Beer noted that viabilities of transplants to rat uteri were extended during pregnancy; Tachi et al reported that phagocytic macrophages populated mammalian uteri; Faulk discovered a lack of rejection-mediating Human Leukocyte Antigens (HLA) on some extraembryonic fetal tissues. From those original observations and many others of note, the field of reproductive immunology was born. Now, more than half a century later, it is well understood that with the initiation of pregnancy, mammalian uteri host an altered variety of interactive immune cells generating an immunosuppressive environment believed to protect the implanted embryo from normal maternal immune responses. Scientists agree that both maternal and fetal factors drive the formation and maintenance of this unique natural environment. In our laboratory we have studied uteroplacental macrophages, have investigated products of both immune cells and placental trophoblast cells for regulatory molecules, and have uncovered unusual biochemical and functional properties of placental HLA. Our findings indicate that particularly in humans, multiple pathways contribute to the safety of the "foreign" fetus as it proceeds to parturition. Funded by the National Institutes of Health, USA.

N1

DOES MALARIA AFFECT PLACENTAL DEVELOPMENT? EVIDENCE FROM AN *IN VITRO* MODEL

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Objectives: In malaria endemic areas such as Papua New Guinea, *Plasmodium falciparum* (*Pf*) malaria during pregnancy is a leading preventable cause of fetal growth restriction (FGR). The underlying pathogenic mechanisms are poorly characterized, but may include impaired placental development. We performed a pilot study to determine if in vitro methods commonly used to assess placental biology could be used to investigate whether maternal malaria infection affects trophoblast invasion.
Methods: We tested serum from PNG women with *Pf* in peripheral blood at their first antenatal presentation (between 16 and 22 weeks gestation) for the ability to inhibit first trimester EVT-cell line invasion and viability *in vitro*. Because invasion is enhanced by a number of hormones and chemokines, and is inhibited by pro-inflammatory cytokines, many of which are dysregulated in malaria in pregnancy, we further compared concentrations of these factors in blood between malaria-infected and uninfected pregnancies.
Results: Compared to controls, serum from malaria-infected women significantly reduced trophoblast invasion ($P = .02$). This phenomenon could not be explained by changes in trophoblast viability ($P = .4$). Serum collected from malaria-infected women had significantly lower levels of invasion promoting factors (Insulin like growth factors -1 and -2, $P = .0001$, $P = .01$ respectively, and IL-8 $P = 0.02$) and higher levels of invasion inhibitory modulators (human chorionic gonadotropin $P = .002$, IL-10 $P = .01$).
Conclusion: Although malaria-induced elevated pro-inflammatory cytokines and reduced fetal growth hormones have been reported at delivery, this study is the first to describe altered levels of such factors early in pregnancy. These inflammatory and hormonal disturbances in early pregnancy may impair placental development. This is a significant advancement in our understanding of the temporal and pathophysiological events that may contribute to FGR due to Pf malaria in pregnancy.

N2

INVESTIGATION FOR TUMORIGENESIS AND NEW MOLECULAR TARGET OF CHORIOCARCINOMA BY INDUCED CHORIOCARCINOMA CELL-1

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Objectives: The molecular mechanisms underlying choriocarcinoma tumorigenesis remain uncharacterized, however, appropriate choriocarcinoma models have not been developed yet. In this study, to overcome above the background, we established induced choriocarcinoma cell-1 (iC^3-1) and performed microarray and bioinformatics analysis using iC^3-1.
Methods: iC^3-1 cells were generated from HTR8/SVneo retrovirally transduced with activated HRASV12. Microarray analysis and quantitative RT-PCR between iC^3-1 and HTR8/SVneo were performed, in addition, bioinformatics analysis that compared the gene profiling of iC^3-1 and normal placental tissue on NCBI Gene Expression Omnibus (GEO) was also performed. The expression of *SOX3*, *HAS2*, *CD44*, *CD68*, *CD163* and *S100* which were focused on by the analyses was examined on clinical samples by immunohistochemistry. To investigate *SOX3* contribution to choriocarcinoma tumorigenesis, we evaluated *SOX3* tumorigenic activity by its expression knockdown with *SOX3*-specific short-hairpin RNA (sh*SOX3*).
Results: Inoculated iC^3-1 cells rapidly generated lethal tumors in nude mice. The tumors contained the two typical trophoblast cell types, syncytiotrophoblasts and cytotrophoblasts, histologically similar to human choriocarcinoma. Gene expression study revealed that matrix metalloproteinase-family genes and epithelial-mesenchymal transition related genes were significantly upregulated in iC^3-1 cells. *SOX3* downregulation by sh*SOX3* markedly attenuated the tumorigenic activity of iC^3-1 cells and inoculated nude mice. *HAS2*, *CD44*, *CD68*, *CD163* and *S100* were significantly upregulated in iC^3-1 compared to control cell by microarray analysis and quantitative RT-PCR. Immunohistochemistry revealed clinical samples showed immunoreactivity with these molecules.
Conclusion: Our established choriocarcinoma model represents a novel tool for studying the tumorigenesis and treatment of choriocarcinoma. Among of focused molecules in this study, *SOX3* upregulation might be involved in choriocarcinoma tumorigenic activity. *S100* and *CD44* were reported that the former expressed several cancers and the latter involved proliferation and metastasis of cancer, especially the marker of cancer stem cell. Thus, these molecules were suggested to be new molecular target in choriocarcinoma.

N3

HYPOXIA MAINTAINS TROPHOBLAST PROGENITOR MARKERS FOLLOWING BMP4 TREATMENT OF HUMAN EMBRYONIC STEM CELLS

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Objective: Early placental growth and development occurs almost exclusively under low oxygen tension. Under such conditions, trophoblast differentiation is inhibited and proliferation is enhanced. Our lab previously identified p63, a member of the p53 gene family, as a specific marker of proliferative cytotrophoblast (CTB) in the human placenta. We have also determined that p63 expression is enhanced during BMP4-induced trophoblast differentiation of human embryonic stem cells (hESC). Here we determined the effects of oxygen tension on trophoblast induction of hESC in order to optimize conditions for maintenance of the trophoblast progenitor state.
Methods: Trophoblast induction of hESC was performed as previously described: feeder-free hESC (WA09) were plated on Geltrex and treated for 8 days with 10 ng/mL BMP4, in the presence of feeder-conditioned medium (FCM). Differentiation was performed under both normoxia (20% O_2) or physiologic hypoxia (5% O_2); the latter was done using an XVIVO hypoxic workstation (Biospherix), which allows cells to be manipulated under desired oxygen tension. Cells and supernatants were collected at days 0, 2, 4, 6, and 8; total cellular RNA was extracted using the miRvana kit (Ambion). Whole genome profiling was performed using the Illumina BeadStation; qPCR was used to confirm a subset of the array results. Secreted hCGbeta was measured using an ELISA kit (Calbiotech).
Results: Microarray analysis showed that hypoxia did not inhibit trophoblast induction of hESC; rather, it did inhibit terminal trophoblast differentiation. qPCR confirmed that under hypoxia, expression of trophoblast progenitor markers, including p63 and CDX2, was enhanced, while expression of differentiation markers GCM1, syncytin A, CGA, and CGB was decreased. Secreted hCGbeta was also reduced under hypoxia.
Conclusion: Low oxygen tension inhibits terminal trophoblast differentiation of BMP4-treated hESC. Further characterization of BMP4-treated hESC under hypoxia may help determine a molecular signature for the human "trophoblast progenitor" state.

N4

CD107A+ CYTOTOXIC NK-CELLS IN DECIDUA AND CIRCULATION IN 3rd TRIMESTER PREGNANCY

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Objective: NK-cells have a specialized role in the uterus during pregnancy where they are involved in regulating maternal immunological responses against fetal cells, and thereby the process of placentation. Our objective was to examine the distribution of subpopulations of circulating and uterine NK-cells from healthy and preeclamptic third-trimester pregnancies, and to quantify their cytotoxic activity by analyzing CD107a expression. CD107a is located in the membrane of intra-cellular lytic granules; upon activation of NK-cells and T-cells these granules are exocytosed and the CD107a molecules are temporarily located on the cell surface where they can be detected by flow cytometry.
Methods: Blood and decidua samples were obtained from women with healthy pregnancies or preeclampsia undergoing cesarean section in the third trimester. Peripheral blood mononuclear cells were isolated from freshly collected blood using gradient density centrifugation. Decidual tissue was harvested by suction from the placental bed after delivery of the placenta, and mononuclear cells were isolated by enzymatic tissue digestion followed by gradient density centrifugation. Cells were fixed and stained with fluorochrome-coupled antibodies towards CD3, CD4, CD8, CD56, CD16 and CD107a and subsequently analyzed by flow cytometry on a BD FACSCanto™II.
Results: We observed a higher percentage of $CD3^{-}CD56^{+}$ NK-cells in decidua compared to blood from third-trimester pregnancies (22.5% vs. 8.3%, P=0.002). Furthermore, we found that decidua has significantly more $CD56^{bright}CD16^{-}$ cells than blood (61.7% vs. 2.84%, $P = < 0.0001$), and that the expression of CD107a on these cells was lower in decidua (5.6% vs. 15.0%, P=0.0099), resulting in a ratio of CD107a/CD56 expression on $CD56^{bright}CD16^{-}$ cells that is 35-fold higher in blood than in decidua ($P<0.001$).
Conclusion: Our results suggest that $CD56^{bright}CD16^{-}$ NK-cells in decidua have lower cytotoxic activity than in the circulation, as shown by surface expression of CD107a+.

N5

VASCULAR SMOOTH MUSCLE CELLS FROM SPIRAL ARTERIOLES PRODUCE CHEMOKINES WHICH REGULATE EXTRAVILLOUS TROPHOBLAST CELL MIGRATION

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Objectives: Early in human pregnancy, extravillous trophoblast (EVT) invades and remodel uterine spiral arterioles feeding the placenta, facilitating increased blood flow to the placenta and fetus. The study objectives were to isolate and characterize uterine spiral arteriole vascular smooth muscle cells (SPAR VSMC) and investigate how factors produced by these cells regulate EVT migration.
Methods: SPAR VSMC was isolated from uterine spiral arteriole segments dissected from myometrial biopsies collected with informed consent following Caesarean section from uncomplicated pregnancies (n=6). Expression of VSMC markers by SPAR VSMC was determined using immunohistochemistry and immunoblotting. Medium collected from SPAR VSMC cultures following 24 hours of serum starvation was analyzed for chemokines using a Multi-Analyte ELISArray (SABiosciences) which gives a positive signal if the chemokine in the sample falls in the range 1-1000pg/ml. Migration assays were performed using the xCELLigence system (Roche).
Results: SPAR VSMC expressed the VSMC markers α-smooth muscle actin, tropomyosin and caldesmon and were negative for the fibroblast marker CD90. SPAR VSMC conditioned medium significantly increased EVT migration 420%±77% over 48 hrs compared to control medium ($p<0.05$, Mann Whitney *U* Test, n=4), indicating the production of chemotactic factors by the SPAR VSMC. Analysis of the conditioned medium for 12 chemokines demonstrated the presence of (in descending order of relative abundance); CXCL8, CCL2, CCL4, CXCL10, CCL22, CXCL11, CXCL9, CCL11, CCL5, CXCL1 and CCL3. CCL17 was not detected. Our previous work has demonstrated that EVT migration is stimulated by CXCL8, CCL2, CCL11, CCL5 and CXCL1 over the range 1-100 ng/ml. Now we show that CCL3, CCL4 and CCL22 over the range 1-40ng/ml all increase EVT migration by an average of 140-670% (n=3).
Conclusion: This study demonstrates that SPAR VSMC produce chemokines which may contribute to EVT migration. The ability to isolate and study SPAR VSMC increases understanding of vessel-EVT interactions in early pregnancy.

N6

THERAPEUTIC EFFECT OF MATERNAL HYDROGEN WATER ADMINISTRATION IN A RAT MODEL OF FETAL BRAIN DAMAGE

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Objectives: Recently, Several studies have shown that molecular hydrogen plays the role of antioxidant. This study was designed to investigate the protective effect of hydrogen-saturated water (HW) using a rat model of Ischemia-Reperfusion (IR) induced fetal brain damage.
Methods: To provoke oxidative stress in fetus, IR operation, in which a bilateral utero-ovarian artery of pregnant Wistar rat was occluded for 30 min and then released, was performed on day 16 of pregnancy. The rats were assigned to three groups: sham group, they underwent laparotomy that IR procedure was omitted; IR group, in which IR operation was performed; IR+HW group, they were given HW from two days before IR operation to delivery. After vaginal delivery, we estimated neonatal growth and collected brain at postnatal day 7. Brain damage was evaluated by hematoxylin-eosin staining and immunohistochemistry of oxidative stress marker was performed. To assess the hippocampal damage, Morris water maze test was carried out. Hydrogen concentration in placenta, amniotic fluid and fetal brain was measured by a gas chromatography.
Results: Neonatal growth was significantly retarded in IR group compared with sham group. HW restored neonatal growth. The degeneration of hippocampal pyramidal cells was observed in IR group. In IR+HW group, the degenerated cells were significantly reduced compared with IR group. Immunohistochemistry showed that 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal were strongly positive in IR group and were attenuated by HW. In Morris water maze test, reference memory was significantly impaired in IR group and improved by maternal administration of HW. Gas chromatography showed that maternal administration of HW significantly increased hydrogen concentration in placenta, whereas no difference was observed in fetal brain and amniotic fluid.
Conclusion: Our results suggest that maternal administration of HW has a potential benefit for cerebral palsy and might be a novel intra-uterine prevention and therapy.

N7

THROMBIN IS A PATHOGENIC FACTOR IN PRETERM LABOR AND PRETERM RUPTURE OF MEMBRANES

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Objectives: Intrauterine or vaginal bleeding is a risk factor for preterm birth and premature rupture of membranes (PROM). Thrombin is a trypsin-like serine protease that plays a major role in hemostasis and in degradation of the extracellular matrix. Here, we investigated the effects of thrombin on (i) pregnancy duration *in vivo* and (ii) matrix metalloproteinases (MMPs) and prostaglandin (PG) synthesis in epithelial and mesenchymal cells of human amnion.
Methods: Thrombin and MMP-1 activities were quantified using FRET methodology. Primary human amnion epithelial and mesenchymal cells were used to quantify *MMP1*, *2*, *9*, and *COX2* mRNA levels by qPCR. Gelatinase activity was assessed by gelatin zymography. PGE_2 levels in conditioned media were assayed by ELISA. To determine the effects of thrombin *in vivo*, precisely timed pregnant mice (d17) were injected in the interface between fetal membranes and uterus with either thrombin (4U/pup) or PBS.
Results: Thrombin activity was increased significantly in amnion from preterm (n=9) compared with term (n=6) deliveries (2.1-fold, $P<0.05$). Treatment of amnion mesenchymal cells with thrombin (2U/ml, 48h) resulted in increased *MMP1* mRNA (6-fold) and enzyme activity (from 8 to 148ng/mg protein, $P<0.01$). Thrombin also increased active MMP-2 (1.5-fold), *MMP9* (8-fold), and pro-MMP-9 (2-fold) in these cells. Using immunohistochemistry, PAR-1, a major thrombin receptor, was localized to human decidua and amnion mesenchymal cells. Moreover, thrombin increased *COX2* mRNA (16-fold, $P<0.01$) and PGE_2 (from 44 to 449ng/μg protein, $P<0.05$). *In vivo*, as expected, PBS-injected control mice delivered at term 48h after injection and all pups survived (n=11). In contrast, all thrombin-injected mice delivered preterm between 17-22h after injection and all premature pups died (n=16).
Conclusion: Thrombin results in increased MMP-1, MMP-9, and PG biosynthesis in amnion mesenchymal cells, not epithelial cells. Collectively, the data indicate that thrombin plays a pivotal role in the pathogenesis of preterm labor and PROM.

N8

DETERMINATION OF A MECHANISTIC LINK BETWEEN ABNORMAL MATERNAL INFLAMMATION AND THE DEVELOPMENT OF INTRAUTERINE GROWTH RESTRICTION AND PRE-ECLAMPSIA

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Objectives: Intrauterine growth restriction (IUGR) and pre-eclampsia (PE) are often associated with abnormal maternal inflammation. Evidence links IUGR/PE with deficient trophoblast-mediated remodeling of uterine spiral arterioles leading to restricted placental perfusion. Decreased placental nitric oxide (NO) bioavailability may also play a role. Deficient placental perfusion can induce IUGR, whereas placental damage can cause the release of factors that induce hypertension, proteinuria and glomerular endotheliosis (GEN). We hypothesize that abnormal inflammation is key to the pathophysiology of IUGR/PE.
Methods: We developed a model of inflammation whereby pregnant Wistar rats are injected with low-dose lipopolysaccharide (LPS) on gestational days (GD) 13.5-16.5. The effects of inflammation on pregnancy complications were determined primarily on GD 17.5. We employed (a) Doppler ultrasound to assess altered hemodynamics; (b) immunohistochemistry to localize trophoblasts, macrophages and nitrotyrosine; (c) light and transmission electron microscopy to assess LPS-induced GEN; (d) protein:creatinine ratio to assess proteinuria, and (e) radiotelemetry to monitor alterations in blood pressure.
Results: LPS administration resulted in IUGR and increased maternal arterial pressure. This effect of LPS was associated with GEN, proteinuria, increased levels of TNF, decreased trophoblast invasion, impaired spiral arteriole remodeling, deficient utero-placental perfusion and increased macrophage infiltration in the decidua and mesometrial triangle. Increased nitrotyrosine immunoreactivity was suggestive of decreased NO bioavailability in utero-placental units of LPS-treated dams. Transdermal administration of an NO mimetic (nitroglycerin, 25 μg/hr) prevented the nitrotyrosine accumulation as well as the LPS-induced IUGR, proteinuria and GEN. Inhibition of TNF activity (etanercept, 10 mg/kg) prevented IUGR and the associated trophoblast, hemodynamic and renal alterations.
Conclusion: These findings demonstrate that maternal inflammation can lead to severe pregnancy complications via a mechanism dependent on increased levels of TNF and decreased NO bioavailability. Our findings provide a rationale for the use of anti-inflammatory agents and NO mimetics in the treatment and/or prevention of pregnancy complications.

N9

SIRT6 REGULATES KEY TERMINAL EFFECTOR PATHWAYS OF HUMAN LABOUR: POSSIBLE THERAPEUTIC TARGET FOR THE MANAGEMENT OF INFECTION-INDUCED PRETERM BIRTH

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Objectives: Infection-induced preterm birth is a major determinant of neonatal mortality and morbidity. The mechanisms that regulate preterm birth are unknown; however, the pro-inflammatory transcription factor NF-κB and its target genes (cytokines; cyclooxygenase (COX)-2; extracellular matrix remodelling enzymes) play an important role in the terminal processes of labour and delivery, including rupture of fetal membranes. In non-gestational tissues, sirtuin (SIRT) 6 exerts anti-inflammatory actions by inhibiting NF-κB and its target genes. The aims of this study were to determine the effect of (1) human preterm labour on SIRT6 expression in human gestational tissue; and (2) SIRT6 inhibition and overexpression on pro-labour mediators in primary amnion cells.
Methods: SIRT6 mRNA and protein expression was determined, by qRT-PCR and Western blotting, on combined fetal membranes from women grouped as (1) preterm no labour: Caesarean section with no labour and (2) preterm labour: after spontaneous labour and normal vaginal delivery. In human primary amnion cells, SIRT6 knockdown was achieved using siRNA and SIRT6 overexpression was achieved using a cDNA clone. After treatment with IL-1β, pro-labour mediators were assayed.
Results: SIRT6 mRNA and nuclear protein expression was significantly lower in fetal membranes from women after preterm labour compared to preterm not in labour. In primary amnion cells, SIRT6 inhibition by siRNA increased IL-1β-induced cytokine expression (IL-6, IL-8, TNF-α), COX-2 mRNA and subsequent PGF2α release, MMP-9 mRNA expression and release, and NF-κB p65 mRNA expression. Conversely, SIRT6 overexpression decreased IL-1β-induced cytokine, prostaglandin, MMP-9 and NF-κB p65 expression.
Conclusion: Spontaneous preterm birth is associated with decreased SIRT6 expression. Functional studies demonstrate an important role for SIRT6 in the regulation of cytokines, prostaglandins and MMPs associated with preterm birth. These actions of SIRT6 appear to be mediated via its effects on NF-κB. Thus, SIRT6 could provide a candidate therapeutic target for the management of infection-induced preterm birth.

N10

PLACENTAL ADAPTATION TO MATERNAL OBESITY: ROLE OF OXIDATIVE STRESS IN ACTIVATION OF AUTOPHAGY AND CELL DEATH

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Obesity during pregnancy is associated with maternal complications, poor perinatal outcome and developmental programming effects on the offspring. Our previous studies show that with increasing maternal BMI there is an overall increase in placental oxidative stress. We hypothesized that the chronic low-grade inflammation of obesity leads to oxidative stress and pathological dysfunction in the placenta.
Objective: The aim of this study was to evaluate oxidative stress and cell death in villous tissue of placenta from lean, overweight and obese women.
Methods: As there is marked sexual dimorphism in placental physiology, we evaluated only placentae from a male fetus. Placental villous tissues were collected from lean (LN: BMI 19-24.9), overweight (OW: BMI 25-29.9) and obese (OB: BMI 30-45) women (n=5 each group) after C-section at term, prior to labor. We employed Western blotting to measure autophagy markers and placental cryosections to measure ROS by Dichlorofluorescein (DCF) staining and apoptosis by TUNEL assay. H_2O_2 levels were measured in placental homogenates by Amplex red assay.
Results: We detected a significant increase in accumulation of the autophagy markers, ATG3 and ATG7 in OB compared to LN and OW groups. This was accompanied by a reduction in p62, a marker for accumulation of misfolded proteins, and of LC3 cleavage associated with formation of autophagosomes. However cathepsin B and LAMP2, markers for completion of autophagy, did not differ across the groups, suggesting defective autophagy. ROS production (DCF staining) was significantly elevated in OW and OB group. H_2O_2 production was 1.8 fold higher in OB villous tissue homogenate compared to LN and OW. TUNEL staining showed 6-fold increase in the number of apoptotic nuclei in both OW and OB compared to LN.
Conclusion: Our data suggests that the obese maternal environment is associated with defective autophagy in the placenta resulting in excessive production of ROS triggering a cascade of pathological events including cell death.

N11

SYNCYTIOTROPHOBLAST EXPRESSION OF THE MINOR HISTOCOMPATIBILITY ANTIGEN HA1 IS INCREASED IN PLACENTAS FROM PREECLAMPTIC WOMEN

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Objectives: Cohorts of maternal T cells reactive to fetal minor histocompatibility antigens, including the autosomally encoded antigen, HA1, expand during pregnancy. We have shown that at HA1 and at least five additional minor antigens are expressed in human placental trophoblast cells. Because the placenta may be a source of fetal antigens to which mothers are sensitized, we sought to determine how placental HA1 is regulated. Specifically, we asked whether HA1 expression is altered in preeclampsia, and whether its expression in trophoblast cells is regulated by oxygen.
Methods: HA1 mRNA and protein expression levels were evaluated in placentas of preeclamptic and healthy matched control women (n=8/group). Real time RT-PCR was used to determine relative expression of HA1 mRNA, and semi-quantitative immunohistochemical analysis was used to evaluate HA1 protein expression in the syncytiotrophoblast. Lastly, purified term cytotrophoblast cells were cultured in 2%, 8% and 21% oxygen for 24 hours, and HA1 mRNA levels were determined using real time RT-PCR.
Results: When compared to normal controls, HA1 mRNA expression in preeclamptic placentas was increased by 3.3-fold (P=0.015). HA1 protein expression was increased in the syncytiotrophoblast of preeclamptic placentas as compared to their matched controls (mean H-score, 35.4 v. 3.0 in preeclamptic and control placentas, respectively; P=0.03). HA1 mRNA was increased in purified term cytotrophoblast cells cultured under 2% oxygen and 8% oxygen as compared to 21% oxygen.
Conclusion: Collectively, these data reveal a novel mechanism by which the maternal immune system may be exposed to enhanced levels of fetal antigens. Increased expression of HA1 in the placenta, together with increased placental deportation of syncytiotrophoblast-derived microvesicles, during preeclampsia may increase fetal antigenic load in the mother. This could potentially alter the maternal adaptive immune response to the fetus in the existing and/or subsequent pregnancies.
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N12

ROLE OF ADENOSINE A_{2A} RECEPTOR AND NITRIC OXIDE-DEPENDENT SIGNALING PATHWAY IN FETAL ENDOTHELIUM MIGRATION AND PROLIFERATION DURING EARLY AND LATE-ONSET PRE-ECLAMPSIA

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Background: Stimulation of A_{2A} adenosine receptor (A_{2A}AR) is associated with either increasing endothelial nitric oxide synthase (eNOS) expression and eNOS activation (i.e., tyrosine 1177 phosphorylation); a mechanism linked to pro or anti-proliferate effects depending of the cell type. Feto-placental endothelial cells from pre-eclampsia exhibit high adenosine extracellular levels but low nitric oxide (NO) formation. **Aim**: Investigate whether NO signaling pathway is involved in fetal endothelium proliferation and migration induced by A_{2A}AR stimulation in early and late-onset pre-eclampsia.
Methods: Human umbilical vein endothelial cells (HUVEC) were isolated from normal (n=15), late-onset pre-eclampsia (n=12) and early-onset pre-eclampsia (n=12). Adenosine A_{2A} expression was evaluated by immunocytochemistry and western blot. Cell proliferation was analyzed using MTS-assay and by direct cell count using haemocytometer, in absence or presence of non-selective adenosine receptor agonist (NECA 10μM), A_{2A}AR selective agonist (CGS-21680, 100nM), and/or the antagonist ZM-241385 (0-100μM) during 24 hours. In parallel, counting of the cells crossing the "wounds" pre-made in dishes of confluent cells, as well as transwell assays were used for assessing cell migration. Besides, NOS inhibitor (L-NAME, 100 μM) was used in co-incubation by either adenosine receptor agonists. Nitrite concentration in the culture medium was measured by Griess reaction and protein nitration was assessed by western blot in cells exposed to CGS-21680 (30min).
Results: Early-onset pre-eclampsia was associated to low (~70%) A_{2A}AR protein abundance compared with normal or late-onset pre-eclampsia. Basally, HUVEC from early-onset showed a low (~42%), whereas late-onset exhibited high (~1.5-fold) proliferation and migration compared to normal pregnancy. Proliferation and migration of HUVEC was increased by CGS-21680 or NECA (~1.5 and 2-fold, respectively) in the three analyzed groups compared with respective control without agonists. Stimulatory effect of CGS-21680 was blocked by ZM-241385 in normal pregnancies (Ki, 25nM) and late-onset (Ki 50nM) but not in early-onset (Ki ambiguous). On the other hand, eNOS protein abundance and eNOS-tyrosine 1177 phosphorylation was reduced (~50%) in early-onset pre-eclampsia compared to late-onset or normal pregnancy. In turn, cells from late-onset pre-eclampsia exhibited high (~2-fold) eNOS phosphorylation compared with normal pregnancy. CGS-21680 (30 min) increased (~2-fold) the eNOS phosphorylation, nitrite and nitrotyrosine formation in normal cells, a phenomena blocked by ZM-241385. In early-onset, but not in late-onset pre-eclampsia, CGS-21680 generates a significant increase in nitrotyrosine formation. L-NAME partially blocked (~25%) the stimulatory effect of CGS-21680 in migration and proliferation of HUVEC observed in late-onset and normal pregnancy.
Conclusion: A_{2A}AR exhibit a pro-angiogenic effect mediated at least partially by NO formation in normal and late-onset pre-eclampsia. Reduced proliferation and migration of HUVEC present in early-onset pre-eclampsia seems related with reduced expression and activation of A_{2A}AR and eNOS.
Supported by FONDECYT 1100684, Conicyt Anillo ACT73.

S1

FETAL ENDOTHELIAL PROGENITOR CELLS IN THE PLACENTA AND PREGNANT UTERUS

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Endothelial progenitor cells (EPCs) are circulating bone marrow-derived cells involved in fetal and adult neovascularisation, angiogenesis and vascular repair. At least two subtypes are described, Circulating Angiogenic Cells (CACs) and Endothelial Colony Forming Cells (ECFCs). Their importance in pre-eclampsia is debated. However, here we present work on fetal EPCs, more specifically ECFCs, and their (i) impact on placental vascularisation, (ii) irregularities in fetal growth restriction (FGR) and (iii) transmigration to the pregnant uterus.

(i) Differential measures of EPCs in arterial and venous human cord blood strongly implied placental sequestration. In confirmation, culture-expanded ECFCs showed full vascular incorporation following ex vivo perfusion into human chorionic placental arteries, whilst similar in vivo transplanted cells, introduced into the mouse fetal circulation, preferentially homed to placental vessels and demonstrated angiogenic/vasculogenic phenomena.
(ii) Compared to uncomplicated pregnancies, human cord-blood EPCs were reduced in FGR pregnancies and their culture-derived counterparts (ECFCs) showed *(i)* diminished proliferative capacity, *(ii)* ineffective tube-formation and *(iii)* unresponsiveness to hypoxia. These disparities likely underpin their observed inability to generated blood vessels de novo, in artificial tissue blocks transplanted into immune-deficient mice.
(iii) Following eGFP and native mouse matings, fetal endothelial-like cells were indentified in murine uterine vessels. *In vivo*, fetal injected human ECFCs were tracked to the mouse uterine endothelium and again confirmed to incorporate. In human studies, fetal cells were observed in the human uterine microvasculature and, through SRY quantitation, estimated to occupy 10% of the total vascular endothelium

These studies confirm that human ECFCs contribute to *de novo* endothelium and vessel formation in the human placenta and suggest their extensive integration into the remodelled pregnant uterus. Intrinsic attenuations in their number and/or function could offer explanation for abnormal placentation of FGR. These anomalies, if persistent, could also hold life-long consequences for the vascular health of affected mothers and babies.

S2

HUMAN UTERINE STEM/PROGENITOR CELLS: IMPLICATIONS FOR UTERINE PHYSIOLOGY AND PATHOLOGY

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The human uterus primarily consists of the endometrium and the outer smooth muscle layer termed the myometrium. The uterus exhibits the unique and remarkable regenerative capacity responsible for cyclical regeneration and remodeling throughout a woman's reproductive life. The endometrium, in particular the functionalis layer, must regenerate, differentiate and regress with each menstrual cycle under hormonal control. These morphological and functional features of human endometrium can be reproduced in murine models in which severely immunodeficient mice are xenotransplanted with dispersed human endometrial cells under the kidney capsule followed by hormonal treatment. The myometrium possesses the similar plasticity of the endometrium. This is demonstrated by multiple cycles of pregnancy-induced enlargement and regression after parturition. Regeneration and remodeling in the uterus are likely achieved through endometrial and myometrial stem cell systems. Indeed, we and other investigators have identified, isolated and characterized these putative stem/progenitor in humans and rodents, providing a new insight into their possible roles in the physiology and pathophysiology of the human uterus. Furthermore, these stem/progenitor cells might be clinically applicable as a novel source of biological material for the reconstruction of not only the human uterus but also other organs.

S3

TRANSPORT ACROSS THE PLACENTA: OF MICE AND WOMAN. A REVIEW

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Placental dysfunction is a major cause of pre-eclampsia (PE) and fetal growth restriction (FGR) and is therefore a target for potential treatments. However, there are no such treatments currently in clinical practice. Here we review work designed to characterise and develop genetic mouse models of human PE and FGR, which could then be used to test new drugs. This has involved (1) comparing and contrasting normal placental function between mouse and woman; (2) characterizing genetic mouse models; (3) testing candidate drugs in the mice.

(1) Myometrial and chorionic plate arteries from women behave similarly to uterine and umbilical arteries from the mouse in the wire myograph. The mouse placenta shows similar, though slightly higher, permeability to inert hydrophilic solutes than the human placenta. Similar transporter systems exist in both mouse and human placentas: for example the system A amino acid transporter is present on the microvillous plasma membrane of both. These data show sufficient similarities to support a programme of work characterizing and using genetic mouse models to test potential therapies.
(2) We have focused on the placental specific *Igf-2* knockout (P0) mouse. Placental weight is reduced in the P0 mice and 97% of these fetuses fall below the 5^{th} centile of wild-type (WT) fetal weights; the P0 placentas have altered system A amino acid transporter activity and lower permeability to hydrophilic solutes. Uterine artery reactivity in the myograph is similar between P0 and WT vessels.
(3) We have begun testing whether the NO potentiating drug sildenafil citrate (S.C) improves placental and fetal growth in the P0 mice. Preliminary data suggest S.C increases placental and, to a lesser degree, fetal weight.

In conclusion, placental function in mouse and woman is similar, and data suggest that mouse genetic models of placental dysfunction might aid the development of therapies for PE and FGR.
Supported by the Medical Research Council (UK).

S4

ADIPONECTIN: THE MISSING LINK BETWEEN MATERNAL ADIPOSITY, PLACENTAL TRANSPORT AND FETAL GROWTH?

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OBJECTIVES, METHODS, RESULTS, CONCLUSION
Fetal growth is positively correlated to maternal adiposity, however the underlying mechanisms remain largely unknown. Adiponectin is the most abundant protein secreted by white adipose tissue and has well-established insulin-sensitizing effects. Pregnant women who are obese or have gestational diabetes typically have low circulating levels of adiponectin, which is correlated to increased fetal growth. Lean women, on the other hand, have high circulating levels of adiponectin. As a result, maternal serum adiponectin is inversely correlated to fetal growth across the full range of birth weights, suggesting that maternal adiponectin may limit fetal growth. Adiponectin abolishes insulin stimulated amino acid uptake in cultured human primary trophoblast cells by modulating insulin receptor substrate phosphorylation. Furthermore, chronic administration of adiponectin to pregnant mice inhibits placental insulin and mammalian target of rapamycin (mTOR) signaling, down regulates the activity and expression of key placental nutrient transporters and decreases fetal growth. These findings have led to the working model that adiponectin binds to the adiponectin receptor-2 on the trophoblast cell and activates p38MAPK and PPAR-α, which inhibits the insulin/IGF-I signaling pathway resulting in down-regulation of placental nutrient transporters and reduced fetal growth. We further hypothesize that accumulation of fatty acid metabolites represent a link between PPARα and the insulin-signaling pathway. These observations suggest that adiponectin causes insulin resistance in the human placenta, in contrast to the well-established insulin-sensitizing effect in skeletal muscle and liver. Regulation of placental function by adiponectin constitutes a novel physiological mechanism by which the endocrine functions of maternal adipose tissue influence fetal growth. These findings may help us better understand the factors determining birth weight in normal pregnancies and in pregnancy complications associated with altered maternal adiponectin levels such as obesity and gestational diabetes.
Supported by NIH HD065007 and HD058032.

S5

MODELLING PLACENTAL AMINO ACID TRANSFER: FROM LAB TO LAPTOP AND BACK AGAIN

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The mechanisms underlying placental amino acid transfer are complex but can now be explained in principle. However, this understanding in principle does not mean that the function of the system as a whole can be predicted. Computational modelling of amino acid transfer may provide an approach which represents the complex interactions between different transporters and allows functional predictions.

Amino acid uptake by the apical microvillous membrane of the placental syncytiotrophoblast requires interaction between accumulative transporters and amino acid exchangers. On the basal membrane amino acid exchangers and facilitated transporters are required to mediate efflux of amino acids. Transporter activity is dependent on amino acid gradients across membranes. These gradients are determined by: individual transporters, interactions between different transporters, maternal and fetal blood flow, mixing of blood within the different compartments and placental metabolism. These factors create a complex interplay between the different transport systems and between transporters and their local environment. This complexity means that while the principles of the system are known, how the system functions in practice cannot be intuitively understood.

Developing an integrated understanding of placental amino acid transport is essential in order to understand amino acid transfer in both normal and pathological pregnancies. The first aim of our approach is to use simple computational models to make testable predictions about placental function. Testing these predictions experimentally will demonstrate the strengths and weaknesses of the model which can be developed with increasing complexity and retested in an iterative fashion. In this way we hope to develop a functional model of amino acid transfer and focus attention on the most important determinants of amino acid transfer.

Once a model is established it will allow exploration of the factors which result in impaired amino acid transfer and development of strategies to optimise placental transfer in intrauterine growth restricted pregnancies.

S6

THE DANGEROUS PLACENTA AND PRE-ECLAMPSIA, A REVIEW

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Normal, third trimester pregnancy and pre-eclampsia are both characterised by systemic vascular inflammation, which is more intense in pre-eclampsia. The danger hypothesis identifies inflammation as a generic and polymorphic response to loss of cellular equilibrium, secondary to various stimuli that include infection, oxidative stress, endoplasmic reticulum stress, trauma and so on. Together these stimuli constitute 'danger'. The evidence indicates that the presence of even a normal placenta is sufficiently destabilising to be 'dangerous' to the mother.

The two stage model of pre-eclampsia predicts that deficient remodelling of maternal spiral arteries causes dysfunctional arterial flow that oxidatively and hydrodynamically damages the placenta, so causing not only the maternal syndrome as a danger response but also fetal growth restriction (FGR). However FGR is not a feature of late onset disease (after 34 weeks) implying that placental function is not impaired in this subtype. This has led to the concept of maternal and placental pre-eclampsia, the former being secondary to the interaction between a normal placenta and an abnormal maternal circulation already predisposed to the vascular inflammation that characterises pre-eclampsia. Mixed presentations, which combine both aspects, are likely to lead to the most severe presentations.

Circulating trophoblast derived molecules are differently affected by maternal and placental pre-eclampsia as predicted by this concept and potentially might discriminate placental from maternal disease.

The pre-eclampsia placenta releases a wide range of danger molecules. This suggests that placental pre-eclampsia is unlikely to be a response to a single factor. Some of these molecules are carried by trophoblast-derived extracellular vesicles. Together they will probably comprise a mix derived from both regulated and dysregulated placental responses to its own 'danger'.

Future research will be facilitated by recognition of these pre-eclampsia subtypes and abandoning the expectation that a single factor causes placental pre-eclampsia.

S7

PREECLAMPSIA, PLACENTAL ACUTE ATHEROSIS AND FUTURE CARDIOVASCULAR DISEASE: IS THERE A LINK?

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Preeclampsia is a potentially lethal pregnancy complication for women and offspring. Sufferers have a long-term augmented risk of cardiovascular disease and premature death and may have risk factors in common with older persons developing cardiovascular disease, which are unmasked by the "stress" of pregnancy. It is also possible that a new risk factor might occur de novo during preeclampsia, and predispose for future cardiovascular disease.

In preeclampsia, lipid deposition in the walls of the spiral arteries of the uteroplacental circulation regularly occurs. These lesions resemble early stages of atherosclerosis (acute atherosis) and are thought to regress after delivery. The mechanisms that contribute to acute atherosis in preeclampsia are largely unknown, but are related to the impaired vascular remodeling of the spiral arteries in the first half of pregnancy. An intriguing and unexplained feature is that acute atherosis seems to be associated with defective remodelling of the spiral arteries that is a consequence of poor placentation. Our data show that spiral artery lipid deposition may also occur in normal pregnancies, which suggests that it may not be confined exclusively to maladapted spiral arteries.

The review will include some recent data of elevated circulating biomarkers that may be linked to cardiovascular disease, which we have found to be present in preeclampsia, and also several years afterwards. Our preliminary findings showing that women with a polymorphism of the *RGS2* (regulator of G protein signalling) gene (C1114G, *RGS2* 1114G allele) are at augmented risk for preeclampsia and placental acute atherosis. RGS2 is a member of a large family of regulators of G protein signalling and is involved in the control of blood pressure. Reduced expression of RGS2 has been associated with hypertension.

Further understanding of the process underlying spiral artery atherosis in the months of pregnancy may help cast light on development of cardiovascular disease later in life.

S8

HOW DOES THE MATERNAL IMMUNE SYSTEM OR AUTOPHAGY SYSTEM CONTRIBUTE TO THE DEVELOPMENT OF PREECLAMPSIA?

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Two stage disorder theory is well accepted for the pathophysiology of preeclampsia. Stage 1 is poor placentation at early stage of pregnancy and the second stage is associated with exaggerated endothelial activation and a generalized hyperinflammatory state. Impaired angiogenesis plays important roles in stage 2, but cause of poor placentation remains largely unknown. Jauniaux's placental oxygen curve shows oxygen concentration in placenta is very low ($\sim$2% O_2). Here, we show that autophagy, which is an intracellular bulk degradation system under stress condition, was observed in extravillous trophoblast (EVT) under hypoxia *in vitro* and *in vivo* clinical samples. Interestingly, invasion and vascular remodeling under hypoxia were significantly reduced in autophagy-deficient EVT that is established by stably transfecting Atg 4B mutant expression vector. We found soluble endoglin (sEng), which increased in sera of preeclamptic cases, suppressed EVT invasion by inhibition of autophagy. High dose of sEng (more than 250 ng/ml) inhibited vascular construction by EVT and HUVEC, meanwhile 100ng/ml of sEng inhibited the replacement of HUVEC by EVT (vascular remodeling). These findings were not observed in autophagy-deficient EVT. We have checked the expression of p62 in EVT which is selectively degraded by autophagy. We found the accumulated p62 in EVT preeclamptic placental biopsy samples showing impaired autophagy.

Epidemiological studies and immunological studies show immune system play some roles for the pathophysiology of preeclampsia. We have shown that granulysin produced by uterine NK cells induced apoptosis of EVT in miscarriage. Serum granulysin is a marker for Th1 type immunity and Th1 type immunity is observed in preeclampsia. We have found that granulysin positive cells were accumulated in preeclamptic placental biopsy samples and intracellular staining of granulysin was observed in EVT of preeclamptic cases but not in normal cases, suggesting that maternal T cells and NK cells attack EVT causing the death of EVT.

TR

TR AWARD LECTURE - SIZE IS EVERYTHING: THE SEARCH FOR A 3-D MORPHOMETRIC MARKER TO PREDICT FETAL GROWTH RESTRICTION

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Objectives: Fetal growth restriction (FGR) is a major cause of perinatal morbidity & mortality, even in term babies. Early placental volume measured with a semi-automated technique has been shown to predict FGR. Placental morphology measured in 2-D in the second trimester may also be correlated with FGR. We sought to explore a novel 3-D method for defining placental shape *in utero* and investigate if any subsequent morphometric indices correlated with FGR.
Methods: We prospectively recruited women with a singleton pregnancy and a BMI of $\leq$35. A 3D ultrasound scan was performed using a Voluson E8 (GE Medical systems) between 11 and 13+6 weeks' gestation. The placental volume, total placental surface area and placental basal plate area were calculated using our previously validated technique. From these we generated dimensionless indices including sphericity (ψ), standardise placental volume (sPlaV) and standardised functional area (sFA) using Buckingham π theorem. FGR was defined as $<$10th customised birthweight centile. Regression analysis examined which morphometric & biochemical measures were independent predictors of FGR. Potential screening performance was assessed with ROC curve analysis. The data were analysed using SPSS (SPSS inc).
Results: Morphometric and biochemical data were collected for 143 women, 20 had FGR babies. Only sPlaV, sFA, PAPP-A and NT were significantly correlated to birth weight ($p<0.001$). Regression demonstrated all dimensionless indices were inter-dependent co-factors. ROC curves showed no advantage for using sFA over the simpler sPlaV. The generated model predicted normotensive, term, FGR with 83% sensitivity for 20% false positive rate.
Conclusion: The dimensionless index of placental volume (sPlaV) is significantly correlated with birth weight. The placental morphometric indices are not independent of volume at this early gestation. A model can be produced combining sPlaV with PAPP-A and NT to predict FGR. This simple process may aid in the development of a screening test for FGR

Than Award Lecture

REGULATING THE REGULATORS: MICRORNA CONTROL OF PLACENTAL GROWTH FACTOR SIGNALLING

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Fetal growth restriction (FGR) and fetal overgrowth (macrosomia) are associated with altered placental growth and development. Using an explant model of human first trimester placenta, we have previously demonstrated that growth factors such as the insulin-like growth factors, are important regulators of placental growth. Most growth factors exert their actions by activating receptor tyrosine kinases (RTKs) to initiate a serious of downstream phosphorylation events within the PI3K and MAPK pathways, thus the ability of the placenta to modulate expression of components of these pathways is important for normal pregnancy outcome. Endogenously, gene expression can be regulated by microRNAs (miRs); the placenta contains high levels of these molecules thus miRs have the potential to regulate placental gene expression. Using a systems biology based approach, we have now affirmed a role for individual miRs in regulating both growth factor signalling and placental growth; these will be discussed in this lecture.

P1.0

CLINICAL RELEVANT HISTOLOGICAL PATTERNS OF CHORIOAMNIONITIS BY AMNIOTIC FLUID INFECTION NOSOLOGY COMMITTEE SYSTEM

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Objectives: Prenatal infections are important aspects of placental pathology. The histological pattern of chorioamnionitis (CAM) by Blanc has been widely used, this scheme has not include necrotizing CAM or fetal inflammatory response. Redline et al. proposed the new histological diagnostic framework of CAM, and showed reproducibility among of pathologists (amniotic fluid infection nosology committee). To assess the clinical relevant histological patterns, we examined the new histological diagnostic framework of CAM.
Methods: We reviewed placentas with 493 singleton births (the mean gestational age: 33 weeks). In this system, maternal stage 1 was defined as acute subchorionitis or chorionitis, stage 2 was acute CAM, and stage 3 was necrotizing CAM. Fetal stage was defined as chorionic vasculitis or umbilical phlevitis, stage 2 was umbilical vasculitis, and stage 3 was necrotizing funisitis. Grade 1 was defined as mild to moderate, grade 2 was severe.
Results: Among of 493 cases, placental examination revealed 112 cases (22%) with CAM. Maternal and fetal inflammatory responses were associated with maternal and infant CRP levels, infant death, sepsis, brain diseases, and lung diseases. Multiple logistic analysis showed maternal inflammatory response was associated with sepsis and brain damage, and fetal inflammatory response was contributed with infant death and brain disease.
Conclusion: Our data suggest that new histological diagnostic framework of CAM is useful for the information in amniotic fluid infection and neonatal prognosis.

P1.1

DOES "BETA" EXIST IN TWIN PREGNANCIES? COMPARISONS OF MONOCHORIONIC AND DICHORIONIC TWINS, AND CORRELATIONS WITH CHANGE IN PLACENTAL METABOLIC EFFICIENCY

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Objectives: We have shown that beta, calculated as ln(PW)/ln(BW), is a measure of placental functional efficiency equal to 0.75 across a range of populations. Do monochorionic (MO) twins, with twins sharing a single placenta, and dichorionic (DI) twins (with each twin with a unique placental mass) also show this relationship? If so, what factors perturb beta?
Methods: 32 MO and 137 DI twins were delivered at New York Methodist Hospital between 2009 and 2011 and had available birth weights (BW), placental weights (PW), gestational age (GA) data and cord insertion site. BW Discordance was calculated as larger weight-smaller weight/larger weight. Beta was calculated as above, and compared between twin types, cord insertions, and BW discordance.
Results: Mean beta for MO and DI were 0.777 ± 0.027 and 0.780 ± 0.032 (NS). Mean GA for MO and DI were 248.4 ± 23.6 and 249.2 ± 19.8 (NS), with mean BW of larger and smaller twins in MO and DI 2311 ± 535g, 2042 ± 534g and 2472 ± 581g, 2192 ± 523g (NS), respectively. Mean discordance in MO and DI twins were $12+8\%$ and $6+7\%$ (NS). Beta varied with BW discordance only in MO twins (MO $r=0.53$, $p=0.002$, DI $r=0.001$, $p=0.99$) independent of GA. Controlling for cord insertion (velamentous, marginal or normal) strengthened the beta - BW discordance relationship ($r=0.65$, $p=0.001$).
Conclusions: MO and DI twins show allometric scaling similar to singletons. Increases in beta indicate reduced placental functional efficiency. Discordant BW is related to greater beta (less efficient placenta) only in MO twins, regardless of GA. The very early events that result in MO twinning can lead to permanent abnormal placental development that may include marginal/velamentous cord insertions and the later development of BW discordance.

P1.2

CHARACTERIZATION OF PLACENTAL GROWTH AS A BIOMARKER OF AUTISM/ASD RISK

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Goal: To determine the correlation between placental growth patterns with diagnosis of autism/ASD as compared to a control group.
Methods: A nested case control study of the Avon Longitudinal Study of Parents and Children (ALSPAC) included archived placentas for 52 children (7 female, 45 male) in the cohort with diagnosed with autism/ASD and for a control group (n=161) with no neurodevelopmental diagnoses, at a 3:1 ratio to cases.
Results:

1. Placental weight was significantly reduced (~100 g) in female autism cases compared to controls, also after adjusting for gestational age ($p<0.05$). This was not the result of outliers in either distribution. No such effects were seen in males.
2. The influence of gestational age on placental weight differed by sex in regression models to predict placental weight by case/control status and gestational age. Gestational age had a strong effect on placental weight for males ($p<0.05$) but not for females; we did not test the interaction due to the small sample.
3. The smaller placental dimension did not significantly differ between autism cases and controls in either gender. However, the difference among females (-1.41 cm) was two-fold that of males (-018 cm).
4. β, a marker of placental functional efficiency and placental fractal structure, did not differ between boys with autism/ASD (0.755+0.0239) and controls (0.753+0.0202). β differed between girls with and without autism/ASD (0.733+0.0354 v. 0.760+0.0161, p=0.001). β differed by gestational age only in boys (point estimate of effect=-0.002, p=0.009 v. females with p=0.53). The association of altered β in girls with autism/ASD persisted after adjustment for gestational age (estimate of effect of autism/ASD "case" status=0.03, p=0.01).

Conclusions: Gestational age effects on autism/ASD risk may be marked by altered placental fractality and altered β. We hypothesize that placental growth patterns are altered in autism/ASD in gender specific fashions which may provide insights into the mechanisms of and differences between autism/ASD frequency and phenotype in females compared to males.

P1.3

ANALYSIS OF PLACENTAL SHAPE AND CORD INSERTION IN A RELATED COHORT OF FAMILIAL AUTISM, THE EARLI COHORT

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Goals: To determine the correlation between placental growth patterns of high risk sibling from families with an older child diagnosed with autism/ASD as compared to a large and well-studied birth cohort.
Methods: 53 well preserved digital photographs of the fetal surface and 51 digital photographs of the sliced placental disk from EARLI high autism risk newborns been processed identically to the University of North Carolina Pregnancy, Infection and Nutrition Study (UNC PIN), extensively analysed by the PI and are treated here as the reference group for the EARLI placentas. Fourier analysis of the shape and cord displacement (calculated as the displacement from the center of the area of the chorionic plate shape) of the two groups were compared using non-parametric correlations with $p<0.05$ significant.
Results: Umbilical cord marginality defined either by the first Fourier coefficient or more directly as the cord displacement differs significantly between autism/ASD cases and the UNC PIN birth cohort. Cord displacement is greater in placentas from siblings of autism/ASD cases; cords are closer to the placental chorionic disk margin. The disks of autism/ASD case siblings are less round, more irregular in perimeter, with significantly larger values of sigma and symmetric difference and measures of placental roundness (each $p<0.0001$). Disk thickness in autism/ASD siblings was also significantly less ($p<0.0001$) as was the linear deviation from the average width (a measure of thickness variability, [2]). This finding was independent on the length of the slice (diameter of placental disk).
Conclusion: These data show early promise of being able to use placental measures to contribute to our understanding of likely pathways of disordered neurodevelopment in the heterogeneous spectrum of autism/ASD.

	Mean ± sd			
	UNC	EARLI	Difference (UNC vs. EARLI)	p-value
Fourier 1 Displacement	3.231 ± 1.817	4.069 ± 2.345	-.838	.015
Displacement/ Diameter	3.455 ± 1.911	4.228 ± 2.515	-.773	.040
Sigma	.164 ± .091	.204 ± .122	-.040	.029
Symmetric Difference	1.106 ± .492	3.104 ± 1.696	-1.998	.000
Average (Avg.) Width	138.815 ± 67.609	3519.491 ± 12086.36	-220.676	.000
Linear Deviation from Avg. Width	2.076 ± .382	1.853 ± .366	.223	.000
Linear Deviation from Avg. Width Relative/Length	.340 ± .111	.368 ± .148	-.028	.000
Average Width/Length	.020 ± .007	.019 ± .008	.001	.005
	.125 ± .029	.100 ± .035	.025	.110

P1.4

IMMUNOHISTOCHEMICAL DETECTION OF MECONIUM IN THE PLACENTA, FETAL MEMBRANE AND UMBILICAL CORD

Naomi Furuta, Chizuko Yaguchi, Hiroaki Itoh, Keiko Muramatsu, Kaori Yamazaki, Kotomi Nagahashi, Naoaki Tamura, Toshiyuki Uchida, Kazunao Suzuki, Kazuhiro Sugihara, Naohiro Kanayama
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Objectives: We often experience meconium-stained amniotic fluid ,one side ,it is known the source of meconium aspiration syndrome. At the time meconium-stained amniotic fluid , it is adsorptioned amnion and englobemented to macrophage. We can observe it as brownish composure at HE stain. But it is difficult to distinguish meconium-stained and hemosiderin, macrophage in inflammation. So we examined useful diagnosis of meconium.
Methods: We previously reported the specific presence of zinc coproporphyrin I (ZnCP-I) in human meconium and demonstrated the possible diagnostic use of an elevation in maternal plasma ZnCP-I levels in cases of amniotic fluid embolism. In this study, we newly developed a specific monoclonal antibody for ZnCP-I and applied it to the immunostaining of meconium in the placenta, fetal membrane, and umbilical cord.
Results: Immunoreactivity of ZnCP-I clearly and specifically identified meconium in the placenta, fetal membrane, and umbilical cord. It was especially useful in cases of sever chorioamnionitis to detect meconium in the macrophages surrounded by numerous neutrophils. Meconium was detected in clear amniotic fluid at delivery, especially in cases of premature rupture of membrane, suggesting previous exposure.
Conclusion: Immunohistochemical detection of ZnCP-I is a highly sensitive histological diagnosis of meconium.

P1.5

IMMUNOHISTOCHEMICAL DETECTION OF MECONIUM IN THE LUNG OF ASPHYXIATED NEWBORNS

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Objectives: The role of meconium as the primary factor contributing to meconium aspiration syndrome is controversial, because autopsy studies have suggested prenatal origins of intrauterine infection and/or chronic hypoxia. We speculate that the inavailability of universal diagnostic criteria for meconium staining in the fetal or neonatal lung is one of the reasons for the confusion.
Methods: We recently developed a specific monoclonal antibody for ZnCP-I and applied it to the immunostaining of meconium in the placenta showing that meconium was mostly present in the CD 68-positive macrophages in the tissues. In the present study, we applied to the immunostaining of meconium in the lung of two newborns complicated with thick meconium obtained at autopsy. One case was intrauterine fetal death at 30 weeks of gestation due to severe hydrops fetalis. Anther case was neonatal death after cesarean delivery at 35 weeks of gestation due to non-reassuring fetal status.
Results: Clear immunostaining of ZnCP-I was detected in the lung tissues; however immunostaining of CD68 was distributed different places. It was suggested that the meconium was present independent of macrophages in the lung tissue of newborns, as a different manner comparing to the placental tissue.
Conclusion: Monoclonal antibody for ZnCP-I would be useful to establish the histological diagnostic criteria for the lung of meconium aspiration syndrome. The study is ongoing.

P1.6

PLACENTAL PATHOLOGY ASSOCIATED WITH FETAL CARDIAC ANOMALIES

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Objectives: To identify placental pathology associated with non-reassuring fetal status(NRFS) and fetal cardiac anomalies(CA) showing fetal hypoxia.
Methods: Placentas and umbilical cords obtained from 41 infants with NRFS and 35 infants suffering from CA including 3 cases with chromosomal anomalies were investigated by histopathology and ultrastructural observations from 2009 to 2011 at Saitama Medical School.
Results:

1) A total of 33 infants with NRFH (80.5%) and 18 infants with CA (51.4%) were delivered by Cesarean section.
2) Premature infants before 35 weeks of gestation were highly found in 60% among CA group, in contrast with 29.3% of the NRFS group.
3) In addition, frequencies of fetal body weight under 2500g and placental weight below 300g were also higher (60%, 54.3%) in the CA group than (29.3%, 31.7%, respectivity) in the NRFS group.
4) As characteristics of placental lesions between both groups, marginal and velamentous insertion of the cord was higher (25.7%) in the CA group than 9.8% in the NRFS group. In particular, SUA cord was highly found (11.4%) in the CA group. On histology, the frequencies of thrombosis and chorangiosis were highly seen in 27.8% and 9.2%, respectively in the CA group.
5) As characteristics of surface ultrastructural findings, cord vessels were affected by thrombotic formation and disruption of endothelium.

Conclusions: Pathological lesions of placenta including cord vessels corresponded closely to the pathogenesis of fetal cardiac abnormality.

P1.7

CORRELATIONS BETWEEN INTRAVILLOUS SCREENING AND PLACENTAL FUNCTIONAL EFFICIENCY: THE INFLUENCE OF VILLOUS CAPILLARY GEOMETRY ONTO OXYGEN TRANSPORT FLUX

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Objectives: We have previously shown that segmented digital photomicrographs can be used as a geometric basis for solving stationary diffusion equations, calculating oxygen flux, with diffusion screening quantified by comparing numerical and theoretical maximum oxygen fluxes. We now test whether our calculated oxygen flux and extensive diffusion screening ("screening factor") are correlated with placental functional efficiency expressed as beta= (ln[placental weight]/ln[birth weight]).
Methods: Multiple digital images were obtained from digitized slides from 22 term placentas selected from a well described birth cohort (www.cpc.unc.edu/projects/pin). Images were traced and segmented as published. Flux and screening factor were calculated for each villus and averaged across each placenta. 10310 capillaries were segmented for this analysis. Nonparametric correlations and curve estimations (SPSS) considered $p<0.05$ significant.
Results: Birth weight, placental weight and gestational age were highly intercorrelated (each $p<0.0001$), but each was uncorrelated with beta ($p>0.07$). Beta was significantly correlated with both mean flux ($r=-0.44$, $p=0.04$), and screening factor (-0.51, $p=0.02$), and for both measures, best fits were logarithmic.
Conclusion: In this small sample at term, diffusion equation solutions from segmented images of routine stained histology slides provide measures that significantly correlate with placental functional efficiency in terms of the grams of fetal weight generated per gram of placenta weight. Both reduced oxygen flux and increased diffusional screening correlates with lower placental functional efficiency, suggesting that poor transport of oxygen in terminal villi results in less mass of fetus per mass of placenta.

P1.8

FRESH VERSUS FORMALIN FIXED HUMAN PLACENTAE: DOES PRESERVATION AFFECT CHORIONIC PLATE MEASURES?

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Goals: We have previously demonstrated that chorionic plate shape is predictive of placental functional efficiency and birth weight in a birth cohort collected at a single institution. The NCS will receive both fresh and formalin fixed placentae. The extent to which information generated from images under these different circumstances is equivalent needs to be quantified.
Materials and Methods: Fresh and fixed photographs of the fetal chorionic surface from more than 50 placentae collected in the National Children's Study were analyzed. Perimeters were extracted manually and using an automated extraction algorithm from both image types. Geometric measures (area, perimeter, radius, radial standard deviation) and Fourier series coefficients were calculated for all four perimeter sets, and values were compared for correlation within image type (automated versus manual) and within perimeter extraction method (fresh versus fixed).
Results: Comparing fresh and fixed placentae, correlations ranged from 0.850 (mean radius)-0.945 (surface perimeter) but was more variable for Fourier coefficients that quantify perimeter irregularity (as low as 0.60). Comparing automated and manually traced fresh and fixed perimeters, the correlations were similar but automated measures consistently overestimated the manually traced measures; image inspection showed this was due to inclusion of deep shadow as disk surface. Automated measures performed equally to manual measures on irregularly shaped chorionic plates.
Conclusions: Fresh and fixed photographs may be used interchangeably for analysis of chorionic plate variables, but fixation alters the apparent perimeter of an irregularly shaped placenta. Automated methods can be a cost-effective alternative to manual tracing in regularly shaped placentae once corrections to the algorithm improve shadow discrimination. Fresh images are most useful for capturing placental shape irregularity. (Supported by NIH-NCS LOI-BIO-2-18)

P1.9

IMMUNOHISTOCHEMISTRY: THROUGH THICK AND THIN

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Insights into the structure and function placental cells are facilitated through the use of immunohistochemistry, which can be coupled with several modalities of microscopy. While routinely employed, there remain a number of limitations associated with these methods which have impeded attempts to describe in detail the architecture of this fundamentally important organ. Immunolabeling of whole mounts of fixed villous tissue, or relatively thick (15 mm) sections derived thereof, is practical for examining the three-dimensional cellar architecture within villi. However, issues relating to antibody penetration and practical limitations of confocal imaging systems limit both resolution and quantitative interpretability. Physical planarization of tissues, achieved by generating ultrathin (50-200 nm) sections, eliminates difficulties arising with staining and imaging efficiency associated with increasing tissue depth, but lacks important volumetric information. Recently, a technique has been developed termed "array tomography", which offers the advantages of very high z-axis resolution with significant improvements in volumetric imaging. This method centers around embedding fixed specimens in a hydrophilic acrylic resin (LR White), which can be cut into ultrathin serial sections that are amenable to immunolabeling, including immunofluorescence microscopy. These serial sections, representing substantial tissue volumes, can then be fluorescently imaged and subsequently reconstructed into three-dimensional renderings, so as to provide spatial context along the optical axis without compromising detailed axial resolution. In addition, these same sections can be imaged using backscatter electron microscopy, which allows for the use of correlative light and electron microscopy. In this workshop report, the practical applications of this procedure to study detailed arrangements of the molecular architecture within placental villi will be discussed.

P1.10

THE IMPORTANCE OF SUB-PROTEOME ANALYSIS IN HEALTH AND DISEASE

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Proteomics analysis is an excellent means to determine the protein complement in a given biological sample. Ideally, one could directly determine the full complement of expressed proteins in an organ such as the placenta. However, in practice this is not feasible due to the large dynamic range of protein expression in a given organ, tissue, or cell. Low abundance proteins, which are often of most interest, tend to be under represented in this sort of analysis. One way to address this problem is to analyze sub-proteomes of the organ, tissue, or cell of interest. We have focused on a specific sub-proteome of the human placenta, namely the apical plasma membrane of the syncytiotrophoblast. We have developed methods to isolate highly enriched preparations of this membrane. Further sub-proteomes were generated from the apical plasma membrane by a series of extraction steps designed to disrupt protein-protein interactions in an effort to obtain a sample enriched for integral plasma membrane proteins. This fraction was suitable for mass spectrometry and proteomics analysis. We identified 340 proteins in which at least two unique peptides were detected for each of these proteins. Forty of these proteins were not previously known to be expressed by the syncytiotrophoblast. The identification of these latter proteins represents unique opportunities in placental disease research. For example, we have studied one of these proteins, dysferlin, and found that there was decreased expression in placentas from severe pre-eclampsia when compared to gestational-age matched controls.

P1.11

CHORIONIC VASCULAR "FIT", CORD CENTRALITY AND FETAL GROWTH RESTRICTION

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Objective: To examine the hypothesis that poor "fit" of the chorionic vessels to the chorionic plate area and asymmetric growth of vessels from the cord insertion are correlated with reduced fetal growth.
Methods: 1001 consecutive consenting mothers delivering singleton live-born infants had placentas collected, and digitally photographed and weighed. The perimeter of the chorionic disk was traced; cord insertion and the sites at which each chorionic vessel dived beneath the chorionic plate were marked. The area and perimeter demarcated by the diving sites was calculated. Dimensionless ratios of chorionic vascular area / chorionic disk area, distance between centroids of the inner and outer areas (inter-centroid distance), and distance from cord insertion to the disk area centroid were analyzed with regression, with $p<0.05$ significant. Beta was calculated as ln(placental weight)/ ln (birth weight). The observed/ expected birth weight ratio (OER) was calculated as the birthweight/ the predicted birth weight based on gestational age and placental weight in regression analysis.
Results: The ratio of the vascular coverage area to the chorionic plate area was significantly correlated with both OER ($r=0.08$, $p=0.016$) and beta ($r= -0,08$, $p=0,009$). The ratio of the irregularities of the vascular areas and chorionic plate areas (symmetric difference ratio) was highly corrected with beta ($r=-0.11$, $p<0.0001$), a diagnosis of preeclampsia ($r=-0.074$, $p=0.019$). Preeclampsia was also associated with a greater ratio of cord displacements in vascular and chorionic surface areas ($p=.014$, $p+0.001$).
Conclusions: How the chorionic plate surface vessels cover the chorionic plate is significantly related to the OER, beta, and also a diagnosis of preeclampsia. Further in preeclampsia, the chorionic vessels are centered differently on the plate, resulting in a greater ratio of cord displacement in vascular area compared to the plate area. The simple measure of chorionic vascular extent may illuminate aspects of placental functional efficiency, and inefficiencies that are seen in pathologies like preeclampsia. (Support – K23 1-MH067857 NIMH)

P1.12

PREGNANCY OUTCOMES AFTER BARIATRIC SURGERY

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Objective: To investigate pregnancy outcomes after bariatric surgery (BS) and the relationship between birth weight (BW) and placental weight (PW) at time of delivery against a control group (CG) of patients of similar gestational age (GA) and BMI who did not undergo BS.
Method: This study is a retrospective chart review of patients in a single community hospital consisting 32 patients who underwent BS prior to conception and who had a subsequent singleton pregnancy. The CG consisted of 39 randomly selected patients with a preconception BMI of >35 (total n=71). The primary outcomes measured were GA, mode of delivery, rates of gestational diabetes (GD) and hypertensive disorder of pregnancy (HDP), and difference in BW, PW, and fetoplacental weight ratio (fpr). ANOVA and chi square tests were used in statistical analysis.
Results: There was no significant difference in GA and the rate of GD and HDP in the BS group vs. the CG. BW was significantly lower in the BS group than the CG (3027g $\pm$ 664g vs. 3417g $\pm$ 701g, p = .006). Neonates were more likely to be SGA in the BS group than the CG (25% vs. 3%, p=.005), and more likely to be LGA in the CG (13%) than the BS group (3%, p>.05). Additionally, the PW was lower in the BS group than the CG (416g $\pm$ 123g vs. 555g $\pm$ 120g, p=.000) and the fpr also differed between the BS group vs. CG (7.62 $\pm$ 2.33 vs. 6.08 $\pm$ 1.02, p=.001).
Conclusion: BS was not associated with reduction in comorbid diseases associated with pregnancy such as GD and HDP, but was associated with an increase in SGA infants and low PW. The weight loss and marked reduction in food intake following gastric bypass surgery does have some effect on the growth of the fetus and placenta. Therefore, we recommend careful monitoring of expectant mothers who have a history of gastric bypass surgery.

P1.13

THE CHORIONIC SURFACE VASCULAR NETWORK IN HUMAN PLACENTAE: WHAT CAN BE MEASURED AND DOES IT MATTER?

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Goal: Chorionic vascular surface networks are well recognized to be highly variable but they have mainly been described in relation to the site of umbilical cord insertion; their description is limited to "magistral" or "disperse", terms of problematic utility for quantitative analysis. However, digital images have rendered these networks amenable to quantitative assessment. What variables can be extracted, and how do they correlate with other established measures of the placental disk?
Materials and Methods: We calculated from chorionic vascular tracings the following variables: arterial, venous and total chorionic vascular area, arterial, venous and total chorionic vascular branch number, and arterial, venous and total branch point numbers. We compared these variables to placental volume, disk thickness and its variability.
Results: Correlations with total vascular surface area and total vascular branch point numbers were strongly correlated with disk thickness, thickness variability but not placental volume. As total chorionic vascular area and branch point numbers increased, there was decreased disk thickness (r= −0.58, −0.85, p=0.04, p<0.001) and decreased thickness variability (r= −0.49, −0.62, p=0.06, p=0.014.)
Conclusions: The architecture of the chorionic surface vessels, laid down by mid gestation, is correlated with the later arborization of villi that results in disk thickness. The more extensive the surface arborization and more extensive the branching, the more uniform the underlying disk and more uniform the villous arborization. Measurement of the surface vasculature may provide insights into the gestational history of stressors germane to fetal well-being. (Supported by NIH-NCS-LOI-BIO-2-18)

P1.14

COST-EFFECTIVE, REPRODUCIBLE, AND RELIABLE AUTOMATED DIAGNOSIS OF INTRAAMNIOTIC INFECTION FOR POPULATION BASED RESEARCH FROM PLACENTAE IN THE NATIONAL CHILDREN'S STUDY

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Goals: Intraamniotic infection is a major risk factor for preterm birth (the greatest single agent of perinatal morbidity and mortality) as well as a contributor to the development of cerebral palsy and other significant childhood diseases. As such, its diagnosis needs to be both reliable (reproducible in the same patient, and across patients and institutions) and valid. Unfortunately, current diagnostic pathology "gold standards" are neither reliable nor have they been validated against measures other than group consensus. We tested our image analysis software to determine the extent to which they may replace the current best practice in routine hematoxylin and eosin (H&E), and provide reliable diagnoses at significant cost savings to the NCS.
Materials and Methods: H&E stained slides of umbilical cord cross sections were digitized and regions of interest (ROIs) submitted for automated image analysis. Receiver operator curve (ROC) analyses determined sensitivity and specificity, with the goal to select a threshold that maximizes both the sensitivity and specificity. Following the image segmentation work of Perkins et al., this work proposes a flexible method for detection of neutrophils.
Results: ROC curve analyses were first performed on a high risk set of cases for which the ROIs had already been read by one of three pathologists. These data were selected because of their higher than average prevalence of infection would provide for a more valid assessment of neutrophil detection. Based on the ROC analysis, the classification distinguished stronger infections (score 3 or 4) with 0.663 area under the curve, however, classification of mild infections (score 2) was unsuccessful with 0.502 area under the curve.
Conclusions: Continued testing will be necessary to improve the performance of the algorithm in a general population to improve cost savings by exclusion of true negatives with reduced false positives. (Supported by NIH-NCS-LOI-2-18)

P1.15

ACCURACY OF 2D AND 3D MEASUREMENT OF PLACENTA SHAPE

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We have shown that placental dimensions apart from weight account for birth weight variance in the National Collaborative Perinatal Project (CPP, 1959–1974). Diameters, qualitative description of shape and a single disk thickness were measured with a ruler. In the National Childrens' Study (NCS), an ongoing study of +100,000 children, we collect photographs of fetal surface and slices and 3D surface scans. Does modern technology improve on the CPP measures?
We measure placental shape in two ways: (1) digital photographs of the fetal surface, and for measurement of thickness, the placenta is sliced and photographed; (2) the 3D shape of the placenta surface is captured using a FlexScan 3D Scanner. We implement the CPP volume for comparison using the diameters and the mean thickness computed from the 3D scan. This thickness measurement is generously accurate, since it can only be crudely estimated with a ruler.
In simulated measurements on the 3D mesh, the CPP volume had average error of 4.92%. Volume computed using the simulated slices had average error of 1.28%. CPP volume error was significantly correlated with the difference between the ellipse and the fetal surface area, which shows that much of the error is due to CPP's elliptic model.
In non-simulated measurements, we again found that volume estimated from slices is more accurate than the CPP method, although both approaches had higher error than when simulated. Volume estimates from the CPP method differed on average by 11.7% from the 3D mesh volume. Estimates from photographs differed on average by 8.6%.
We believe that our precise and robust measures will provide greater clarity as related to cognitive and somatic outcomes with placental growth. (Supported by NIH-NCS-LOI-BIO-2-18)

P1.16

HOW TO SLICE THE PLACENTAL DISK - ONE-PROTOCOL-FITS-ALL OR A SPECIMEN-SPECIFIC APPROACH?

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Goals: Conventionally, placentas have been suggested to be sliced at 1cm intervals, but are often impractical. We implemented a simpler method of slicing in eighths. Placentas with variable shape may have the most problematic intrauterine environments, and thus be of greatest interest to the NCS. We sought to determine, using virtual slicing of 3D volume scans, the robustness of the current one-size fits-all grossing protocol , and the effect of placental size and shape variability on estimates.
Materials and Methods: 3D scans from 70 placentas collected as part of the NCS Morphology Project were used. Placental volume estimates from virtual slices obtained at 8 and 12, and at decreasing intervals 1cm and 0.5 cm, were compared to the gold standard from the 3D volume scan.
Results: Volume estimates from 8 slices are on average 1.23% difference with the gold standard and thickness mean on average have 2.31% difference. Variable placental shapes, those likely of most interest to the NCS due to the gestational struggles altered shape implies, show a difference with the gold standard with $<$3.31% in volume and $<$9.42% in the mean thickness. As the slices number increase, the errors decrease both in volume and thickness mean. However, in practice, it becomes more challenge to cut placentas into too many thin slices.
Conclusions: As the number of virtual slices of 3D shapes increases, the correlation of volume estimates with volume from a 3D volume scan approaches 1.00. However, in practice, small number of slices is easier to cut and process. After simulating slicing placentas in different numbers with 70 3D volume scan data, we found that our current protocol (8 slices) is a consistent and reliable method for volume estimation and thickness regardless of placental size and shape. (Supported by NIH-NCS LOI-BIO-2-18)

P1.17

IMMUNOHISTOCHEMICAL PECULIARITIES OF PLACENTAL ONCOMARKERS BY PATHOLOGICAL CHANGES

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Objectives: The objective of this study was to evaluate immunohistochemical peculiarities of placental oncomarkers in women with benign tumors and cancer
Methods: Women (aged 24 to 30 years) were with benign tumors of the uterus- I group, after surgical treatment of papillary thyroid carcinoma- II group, with physiological pregnancy and delivery- III group. It was immunohistochemical evaluation of Ki-67, Bax, Bcl-2, p53, CEA, Cytokeratin AE1/AE3 and Vimentin expression in placenta (39-40 weeks of gestation)
Results: Intensive nuclear expression of Ki-67 was found in cytotrophoblast and syncytiotrophoblast and less intensive in stromal cells, vascular endothelium with reliable of proliferative index in II group of investigation. CEA cytoplasmic expression of stromal cells was found in all cases of II group, 2 cases – of I group and was absent in all cases of III group. Nuclear p53 expression was focal in extravillous trophoblast of II group. Strongly marked cytokeratin expression was evaluated in epithelial structures of villi with abnormal architectonics in the II group.
Conclusion: Immunohistochemical markers of Ki67, CEA, p53 were strongly marked in placenta from women with papillary thyroid carcinoma.

P1.18

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PECULIARITIES OF PLACENTA AFTER IVF TREATMENT WITH OOCYTE DONATION

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Objectives: The objective of this study was to evaluate morphological and immunohistochemical peculiarities of placenta after IVF treatment with oocyte donation.
Methods: The I group - 55 women after IVF treatment with oocyte donation, the II group – 60 patients after IVF treatment with own oocytes, the III – 53 women after natural fertilization. Morphological investigation was done on paraffin-embedded sections with hematoxylin-eosin and Van-Gieson stain. Immunohistochemical evaluation of TNF and CD 31 were performed.
Results: Focal pathological immaturity of villous tree as intramediate differentiated villi and disorder sclerosed villi was evaluated in 84,6% of the I group. Hypoplasia of decidua with dystrophic changes was found in the most cases of the I group. Intensive and moderate TNF-α expression was revealed in the stroma of villi and in epithelial cells. The level of TNF-α expression in endothelium and decidual cells was the same as in III group. Strongly marked expression of CD-31 was evaluated in endothelial cells in placenta of the II group.
Conclusion: Morphological and Immunohistochemical (TNF-α and CD-31) changes were found in placenta of the I group.

P1.19

PROGRESSION OF VUE IS REPRESENTED WITH TERMINAL VILLOUS INFLAMMATION OF CYTOTOXIC T CELLS AND ASCENDING INFLAMMATION OF INTRAVILLOUS SPACE PROGRESS TO VASCULOPATHY

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Objectives: Chronic villitis is defined by a chronic inflammatory infiltrate in the chorionic villi and divided into two groups, infectious villitis and so-called villitis of unknown etiology (VUE). VUE is associated with various problems such as fetal growth restriction, recurrent abortions, prematurity, abnormal neurologic development, and intrauterine fetal demise. Two suggestions have been presented the cause of VUE. First, VUE is an infectious disease caused by a yet recognized agent or an agent that cannot be identified from placental examination. The second theory is that VUE is an immune reaction between mother and fetus.
Methods: To clarify the process of villous inflammatory process in VUE, we examined six placentas with VUE.
Results: Then we find out the three inflammatory phases of chronic villitis. First phase is the “perivillous inflammation phase”. At this stage CD8 positive cytotoxic T lymphocytes “wrap” the terminal villous tissue and destroy the basement membrane. Second phase is “intravillous phase”. At this phase CD8 positive T lymphocytes exist at the intravillous space. And those cells migrate within the intravillous space. There is no villous surface inflammation. Finally inflammation progress to “vasculopathy stage”. At this stage fetal vessels are deranged and occluded. According to the vasculopathy villous stromal become fibrotic. All of T lymphocytes are positive for CD8 and also express the pore-forming protein “perforine” and apoptosis-inducing “granzyme B”.
Conclusion: These cytotoxic T cells destroy the musculature of fetal vessels and occlude the vasculature.

P1.20

AN ANATOMICAL RECORDS OF MONOCHORIONIC-MONOAMNIOTIC TWIN PLACENTA IN LATE 18TH CENTURY JAPAN

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Objectives: Placentas of mammalian animals are known since ancient eras. Aristotle recorded natures of human placentas obtained through normal deliveries However, their structure and functions are not known until 19th century. Leonardo da Vinci and Vesalius confused human placentas to those of carnivores. In order to examine anatomical understanding of placenta by Japanese physicians during Edo period (1603-1868 CE, an anatomical records of female cadaver was examined.
Methods: We observed detailed anatomical records of twin placentas in colored hand-writing original sketch in A.D.1800 Japan owned by Nihon University library. The sketch was one of the oldest anatomical records in feudal Japan and the only case of pregnant woman. Two physicians of Western medical school, namely OYA-SHOSAI, FUSHIYA-SOTEKI, and an orthopedic surgeon KAGAMI-BUNKEN performed autopsy of executed female cadaver under official permission of shogunate regime.
Results: The name and guilt of the female criminal was unknown but she was 37 years old. The records show healthy thoracic, abdominal and pelvic organs including pregnant uterus. Uterine content was illustrated in detail. A monochorionic-monoamniotic twin placenta located at uterine fundus with detailed blood vessels, umbilical cords with three vessels and fetal membranes. They also described two near term fetal findings. One looked healthy while the other was degenerated. Since the presence/or absence of fetal membranous septum and vascular anastomoses were not described until late 18th century European textbooks, we believe the findings of placenta in this record is based on their original observations. Shogunate government of Japan permitted autopsy of dead bodies in 1755. The first Japanese textbook of anatomy translated from Dutch "Tafel-anatomie" was published in 1774.
Conclusion: The presented anatomical record of pregnant uterus and feto-placental unit by late 18th century Japanese doctors were accurate and we can make some pathological speculations from their observations.

P1.21

DEVELOPMENT OF BOVINE EMBRYOS DERIVED FROM REPRODUCTIVE TECHNIQUES

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Assisted reproduction techniques have improved agricultural breeding in bovines. However, the development of embryos and fetal membranes may differ from the situation in *vivo* and there is a high mortality rate during the first trimester of gestation.
Objectives: In order to better understand the impact of these methods, we investigated the development of embryos and fetal membranes derived from fixed-time artificial insemination (FTAI), *in vitro* fertilization (IVF), and nuclear transfer (NT).
Methods: A total of 20 embryos were collected following FTAI at 25, 27, 29, 30, 40, and 45 days of gestation. For IVF were used 11 embryos on days 24, 28, 32, 36, 40, and 44 of gestation and 9 embryos derived from NT at 9, 28, 32, 36, 40, and 43 days of gestation. Embryos were collected following FTAI, IVF, and NT. The methodologies included macroscopic and morphometric studies, as well as light and electron microscopic procedures.
Results: The onset of yolk sac development was not normal in cloned embryos. Latter steps differ from the condition in *vivo* in all three groups; the yolk sac was yellowish and juxtaposed with the amniotic membrane. The vascularization of the chorioallantoic membrane was low in NT gestations, but normal in the other groups. The overall development of the embryos was normal in FTAI and IVF pregnancies, whereas in some NT embryos the liver and other organs completely occupied the abdominal cavity. In addition, the cloned embryos exhibited abnormalities in heart development.
Conclusion: In conclusion, especially the yolk sac seems to be vulnerable to severe morphogenetic alterations. This may be responsible for the high incidence of embryonic, fetal, and postnatal mortality, especially in clones.

P1.22

THERE IS NO ROYAL ROAD FOR PLACENTAL EXAMINATION

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Purpose: Not only am I writing this report merely in terms a pathological diagnosis, but also, in regard to the work of the pathologist. Many pathologists already recognize that pathologic education and enlightenment are important, extending this awareness has become my mission, and I have conveyed the significance of the examination of placenta to obstetricians for the last four years. The content of my work consists of three fundamental questions. 1. Why should we examine placenta? 2. How to examine the placenta ? 3. Which placenta should we examine ?
Materials, method: During these four years I have examined placentas as part of my routine works. We have held over 20 meeting in the department of obstetrics in order to inform and convey my findings in this field.
Results: The rate of placental examination to the number of deliveries from 20% grew to 50%. There have been many public presentations about the placenta in the last two years by obstetrician. The IgM was 1mg/dL, which is normal range, but the clinical records by neonatologist shows neonatal chronic lung disease Type III (related with inflammation) due to inflammation of the placenta.
Discussion: 1. "Why" Examinations of placenta were in the best interest of the patients. 2. "How" Obstetrician should observed placentas after delivery before pathologic examination. If there is inflammation it is important to start treatment as soon as possible. Inflammation is indicated by the color of the placenta being whitish and having thick chorion and amnion. 3. "Which" All placentas. If there is even the slightest chance of some unknown problems, we need to know in advance.

P1.23

DEVELOPMENTAL TRAJECTORIES OF MICROSCOPIC PLACENTAL ARCHITECTURE AND FETAL GROWTH IN THE VERVET MONKEY (*CHLOROCEBUS SABAEUS*)

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Objective: Nonhuman primate models of placental and fetal development are rare but have specific relevance to human growth and development. Most data comes from term samples, but less is known about trajectories of placental and fetal growth across gestation.
Methods: A series of 50 vervet monkey (*Chlorocebus sabaeus*) placentas from the St. Kitts Biomedical Research Foundation was characterized in terms of microscopic morphology and shifts in efficiency across the latter half of a species-typical 167-day gestation. Architecture was analyzed via stereology.
Results: Both fetal mass and placental mass increased significantly with gestational age (Pearson's correlations: $R=0.85$, $P<0.00001$; $R=0.64$, $P<0.00001$, respectively) but the size of the placenta relative to fetal mass decreased significantly between period 1 (d. 83-130) and period 2 (d. 131-159) (T-test: $T=-3.60$, $P<0.00001$). Placental mass accrual slowed at day 130 while fetal mass continued to increase. Though relative placental size decreased between period 1 and period 2, the surface area of the placental villi – the site of nutrient transport from mother to fetus – increased significantly, both in terms of volume (T-test: $T=-4.49$, $P<0.00001$) and surface area (T-test: $T=-5.33$, $P<0.00001$). The surface area expanded significantly more than the underlying volume, suggestive of increases in topographical complexity.
Discussion: These changes suggest there is an important shift in the metabolic capacity of the placenta, via an expansion of the microscopic surface area of the villi to support the energetic burden of late gestation brain and somatic growth. Future work in this sample will incorporate analyses of GLUT1 expression to characterize aspects of the transport of glucose, the primary substrate for brain growth. A better understanding of how the placenta drives and constrains fetal and brain growth in anthropoid primates is thus directly relevant to developmental models of human brain evolution.

P1.24

LEVELS OF ANGIOGENIC FACTORS ARE ASSOCIATED WITH HYDATIDIFORM MOLAR PREGNANCY

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Objectives: Hydatidiform mole is an abnormal pregnancy, and is a type of gestational trophoblastic disease with over proliferation of the placenta, which causes the dysfunction of placenta. It is classified with partial hydatidiform moles and complete hydatidiform moles. Although more than 80% of hydatidiform moles are benign with good outcome, hydatidiform moles are associated with very early onset preeclampsia. Preeclampsia is associated with altered levels of angiogenic factors, such as vascular endothelial growth factor (VEGF), soluble Endoglin (sEndoglin) and soluble Flt-1 (sFlt-1). A recent study suggested that levels of Flt-1/sFlt-1 were increased in hydatidiform molar placentae (1). The aims of this study were to investigate the levels of Endoglin and VEGF in hydatidiform molar placenta.
Methods: 17 placentae from complete hydatidiform mole, 8 placentae from partial hydatidiform mole and 16 placentae from gestation matched first trimester were collected and the levels of Endoglin (CD105) and VEGF expression were measured using immunohistochemistry and western blotting.
Results: Immunohistochemical staining measured by semi–quantification demonstrated that levels of Endoglin expression in hydatidiform molar placentae was significant decreased ($p=0.04$), while the levels of VEGF expression were significantly increased ($p\leq0.05$) in hydatidiform molar placentae, compared to normal gestation matched placentae. These findings were also confirmed by western blotting. In addition, there was no difference in Endoglin and VEGF expression between placentae with complete hydatidiform mole or partial hydatidiform mole.
Conclusion: In this study our data show that the levels of Endoglin were significantly reduced, while the levels of VEGF were significantly increased in first trimester placentae with hydatidiform moles. The altered levels of angiogenic factors may link to the mechanism of developing hydatidiform moles.

Reference

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P1.25

ISOLATION AND CHARACTERIZATION OF HUMAN TROPHOBLAST PROGENITOR CELLS IN PRIMARY VILLOUS CYTOTROPHOBLASTS AND HTR-8/SVNEO CELL LINE

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Objectives: Recently increasing studies have demonstrated that immature cell population including stem cells and progenitor cells can be found in "side-population" (SP) cells. Although SP cells isolated from some adult tissues have been reported elsewhere, isolation and characterization of human trophoblast SP remained to be reported. In this study, we intended to isolate and characterize human trophoblast progenitor cells.
Methods: HTR-8/SVneo cells and human primary villous cytotrophoblasts (vCTBs) were stained with Hoechst 33342 and SP and non-SP (NSP) fractions were isolated using a cell sorter. Expression analyses were shown by immunohistochemistry, western blot and real-time RT-PCR.
Results: HTR-8/SVneo cells are heterogenesis in nature including vCTB, STB and EVT cells. A small population of SP cells was identified from HTR-8/SVneo cells and in vCTBs. SP cells expressed several vCTB-specific markers and failed to express syncytiotrophoblast (STB) or extravillous cytotrophopblast (EVT)-specific differentiation markers. SP cells formed colonies and proliferated on mouse embryonic fibroblast (MEF) feeder cells or in MEF conditioned medium supplemented with heparin/FGF2, and they also showed long-term repopulating property. SP cells could differentiate into both STB and EVT cell lineages and expressed several differentiation markers. Microarray analysis revealed that IL7R and IL1R2 were exclusively expressed in SP cells and not in NSP cells. vCTB cells sorted as positive for both IL7R and IL1R2 failed to express trophoblast differentiation markers and spontaneously differentiated into both STB and EVT in basal medium.
Conclusion: These features shown by SP cells suggest that IL7R and IL1R2 are available markers to detect SP cells, implicating that vCTB progenitor cells and trophoblast stem cells are involved in SP cell population.

P1.26

FATTY ACID BINDING PROTEIN 3 (FABP3) AS A CELLULAR REGULATOR OF FATTY ACID TRANSPORT FROM MOTHER TO FETUS IN RODENT PLACENTA

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Objectives: Malnutrition of mother during pregnancy is now thought to contribute to the aetiology of various metabolic and/or neural disorders that manifest throughout life. Poly unsaturated fatty acids (PUFAs) are being focused in connection with neurodevelopmental disorders from the concept of Developmental Origin of Adult Health and Disease (DOHaD).Placenta is the key organ for regulating the transfer of various nutrients including fatty acids from mother to fetus. In this study, we examined the localization of fatty acid binding proteins (FABPs), cellular chaperons of fatty acids, in the mouse placenta and its functional significance.
Methods: Expression of FABPs in the mouse placenta on embryonic day (E) 11, E15 and E18 was examined by RT-PCR, western blotting and immunohistochemistry. Radio-labelled fatty acid was administrated into the female mice at embryonic day (E) 18, and its transfer to the fetus was measured by liquid scintillation counter.
Results: In RT-PCR, gene expression of FABP3, 4 & 5 was detected in the mouse placenta at different stages, among FABPs (1, 2, 3, 4, 5, 7, 8 & 9) examined. The protein expression of FABP3, 4 & 5 was also confirmed by western blot analysis. In immunohistochemistry, FABP3 was found to be highly expressed in the labyrinthine (transport) zone of mouse placenta; FABP4 was highly expressed in decidua basalis zone; FABP5 was weakly and widely distributed in the labyrinthine, decidua and spongiotrophoblast zone. FABP7 was identified in the maternal vessels of labyrinthine zone. In FABP3KO placenta, transportation of long chain polyunsaturated fatty acids (LCPUFA)[ω-6 & ω-3] were found to be significantly decreased compared to wild-type.
Conclusion: FABPs were expressed in the mouse placenta with spatial differences. FABP3 was strongly suggested to be involved in regulation of cellular transfer of fatty acids through the trophoblast.

P1.27

THE CORRELATION WITH SYNCYTIN-1 AND SYNCYTIN-2 FOR THE CELL FUSION ACTIVITY IN HUMAN PLACENTAL BEWO CELLS

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Objectives: Two human endogenous retroviral proteins, HERV-W (syncytin-1) and HERV-FRD (syncytin-2) have been found to be relevant to human trophoblast cell fusion (or syncytialization). Each has a different localization in placenta, syncytin-1 is located in syncytiotrophoblast predominately, and syncytin-2 in cytotrophoblast. The enhancement of intracellular cAMP levels, via not only classical PKA but EPAC pathway, causes cell fusion in the trophoblast derived human choriocarcinoma BeWo cells. The interaction between these proteins and the mechanisms of cell fusion remains less well understood.
The aim of our study is to investigate the role of syncytin-1 and syncytin-2 on cell fusion after stimulation by cAMP analogues.
Methods: BeWo cells was treated with siRNA for syncytin-1 and/or syncitin-2 and then incubated with cAMP analogues to induced cell fusion. The fusion associated factor, such as placental protein 13 and beta-hCG, was evaluated by mRNA expression by means of real-time RT-PCR.
Results: The mRNA expression of the fusion associated factors was reduced especially in the both of syncytin reduction group.
Conclusion: There are many kinds of factor to correlate with syncytialization, but syncytin-1 and 2 have great involvement with the cell fusion. We report further investigations in this point.

P1.28

HEPARIN/HEPARAN SULPHATE (HS) /CD44V3-MEDIATED SIGNALING ENHANCES THE STRUCTURAL REPAIR OF TROPHOBLAST CELL LAYER DAMAGED WITH MECHANICAL SCRATCHING AND LUPUS ANTICOAGULANT-POSITIVE PLASMA

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Objectives: The main treatment of pregnant women with anti-phospholipid syndrome (APS) is the anticoagulation with heparin/heparan sulphate (HS) for the prevention of thrombin generation caused by the hypercoagulable state in the intervillous space. Enhanced thrombin generation induces the fetal growth restriction in animal models. However, the mechanism of treatment with heparin still remains unclear in the patients with APS. The function of heparin/HS /CD44v3 was investigated during trophoblast cell migration to identify its role in the repair of syncytial layer damage caused by increased hemodynamic turbulence and Lupus anticoagulant (LA)-positive plasma and in the maintenance of syncytial integrity in the intervillous space as in vitro model in APS.
Methods: We evaluated the effect of heparin/HS /CD44v3-mediated processes during scratch wound closure in monolayer immortalized human trophoblast cells derived from term placenta (TCL-1).
Results: Western blot analysis showed that these cultured human trophoblast cells express CD44v3. *In vitro* scratch wound healing assay showed dose-dependent enhanced migration of trophoblast cells in the presence of heparin compared with control when culture under serum-free conditions. Conversely, an anti-CD44 function blocking antibody, CD44siRNA and LA positive-plasma suppressed the migration of cells in the presence of heparin in a similar scratch assay. Furthermore, both heparin treatment and *in vitro* scratch wounding induced the phosphorylation of p21-activated kinase 1 (PAK1), while the anti-CD44v3 antibody suppressed the heparin-induced phosphorylation of PAK1 in trophoblast cells.
Conclusion: These results suggest that heparin/HS /CD44v3-mediated signaling enhances the repair of the trophoblast cells layer damaged with mechanical scratching and LA.

P1.29

PLACENTAL RHO KINASE2 EXPRESSION IS SUPPRESSED IN PRE-ECLAMPSIA

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Objectives: Rho kinase (ROCK), including the ROCK1 and ROCK2 isoformes, belongs to a family of serine/threonine kinases, which is down stream effector of Rho A GTP ase. ROCK involves in a wide range of fundamental cellular functions, such as contraction, adhesion, migration and proliferations. Recent studies have shown that ROCK2 is localized in syncytiotrophoblast (ST) of the human placental villi in third trimester. Furthermore, it have been reported that ROCK inhibitor Y27632 inhibits prostaglandin E2 mediated migration of extravillous trophoblast (EVT) cell line, HTR-8/SV neo. ROCK2 might be an essential factor for EVT migration. Impaired trophoblast invasion and spiral arterial remodeling, which results in poor placentation during early pregnancy, leads to preeclampsia. We therefore have investigated the expression and significance of ROCK 2 in normal and preeclamptic placenta.
Methods: ROCK2 protein levels in villous tissue obtained from first, second, third trimester, and third trimester complained with preeclampsia were assessed by Western blot and immunohistochemistry (IHC).
Results: IHC demonstrated that ROCK2 expressed in cytotrophoblast (CT) in all pregnancy period, but not in ST. ROCK2 protein levels were not changed significantly with the period of pregnancy. But in preeclamptic placentas, ROCK2 protein levels were significantly lower compared to normal placenta.
Conclusion: These observations suggest that ROCK2 may subsequently develops preeclampsia.

P1.30

INHIBITION OF SFLT-1 INDUCTION CAUSED BY SCRATCHING IN THE HUMAN IMMORTALIZED TROPHOBLAST CELL LINES. (HTR-8 SV/NEO CELLS AND TCL-1 CELLS).

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Objective: It is known that enhanced production of sFLT1 is associated with fetal growth restriction and preeclampsia (PE). The production and secretion of sFLT1 are very important because it acts as a potent endogenous soluble inhibitor of VEGF- and PlGF-mediated various biological functions. However, it remains unknown how the release of sFLT1 are regulated into the intervillous space from the trophoblast cells.
Methods: We induced migration by scratch wounding in the human immortalized trophoblast cell lines (HTR-8/SV neo cells and TCL-1 cells) and examined the changes in sFLT1/FLT1/VEGF expression. The concentrations of supernatant were measured by ELISA. The expression and production of sFLT1 in cells were examined by Western blotting and real-time PCR. The changes were compared with controls in the presence or absence of scratching and serum in culture medium.
Results: In HTR-8 SV/neo cells, sFLT1 was detected in the membrane fraction, however, it was undetectable level in supernatant, regardless of the presence or absence of serum and scratching. In contrast, sFLT1 in the supernatant from TCL-1 cells was increased for 24 hours and decreased in the absence of serum in culture medium. In the presence of serum in culture medium, sFLT1 in the supernatant was suppressed and remained unchanged. The production of sFLT1 and the expression of sFLT1 mRNA in TCL-1 cells were not changed significantly in the presence or absence of scratching and serum by Western blot analysis and by real-time PCR, respectively.
Conclusion: These results suggest that the secretion of sFLT1 into the circulation is composed of production and release processes. Furthermore, the release process of sFLT1 from membrane is regulated on the cells membrane.

P1.31

HLA-G EXPRESSION IS REGULATED BY MIR-365 IN TROPHOBLAST UNDER HYPOXIC CONDITION

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Objectives: Hypoxia occurs during the development of placenta in the first trimester and is implicated in trophoblast differentiation. Intervillous blood flow increases after 10 weeks of gestation and results in exposure of trophoblast to oxygen. Before this time, low oxygen appears to prevent trophoblast differentiation toward an invasive phenotype. MicroRNAs (miRNA) are noncoding single-stranded RNAs modulating gene expression by targeting mRNA, which play a key role in various pathological and physiological discordants. We hypothesize that trophoblast in hypoxic condition have a unique miRNA profile, which may play a critical role in placental development.
Methods: Total RNA was extracted from human trophoblast cell line (HchEpC1b) exposed to normoxia or hypoxia (1% O2) for 24 hours. We first examined the miRNA expression profile using MERCURY LNA microRNA Array. Several differential miRNAs were selected and validated using real-time RT-PCR. We search potential targets of these miRNAs using in silico analysis (miRBLAST). We confirmed a potential target protein by western blot analysis and luciferase assays.
Results: We found that the expression of miR-365 was significantly higher under hypoxia. According to the results of in silico analysis, miR-365 targeted human leukocyte antigen G (HLA-G). Hypoxic condition and overexpression of miR-365 inhibited the HLA-G protein expression. Also, overexpression of miR-365 decreased the activity of luciferase-reporter containing the 3'-untranslated region (UTR) of HLA-G with the predicted miR-365-binding site.
Conclusion: HLA-G is a non-classical HLA class-Ib molecule expressed mainly in the extravillous cytotrophoblasts (EVT) and plays a key role in maintaining immune tolerance at the maternal-fetal interface. Our results indicate that miR-365 targets HLA-G 3'UTR to repress its expression. The expression of miR-365 may play an important role in human placental development and immunoprotection to the semiallogenic embryo.

P1.32

ACTIVATION OF INTERMEDIATE CONDUCTANCE CALCIUM ACTIVATED POTASSIUM CHANNELS (IK_{CA}) IMPAIRS CYTOTROPHOBLAST SYNCYTIALISATION *IN VITRO*

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Objectives: Regulation of syncytiotrophoblast (STB) renewal by cell migration, aggregation/fusion and differentiation is essential for successful pregnancy. In several tissues, cell migration/fusion is regulated by IK_{Ca}. We used cytotrophoblasts in primary culture to test the hypothesis that IK_{Ca} participate in the formation of multinucleate STB-like cells.
Methods: Cytotrophoblasts were isolated from normal term placentas (n=6) and cultured for 66h. This preparation recreates STB formation *in vivo*, as mononucleate cells (15h) fuse into multinucleate syncytia (66h) concomitant with elevated secretion of human chorionic gonadotropin (hCG). Cells were untreated (control) or treated at 3, 15 and 42h with IK_{Ca} inhibitor TRAM34 (10μM) or activator DCEBIO (100μM). At 15, 42 and 66h, culture medium was collected to measure hCG (mIU/ml/mg protein) and cells fixed for immunofluorescence with anti-desmoplakin and anti-IK_{Ca} antibodies to assess multinucleation (syncytial nuclei as % of total nuclei) and IK_{Ca} expression respectively. Data are median+/-IQR.
Results: Cytotrophoblast multinucleation increased 11-fold ($p<0.05$), and hCG secretion 45-fold ($p<0.05$), between 15 and 66h. Compared to controls at 66hr, DCEBIO reduced multinucleation by 50% (26.6 30/16 vs. 13.8 17/9%; $p<0.05$) and hCG secretion by 95% (2497 4030/448 vs. 145 401/80; $p=0.03$). This inhibition of differentiation was not caused by cytotoxicity as total nuclei and cell protein with DCEBIO did not differ from controls. TRAM34 did not affect cytotrophoblast multinucleation or hCG secretion. IK_{Ca} staining was evident in the nucleus, cytoplasm and surface of mono- and multinucleate cells.
Conclusion: Activation of IK_{Ca} inhibits formation of multinucleate cytotrophoblasts *in vitro*. We reported previously that cytotrophoblast IK_{Ca} are activated by peroxynitrite (1). As pre-eclampsia is associated with nitrative stress, we speculate that activation of IK_{Ca} through nitration could contribute to dysregulated STB renewal in this pregnancy complication.
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P1.33

ANALYSIS OF THE GALECTIN 4 EXPRESSION DURING TROPHOBLAST DIFFERENTIATION IN RAT PLACENTATION

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Objectives: Galectins comprise a family of proteins which shares binding affinity for b-galactosides. Recently, it has been suggested that some members of Galectin family have regulatory roles in the differentiation of trophoblast cells. In order to understand how Galectins are involved in the trophoblast differentiation, we have analysed the expression of Galectins in the rat placental cell line Rcho-1 and in the rat placenta.
Methods: The differentiation of Rcho-1 cells was induced by exchanging the growth medium into low-nutritional medium when the cells were reached to confluent. The expression of mRNA was analysed by DNA micoarray and RT-PCR. The distribution of Galectin 4 in the rat placenta was analysed by immunohistochemistry.
Results: The DNA microarray analysis revealed that *Galectins 1, 3, 4, 5, 7, 8, 9 and 12* were expressed in Rcho-1. Of these, the expression of *Galectin 4* was down-regulated during the differentiation of Rcho-1 cells, and the down-regulation began within 24 hrs after the induction of differentiation. Once the expression of *Galectin 4* was inhibited, it did not recover even if the cells were returned back into the growth medium. Also, the expression of *Galectin 4* was not affected in HT29, the colon cancer cell line, cultured with the low-nutritional medium. These results suggest that the down-regulation of *Galectin 4* expression is specifically associated with the differentiation of Rcho-1 cells. During rat placentation, we have detected the Galectin 4 protein in the decidua, trophoblast giant cells and spongiotrophoblasts.
Conclusion: Galectin 4 had been thought to have roles specifically in the epithelial cells of digestive tract. However, the results of present study suggested that Galectin 4 is also associated with the differentiation of trophoblasts during rat placentation.

P1.34

REGULATION OF CCN1 AND CCN3 IN AN *IN VITRO* CO-CULTURE MODEL OF HUMAN TROPHOBLAST AND ENDOMETRIAL STROMAL CELLS

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Objectives: The matricellular proteins CCN1 and CCN3 are expressed in extravillous trophoblast cells invading the maternal decidua. For successful placentation, a balance of invasion and proliferation is crucial since proliferation of trophoblast cells provides a continuous supply of invasive cells. Pathologies like preeclampsia coincide with hypoinvasion of trophoblast into the decidua. Our finding that the levels of both proteins were decreased in early-onset preeclamptic placentas underlines the importance of CCN regulation. Here we focus on the role of CCN1 and CCN3 in migration and proliferation properties of trophoblast cells into the maternal decidua using an *in vitro* co-culture model.
Methods: To mimic the stromal compartment the human endometrial stromal cell line T-HESC has been decidualized *in vitro* by a combination of inducers (cAMP, MPA, E2). For the invasive extravillous trophoblast we use the benign trophoblast cell line SGHPL-5, originated from first-trimester placental tissue.
Results: *In vitro* decidualized T-HESCs showed an upregulation of the typical marker genes prolactin and IGFBP-1. Undifferentiated as well as decidualized T-HESCs express both CCN1 and CCN3, but only CCN1 levels increased upon the decidualization process. SGHPL-5 cells also strongly express CCN1 and to a lesser extent CCN3. Thus, both partners exhibit predominantly CCN1. Co-culturing SGHPL-5 with T-HESCs revealed migration of single trophoblast cells into the border of confluent T-HESCs. To test proliferation and migration properties upon elevated CCN3 levels we first cultured only SGHPL-5 in the presence of recombinant human CCN3. To our surprise SGHPL-5 then showed a significantly reduced proliferation. These results point out that CCN3 levels may regulate the proliferation capacity of extravillous trophoblast which is therefore reduced in the SGHPL-5 cell line.
Conclusion: The two cell lines represent an excellent tool to manipulate CCN expression levels and to study the roles of CCN1 and CCN3 for proliferation, invasion and migration properties of trophoblast cells into the maternal compartment. In this matter CCN3 seems to reduce proliferation of SGHPL-5, possibly to shift the trophoblast into the invasive phenotype.

P1.35

EFFECTS OF AHR ON PROLIFERATION AND MIGRATION OF HUMAN TROPHOBLAST CELL LINES

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Objectives: Aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, mediates a variety of biological processes including successful pregnancy establishment, vascular formation, and immune system homeostasis. Recently, we have reported that AhR protein is highly expressed in human placentas, and primarily presented in syncytiotrophoblasts within anchoring villi. However, the functional roles of AhR in the trophoblost are still unknown.
Methods: In this study, both mRNA and protein expression levels of AhR in human trophoblast cell lines JEG-3 and JAR were examined by RT-PCR and Western blotting, respectively. Inducibility of CYP1A1 by the AhR endogenous ligand [2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) was detected using RT-PCR. Cell proliferation and migration in response to ITE treatment were determined by crystal violet and Transwell chamber assays, respectively. Also, flow cytometry was used to determine cell cycle phase distribution.
Results: AhR is moderately expressed in JEG-3 cells but is undetectable in JAR cells on both protein and mRNA levels. ITE time-dependently ($p<0.05$) induced CYP1A1 expression in JEG-3 cells, but not in JAR cells. ITE significantly ($p<0.05$) inhibited JEG-3 cell proliferation in a dose-dependent manner characterized by S cell cycle phase arrest, but did not change JAR cell proliferation as well as cell cycle phase distribution. Furthermore, no changes were observed on JEG-3 and JAR cells migration in response to ITE treatment.
Conclusion: ITE significantly inhibited human trophoblast cell proliferation by arresting the cell cycle at the S phase. This inhibitory effect is mediated via AhR activation. These findings suggest that AhR might play a critical role in mediating trophoblast functions, particularly in the cell proliferation. Thus, ITE can potentially be used for therapeutic intervention of human placental choriocarcinoma.

P1.36

NOTCH-DEPENDENT RBPJκ IS A CRUCIAL TRANSCRIPTION FACTOR IN TROPHOBLAST INVASION

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Objective: Canonical Notch signalling is fundamental in cell fate decision and differentiation of several cell types and has recently been shown to play a critical role in murine trophoblast invasion and vessel remodelling. However, the role of this conserved pathway and its central transcription factor, Recombining Binding Protein-Jκ (RBPJκ), in differentiation and biological function of invasive human trophoblasts has not been elucidated. Hence, placental distribution pattern of RBPJκ and its co-activators, the Mastermind-like proteins (MAMLs), as well as the role of RBPJκ in trophoblast proliferation and invasion was investigated.
Methods: To study expression and distribution of RBPJκ and MAMLs in different trophoblast subtypes, immunofluorescence of first trimester human placentae and decidual tissues were performed. Gene silencing of RBPJκ was accomplished in trophoblastic SGHPL-5 cells after transfection of siRNA. Protein and mRNA were isolated from cultures and analyzed by Western blotting and quantitative RT-PCR, respectively. Fibronectin-coated transwells were used in invasion assays. Proliferation was measured by analyzing cumulative cell numbers.
Results: Immunofluorescence of tissues revealed ubiquitous expression of RBPJκ in decidual cells and all trophoblast subtypes except the syncytium. Similarly, MAML 2 and MAML3 were present in villous and extravillous cytotrophoblasts but decreased during trophoblast cell fusion. MAML1 was less detectable in placenta and decidua. Lipofection of siRNA against RBPJκ completely abolished its protein expression within 16 hours. Even 5 days after treatment RBPJκ remained undetectable suggesting efficient, long-term gene silencing. Realtime-time PCR revealed changes in mRNA expression of the canonical Notch target HES1 upon RBPJκ gene silencing. Compared to controls, invasion of RBPJκ knock-down cells decreased to 40%, but proliferation was not affected.
Conclusion: Ubiquitous expression of RBPJκ and MAML proteins in cytotrophoblasts suggest that Notch signalling could play a role in trophoblast maintenance and differentiation into the invasive lineage, whereas down-regulation of these proteins could promote syncytialization. Furthermore, RBPJκ has been identified as a novel, positive regulator of trophoblast invasion.

P1.37

GCM1 ALTERS THE DIFFERENTIATION PATTERN OF EQUINE CHORIONIC GIRDLE TROPHOBLAST CELLS

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Objectives: Around day 30-31 of equine pregnancy, rapidly proliferating uninucleate trophoblast cells of the chorionic girdle begin to differentiate into specialised binucleate cells. Similarly to human syncytiotrophoblast cells, equine binucleate trophoblast cells secrete Chorionic Gonadotrophin (CG) and, in the horse, this is dependent on binucleate trophoblast differentiation. We recently showed the transcription factor Glial Cells Missing 1 (GCM1) is rapidly induced *in vivo* during differentiation of equine binucleate trophoblast. The aim of this study was to determine GCM1 function during *in vitro* differentiation of binucleate trophoblast.
Methods: Pure populations of chorionic girdle trophoblast cells were isolated from day 31-35 equine conceptuses. To induce GCM1 expression, cells were grown in the presence/absence of 25-100 μM forskolin (0.02% DMSO) for up to 72h. Differentiation was determined following labelling of the cells with CellTrace™ BODIPY® TR methyl ester and the nuclear stain Hoeschst. The number of uni-, bi- and multinucleated cells was quantified in 5 randomly selected fields under an inverted fluorescent microscope. GCM1 and eCGβ mRNA expression was determined by qRT-PCR. The concentration of eCG was determined using an eCG enzyme linked immunoassay.
Results: In contrast to untreated/vehicle treated cells, qRT-PCR experiments confirmed an induction of both GCM1 and eCGβ mRNA at higher concentrations of forskolin treatment (75-100 μM), despite a total absence of eCG secretion determined by immunoassay. Forskolin significantly decreased total cell number at 50μM ($p<0.05$), 75 and 100μM ($p<0.001$) and resulted in a 4-fold increase ($p<0.01$) in multinucleated cells. In contrast to the heterogenous population of uni- and binucleate cells in control cells, forskolin induced differentiation that occurred either directly around the edge of a uninucleate cluster (25μM) or in discrete zones ($\geq$50μM).
Conclusion: In conclusion, forskolin treatment induced GCM1 mRNA expression, either inhibited cell proliferation or promoted cell apoptosis and resulted in an altered differentiation pattern of equine chorionic girdle trophoblast cells.

P1.38

DETECTION AND ANALYSIS OF THE ROLE OF GALECTINS IN TROPHOBLAST FUNCTION, DIFFERENTIATION AND SIGNAL TRANSDUCTION

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Objectives: Galectins are lectins with the ability to bind β-galactosides through a conserved carbohydrate recognition domain. Galectins exert their biological effects by binding galactose containing glycan ligands on proteins involved in cell adhesion and growth regulation. Within this study we investigated the expression and function of galectins in normal, abortive, preeclamptic and HELLP placental tissue. In addition, *in vitro* fusion and signal transduction experiments were performed.
Methods: Frozen and paraffin embedded placental tissue of patients with single and recurrent abortion, preeclamptic patients, HELLP patients and normal first trimester and term placentas were obtained from the Department of Obst. & Gyn. and used for this study. For immunohistochemical analyses, tissue slides were incubated with monoclonal and polyclonal antibodies against gal-1, gal-2, gal-3, gal-4, gal-7,gal-8, gal-9, gal-10 and gal-12. The mRNA was isolated from frozen material and real-time RT-PCR was performed for quantification of the same set of galectins. The role of gal-1 in trophoblast cell fusion and signal transduction was analysed by immunocytochemistry and phosphorylation array technology.
Results: Gal-1 and gal-3 were up regulated EVT in preeclamptic placentas. Gal-1 is significantly up regulated in decidual tissue of preeclamptic placentas and villous trophoblast of HELLP placentas. Gal-1 and gal-9 expression is upregulated and gal-2 expression is down regulated in abortive decidual tissue. Gal-2, gal-4, gal-7, gal-10 and gal-12 are down-regulated in abortive syncytiotrophoblast. Gal-1 stimulates the syncytium formation in choriocarcinoma cells BeWo and primary trophoblast cells *in vitro.* In addition, gal-1 influenced the activity of receptor tyrosine kinases and mitogen-activated protein kinases in BeWo cells *in vitro*.
Conclusion: A total of 9 human galectins as galactose binding lectins were investigated in this study and were found to be expressed in human placental tissue. In addition, we demonstrated that gal-1 induces cell differentiation processes on BeWo cells, including RTKs

P1.39

KISSPEPTIN INHIBITS CELL MIGRATION IN HUMAN TROPHOBLASTS VIA GPR54

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Objectives: To determine the signalling pathways and gene expression induced by Kisspeptin (KP) and its receptor GPR54 in first trimester human trophoblast primary cultures.
Methods: Trophoblasts were isolated from first trimester elective terminations using a Percoll gradient. RNA was extracted using TRIZOL (Invitrogen). Transcript expression was determined by real-time PCR (RT-PCR). Protein expression was assessed by Immunocytochemistry (ICC) and Western Blot. Receptor activity was determined by Inositol Phosphate (IP) assay. Wound-healing assay was performed to assess migration capacity.
Results: Trophoblasts were isolated and expression of Cytokeratin-7, a positive marker of trophoblasts, was determined by ICC. Trophoblasts express higher amounts of GPR54 than the immortalized trophoblast cell line HTR8SVneo. When treated with KP, ERK1/2 becomes phosphorylated. This demonstrates that the receptor can be activated. Surprisingly, no IP response was observed. Treatment of trophoblasts with KP reduces the ability of these cells to migrate in a wound-healing assay. Employing RT-PCR we have determined that KP treatment reduces the expression of Matrix Metalloproteinase (MMP) 3, 9 and 10 and it increases expression of Tissue Inhibitor of Metalloproteinase (TIMP) 1 and 3. ERK1/2 phosphorylation, migration and gene expression regulation are specific to GPR54, given that treatment with p356, a GPR54 antagonist, can revert all of these effects.
Conclusion: These results confirm that KP can inhibit trophoblast migration and demonstrate that primary cultures of placenta are the best model to study the molecular mechanisms underlying trophoblast invasion. We plan to perform a microarray to discover new genes regulated by KP treatment in trophoblasts.

P1.40

THE EXPRESSION OF HISTONE VARAINT H2AFZ IN THE DEVELOPING MOUSE PLACENTA

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Objectives: The dynamic incorporation and expulsion of histone variants from chromatin along with chromatin remodeler activity can establish both permissive and repressive chromatin environments. The role of histone variant H2afz in this phenomenon is particularly interesting due to its localisation at both transcriptional start sites and in heterochromatin. This implies that H2afz is a master controller of chromatin mediated transcriptional regulation and assists in mediating cell plasticity. Interestingly, in mouse blastocysts, H2afz is present only in the trophectoderm and to date; the potential role of H2afz in the extra-embryonic tissue development has not been investigated. We aimed to investigate the expression of *H2afz* during mouse placentation, hypothesising that *H2afz* is limited to uncommitted trophoblast cells and may be involved in controlling trophoblast differentiation.
Methods: To investigate *H2afz* expression *in vivo*, we performed *in situ* hybridizations with DIG-labelled *H2afz* probes on mouse placental sections ranging from embryonic day (E)6.5 to E18.5. To investigate the expression patterns of *H2afz* during trophoblast differentiation *in vitro*, qRT-PCR and western immunoblotting was performed on undifferentiated and differentiating trophoblast stem (TS) cells.
Results: *In situ* hybridizations showed that *H2afz* was widely expressed in proliferating, progenitor cell populations within the ecto-placental cone and chorion (E7.5 – E10.5). As mouse placentation continued, *H2afz* became restricted to the labyrinth in small clusters of cells that did not exhibit any morphological characteristics of committed trophoblast cells (E14.5 to E18.5) ($n \geq 3$). qRT-PCR revealed *H2afz* was expressed in undifferentiated TS cells *in vitro* and that levels declined following differentiation (ANOVA, $P < 0.05$, $n \geq 3$). Western immunoblotting confirmed these results (qualitative data, $n \geq 3$).
Conclusion: This study is the first to demonstrate that *H2afz* is expressed in the mouse placenta and is limited to progenitor-like trophoblast cells. This data lends support to the hypothesis that H2afz may be involved in the control of differentiation in mouse placentation.

P1.41

SYNCYTIAL FORMATION REDUCES EXOSOME DEPORTATION FROM TROPHOBLAST CELLS

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Objective: Exosomes are released from human trophoblast cells and are involved in diverse biological functions, such as cell-cell communication, immunological response and carry a variety of proteins as mRNA and microRNA. The aim of this study was to determine the effect of syncytial formation on the release of exosomes from trophoblast cells.
Methods: An *in vitro,* two–sample cell culture cells experimental design was used to characterize the release of exosomes from trophoblast cells. Trophoblast cells were purified from term placenta ($n=2$) using a trypsin-deoxyribonuclease-dispase/Percoll method and characterized by specific markers anticytokeratin-7 and antivimentin. Trophoblast cells were cultured (in duplicate) for 4 days (DMEM, 1% FCS 5% CO_2 in T75 flasks) and 10 days for syncytiotrophoblast. Cells were washed and then incubated in FCS-free medium for 48 hours. The supernatant was collected and exosomes were isolated using a commercially available kit (ExoQuick™, System Biosciences, Mountain View, CA) according to manufacturers instructions and characterized by flow cytometry and Western blot using exosome markers (CD63, CD81 and CD9). Finally, the exosome pellet was resuspended in RIPA buffer and protein content determined. Exosome release was expressed as the ratio of exosomal protein/total cell protein.
Result: The protein content of the exosomes determined by flow cytometry and Western blot showed a positive population and the presence of CD63, CD81 and CD9, respectively. The release of exosomes from trophoblast cells incubated *in vitro* was 626-μg/48 h. Following syncytialisation, exosome release was 115 μg/48 h. When normalized for total cell protein, exosome release was 3.6 fold higher in trophoblast cells than following syncytialisation.
Conclusions: The role of exosomes during normal and pathological pregnancies has yet to be clearly established, however, it is tempting to speculate that during normal pregnancy the syncytium limits the deportation of exosomes that may cause maternal endothelial cell damage.

P1.42

REGULATION OF CHORIONIC GIRDLE TROPHOBLAST DIFFERENTIATION BY TGFβ SIGNALLING PATHWAYS

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Objectives: The chorionic girdle (CG) is a discrete annular structure of the early equine conceptus that gives rise to the endometrial cups. Beginning at around day 31 of pregnancy, the rapidly proliferating trophoblast cells of the CG terminally differentiate into eCG-secreting binucleate trophoblast. This study investigated the role of TGFβ signalling molecules in terminal differentiation of binucleate trophoblast.
Methods: CG and chorion tissue was isolated from day 30 to 35 equine conceptuses by established methods. A 44K gene probe equine expression array and RT-PCR was used to compare Type I and Type II serine/threonine kinase and accessory receptor expression between Day 34 CG and chorion tissue. Cultured CG cells were supplemented with 1-100 ng/ml human BMP4 or PBS/BSA as a control for up to 10 days. Differentiation was determined following labelling of the cells with CellTrace™ BODIPY® TR methyl ester and the nuclear stain Hoeschst. The number of uni-, bi- and multinucleated cells was quantified in 5 randomly selected fields under an inverted fluorescent microscope. The concentration of eCG was determined using an enzyme linked immunoassay.
Results: TGFβ receptor expression in the CG was tightly regulated. CG preferentially expressed Type I, Type II and accessory receptors, Bone Morphogenetic Protein Receptor Type 1A (ALK3), Bone Morphogenetic Protein Receptor 2 (BMPR-II), Dragon and Bambi that bind the ligand BMP4. ALK1, ALK5, ALK6, ActRIIB, endoglin and betaglycan mRNA was not detected in the CG. Stimulation of chorionic girdle cells with 1-100 ng/ml BMP4 for 4 days resulted in up to a 2-fold increase in percentage of binucleate cells ($p<0.001$). Furthermore, stimulation of cultured CG cells with BMP4 for 10 days resulted in an increase in eCG concentration.
Conclusion: Our findings support a role for TGFβ signalling in regulation of chorionic girdle differentiation via BMP4 binding to BMPR-II, ALK3, Dragon and Bambi.

P1.43

DOES PHOSPHOLIPID SCRAMBLASE 1 PLAY A ROLE IN VILLOUS TROPHOBLAST FUSION?

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Objectives: Villous cytotrophoblast fusion with the overlying syncytiotrophoblast layer is thoroughly coordinated by a set of factors. One of the crucial aspects for successful fusion is the transient deregulation of regular membrane asymmetry leading to externalization of phosphatidylserine to the outer membrane leaflet. Initial studies revealed phospholipid scramblase 1 (PLSCR1) to be the strongest expressed enzyme among all transporters involved in regulation of membrane architecture in the trophoblast. The aims of this study were to analyze the spatio-temporal expression of PLSCR1 in the placenta and to further elucidate its putative role in syncytialization.
Methods: Immunostaining for PLSCR1 was performed using placental tissues of different gestational ages and the trophoblast fusion model BeWo. Expression of PLSCR1 was analyzed in BeWo cells by Western Blot and qPCR. RNA interference approach and an inhibitor for scramblases (R5421, ethaninidothioic acid) were used to define the functional role of PLSCR1 in forskolin induced BeWo cell fusion. Fusion efficiency was evaluated and correlated with beta-hCG.
Results: While PLSCR1 was strongly expressed in syncytiotrophoblast, only slight staining was detected in villous cytotrophoblasts. In BeWo cells no significant changes in PLSCR1 mRNA and protein expression were observed during cell fusion. At the same time, a subset of specific BeWo syncytia showed double immunostaining for beta-hCG and PLSCR1. Incubation with scramblase inhibitor R5421 or siRNA mediated knockdown of PLSCR1 had no significant effect on forskolin induced beta-hCG secretion and fusion of BeWo cells.
Conclusion: Although PLSCR1 is located at the right place, i.e. the villous trophoblast, cell culture experiments so far do not support a direct involvement of PLSCR1 in trophoblast fusion. However, we cannot exclude a role of PLSCR1 in the fusion process since it may well be effective on the syncytial rather than the cytotrophoblast side of the fusion process.

P1.44

ROLE OF MAPKS PATHWAY IN TROPHOBLAST DIFFERENTIATION REGULATED BY HYPOXIA OR PPAR

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OBJECTIVES, METHODS, RESULTS, CONCLUSION

Objectives: A successful mammalian pregnancy depends on two major steps in placental development that are related to trophoblast differentiation. First, the differentiation of extravillous cytotrophoblast, responsible for the invasion of maternal uterine stroma. Second, the increased level of maternal blood flow to the placenta is associated with increased nutrients, gazes and waste exchange between the mother and the foetus. This latter function is associated with the differentiation of specific structure: the syncytiotrophblast (STB) which arises through the differentiation and fusion of the relatively undifferentiated mitotically active villous cytotrophoblast (CTB). Mechanisms that control syncytiotrophoblast differentiation are still poorly understood. We have previously shown the implication of mitogen-activated protein kinases (MAPK) and Src family kinases (SFK) in trophoblast differentiation. Moreover, we and others showed that hypoxia and peroxisome proliferator-activated receptor (PPAR) pathways play a critical role in trophoblast differentiation. The aim of this study is to elucidate the implication of MAPKs pathways in the regulation of trophoblast differentiation either by hypoxia or PPAR ligands.
Methods: To address this, we are using a placental cell line (BeWo) capable of differentiating from a CTB phenotype to a STB phenotype *in vitro*. The role of MAPK pathway will be evaluated using specific MAPK inhibitors, transfection assays, SiRNA and reporter assays.
Results: These experiments are underway now and our preliminary data suggest that ERK and p38 modulate how hypoxia and PPAR regulate trophoblast differentiation.
Conclusion: The accomplishment of this work has implication in pregnancy related diseases such as pre-eclampsia and intra uterine growth retardation (IUGR).

P1.45

IS SYSTEM ASC OR SYSTEM XC^- MEDIATING GLUTAMATE EXCHANGE ACROSS THE HUMAN PLACENTAL SYNCYTIOTROPHOBLAST INTO THE FETAL CIRCULATION?

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Objectives: Glutamate concentrations within the placental syncytiotrophoblast are high at around 5 mmol/l but the role of this glutamate within the cell is uncertain. Glutamate efflux from the syncytiotrophoblast has been observed but the mechanism behind this is unclear. This study aimed to investigate whether glutamate efflux from the syncytiotrophoblast is mediated by amino acid exchange transporters.
Methods: Ethical approval for this study was granted by the Southampton and Southwest Hampshire Regional Ethics Committee. Human placentas were collected following uncomplicated term pregnancies. Isolated perfused human cotyledons ($n = 10$) were perfused with Earle's bicarbonate buffer. ^{3}H-proline, ^{14}C-glutamate and 1.8 mM creatinine were perfused into the maternal arterial circulation. Boluses (16 μmol) of glutamate, aspartate, alanine, serine and N-acetylcysteine (NAC) were injected into the fetal circulation to stimulate exchange. A 0.1 mol maternal bolus of NAC was also administered. Radioactivity in maternal and fetal venous samples collected were determined via liquid scintillation counting and creatinine levels were determined using an enzymatic assay. Western Blotting ($n = 5$ blots, 45 μg basal membrane (BM) and microvillous membrane (MVM) protein) and PCR was used to identify whether Xc^- is expressed on the BM or MVM.
Results: In the fetal circulation, a bolus of glutamate stimulated release of glutamate but not proline. No other substrates stimulated release of glutamate or proline in the fetal circulation. In the maternal circulation the bolus of NAC stimulated release of glutamate from the placenta consistent with activity of the amino acid exchanger Xc^-.
Conclusion: This study demonstrated amino acid exchange for glutamate, but not for proline, in both the maternal and fetal circulations. Exchange into the maternal circulation is likely to be mediated via Xc^-. These results suggest that a high concentration of glutamate in the placenta creates a gradient driving cystine uptake for glutathione synthesis.

P1.46

TRANSPLACENTAL TRANSFER OF URIC ACID

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Objectives: Serum uric acid (UA) concentrations are grossly elevated in pregnancy-induced hypertension (PIH) and twin pregnancy. Hyperuricemia is one of the characteristic findings in PIH. In clinical practice, UA determination is considered to be a part of the course in women with PIH to monitor disease severity and aid management of these women. However, UA handling in placenta has not been extensively evaluated.
Methods: UA levels of maternal blood, umbilical venous and arterial blood on cesarean section in 50 normal singleton pregnancies, 21 twin pregnancies, and 13 singleton pregnancies with PIH were measured. Primary cultured trophoblasts were established from the villous tissues from patients underwent legal, elective termination of pregnancy. All patients gave informed consent for collection and investigational use of tissues. This study protocol was approved by ethics committee of Kyorin University. To clarify transportation of UA in placenta, RT-PCR, immunohistochemistry and [14C] UA uptake of p cultured trophoblasts and BeWo cells were performed.
Results: UA levels were virtually identical among maternal blood, umbilical artery and vein, suggesting that UA can pass through blood placental barrier. Among possible UA transporters, OAT4, OAT10, URATv1, and ABCG2 were identified in placenta by both RT-PCR and immunohistochemistry. Surprisingly, [14C]UA was not taken up in primary trophoblasts and BeWo cells, which expressed same sets of UA transporters in placenta, To clarify paracellular transport of UA via basolateral and apical transport, BeWo monolayers were utilized. BeWo monolayers showed that [14C]UA, was transported bidirectionally at 4°C to basolateral and apical side using double chamber system.
Conclusions: Taken together, there was no active transport of UA in trophoblasts and BeWo cells. These data suggest that there was no active transport of UA in trophoblasts and BeWo cells, and that paracellular route is a major pathway of UA across blood placental barrier.

P1.47

PLACENTAL TAURINE TRANSPORT IS REDUCED IN FIRST TRIMESTER IN WOMEN WITH RAISED BMI

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Objectives: The prevalence of maternal obesity is rising and increases the risk of pre-eclampsia, stillbirth and abnormal fetal growth. These complications are associated with altered placental development and function. Syncytiotrophoblast taurine transporter (TauT) activity is lower in women with raised body mass index (BMI) than women of ideal weight at term (Ditchfield et al 2010). In vitro knockdown of TauT in trophoblast cells compromises their differentiation (Parsons et al 2009) and increases cytokine-induced apoptosis (Desforges et al 2010). It is possible that reduced syncytiotrophoblast TauT activity in early gestation links raised BMI to placental pathology. We tested the hypothesis that syncytiotrophoblast TauT activity and expression is reduced in women with raised BMI in the first trimester.
Methods: Placentas were collected from elective medical or surgical terminations of pregnancy (7-12 weeks gestation) and women grouped as ideal weight (BMI 18.5-24.9), overweight (25-29.9) or obese (>30). Syncytiotrophoblast TauT activity was measured as Na^{+}-dependent uptake of ^{3}H-taurine into villous fragments over 90minutes at 37C. TauT protein intensity in syncytiotrophoblast was scored by two independent blinded observers following detection by immunohistochemistry.
Results: TauT activity in ideal weight (n=3), overweight (n=3), and obese (n=4) women was 2984-4572, 1787-2474, 1208-2291 fmol/mg protein/90min respectively. TauT activity was significantly lower in obese than ideal weight women ($p<0.05$, Kruskal-Wallis; Dunn's post hoc test), negatively related to maternal BMI (Figure 1), but unaffected by gestation. Comparison of syncytiotrophoblast TauT staining intensity in ideal weight (n=20), overweight (n=10) and obese women (n=11) revealed no significant difference.

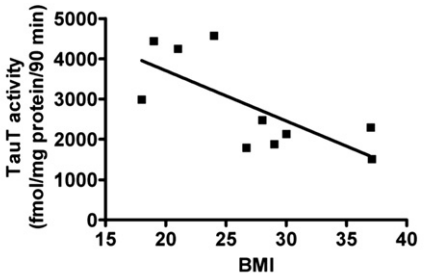

Figure 1. Negative relationship between placental TauT activity and maternal BMI in the first trimester. Least squares linear regression; $r^2 = 0.516$; $p<0.02$.

Conclusion: Raised maternal BMI is associated with reduced placental TauT activity, but not expression, in the first trimester. This could increase syncytiotrophoblast susceptibility to damage by inflammatory cytokines that are elevated in maternal obesity, predisposing to development of pathology as pregnancy progresses. The cause of reduced placental TauT activity in maternal obesity requires further investigation.

P1.48

CHARACTERIZATION OF SODIUM DEPENDENT BETAINE UPTAKE IN HUMAN PLACENTAL BRUSH-BORDER MEMBRANES

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Objectives: The principal physiological role of betaine is as an osmolyte and methyl donor and thereby helps to protect fetus and placenta against osmotic stress and hypomethylation. The uptake system of betaine from the maternal blood in placental brush-border membranes is a key step for supplying betaine to the fetus. The purpose of this study is to clarify the transport mechanism of betaine in human placental brush-border membranes.
Methods: Brush-border membrane vesicles (BBMVs) were isolated from human term placenta using magnesium precipitation technique. The uptake of [14C]betaine was characterized using human placental BBMVs and rat placental TR-TBT 18d-1 cells. The protein expression level in cells was evaluated by western blot and immunocytochemical analyses.
Results: A transient overshoot phenomenon of [14C]betaine uptake by human placental BBMVs was observed in the presence of inwardly directed gradients of sodium, suggesting the presence of sodium-dependent betaine uptake process(es). The uptake of [14C]betaine by TR-TBT 18d-1 cells was significantly inhibited by 2-methylaminoisobutyric acid (MeAIB), a specific substrate of system A sodium-dependent neutral amino acid transporters (SNAT), but not by GABA, a substrate of sodium-dependent betaine/GABA transporter (BGT)-1/SLC6A12. Enhanced expression of SNAT2/SLC38A2 protein under hypertonic conditions was observed in TR-TBT 18d-1 cells and was accompanied by an increase in the MeAIB-sensitive uptake of [14C]betaine. The initial uptake rate of [14C] betaine in human SNAT2-tranfected cells was greater than that in mock-transfected cells.
Conclusion: Human SNAT2 recognizes betaine as a substrate and appears to be involved in osmosensitive transport of betaine in placental brush-border membranes.

Reference

Nishimura et al., Placenta 31, 1003-1009 (2010).

P1.49

EXAMINING THE ROLE OF SLC10A3 IN THE PLACENTA

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Objectives: SLC10A3 is an orphan transporter belonging to sodium/bile acid cotransporter (SLC10A) family. The observation that SLC10A3 is most abundantly expressed in the placenta warrants vigorous investigation on placental SLC10A3 for better understanding of its potential role. The purpose of this study is to examine the cellular and subcellular localizations and function of SLC10A3.
Methods: Placental brush-border membranes were isolated from human term and rat GD19 placenta by magnesium precipitation technique and subjected immunoblot analysis. Rat *Slc10a3*-transfected cells were used for examining taurocholic acid (TCA) uptake activity and subcellular localization of Slc10a3. The cellular localization of Slc10a3 was determined by immunohistochemical analysis using rat placental frozen sections.
Results: SLC10A3 protein was detected in human and rat placental homogenate, but the expression level of SLC10A3 protein in placental brush-border membranes was low compared with placental homogenate. In rat placental sections, immunostaining of Slc10a3 was observed in the trophoblast layer close to that of P-glycoprotein, which is known to be expressed in the brush-border membrane of the syncytiotrophoblast, but not overlapped that of CD31, an endothelial cell marker. In rat Slc10a3-transfected cells, Slc10a3 immunoreactivity was intense at the cytoplasm and colocalized with mitochondria and ER markers, but present to a lesser extent at the plasma membrane. No signal was seen in the mock-transfected cells. Rat *Slc10a3*-transfected cells exhibited no significant increase in TCA uptake compared to mock-transfected cells.
Conclusion: Placental SLC10A3 appears to be localized in the trophoblast layer, except for its brush-border membrane. SLC10A3 appears to be distributed mainly in intracellular compartments rather than plasma membranes, which might cause no significant effect on cellular uptake of bile acids.

P1.50

ROLE OF ORGANIC ANION TRANSPORTER (OAT) 4 ON ESTRIOL SYNTHESIS IN THE PLACENTAL SYNCYTIOTROPHOBLASTS

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Objectives: Estriol (E_3) is synthesized from fetal-derived 16α-hydroxy dehydroepiandrosterone sulfate (16α-OH DHEAS) in the syncytiotrophoblast constituting the placental barrier. Therefore, placental syncytiotrophoblasts must be equipped with 16α-OH DHEAS transporter(s) at the basolateral (fetal side) membrane for E_3 synthesis. The purpose of this study is to clarify transporter mechanism of 16α-OH DHEAS at the basolateral membrane of the placental syncytiotrophoblasts.
Methods: [^{3}H]16α-OH DHEAS was converted from [^{3}H]DHEAS using recombinant human CYP3A7 and subjected to the cellular uptake study. The secretion of E_3 from cells was measured by ELISA.
Results: The uptake of [^{3}H]16α-OH DHEAS by forskolin-induced differentiated JEG-3 human choriocarcinoma cells, used as model cells of the placental syncytiotrophoblasts, was significantly inhibited by 1 mM DHEAS, estrone sulfate (E_1S), and bromosulfophthalein (BSP), but not by tetraethylammonium (TEA), suggesting the involvement of organic anion transport system on 16α-OH DHEAS uptake. The secretion of E_3 from differentiated JEG-3 cells was detected by incubating with 16α-OH DHEAS for 8 hrs but is also inhibited by co-incubating with 50 μM BSP. Organic anion transporter (OAT) 4 (SLC22A11) and organic anion transporting polypeptide (OATP) 2B1 are known to be present at the basolateral membrane of the placental syncytiotrophoblasts. Human OAT4-transfected COS-7 cells exhibited [^{3}H]16α-OH DHEAS uptake activity with a K_m of 7.4 μM, and moreover, this uptake was also inhibited by DHEAS, E_1S, and BSP, but not by TEA. On the other hand, the OATP2B1-mediated uptake of [^{3}H]16α-OH DHEAS was not observed.
Conclusion: OAT4 appears to play a principal role in the uptake of 16α-OH DHEAS and contribute to the E_3 synthesis during pregnancy.

P1.51

INCREASED PLACENTAL NITRATION IS ASSOCIATED WITH REDUCED TAURINE TRANSPORTER ACTIVITY

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Objectives: Pre-eclampsia (PE) is associated with increased nitrative stress, abnormal syncytiotrophoblast renewal and fetal growth restriction. We have shown that syncytiotrophoblast taurine transporter (TauT) activity is significantly lower in PE than normal pregnancy (NP) (1). Nitrative stress alters protein function by nitration of tyrosine residues. We propose that post-translational modification of TauT through tyrosine nitration could down-regulate transporter activity in PE. We tested the hypothesis that nitration is higher in PE placentas, previously shown to have reduced TauT activity, than in NP and inducing placental nitrative stress reduces TauT activity.
Methods: Placentas were collected from NP and PE (blood pressure >140/90mmHg after 20 weeks gestation in previously normotensive women plus proteinuria >300 mg/L in a 24-hour collection). Syncytiotrophoblast nitrotyrosine was detected by immunohistochemistry and staining intensity scored by two independent blinded observers. Nitrative stress was induced in vitro by treating placental villous explants from NP with SIN-1 (1mM), a generator of nitrative stress, for 48 hours. Syncytiotrophoblast nitrotyrosine staining intensity was greater following SIN-1 treatment than in untreated controls, confirming induction of nitrative stress. TauT activity was measured as initial rate Na^+-dependent ^{3}H-taurine uptake into treated and control explants.
Results: In the PE placentas previously shown to have reduced TauT activity (1), intensity of syncytiotrophoblast nitrotyrosine staining was greater than in NP (Figure 1a). SIN-1 significantly reduced syncytiotrophoblast TauT activity compared to the corresponding control (Figure 1b).

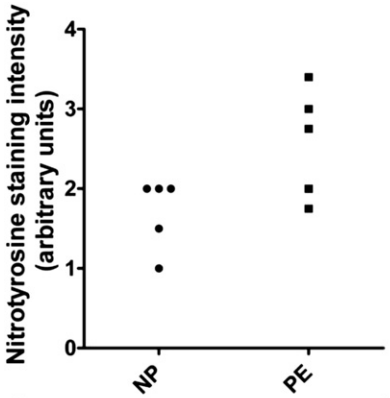

Figure 1a: Nitrotyrosine staining intensity in pre-eclampsia (PE; n=5) and normal pregnancy (NP; n=5)

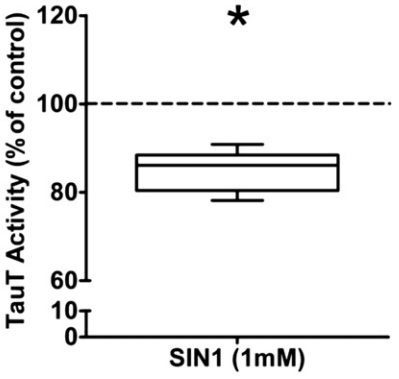

Figure 1b: SIN-1 significantly reduces TauT activity in placental villous explants (Box and whisker plot; Wilcoxon signed rank test *p<0.05 vs control: 100%)

Conclusion: Induction of nitrative stress with SIN-1 significantly reduced placental TauT activity. Initial studies suggest that syncytiotrophoblast nitrotyrosine is greater in PE than NP and nitration of TauT could down-regulate activity in PE. *In vitro* knockdown of TauT activity in trophoblast inhibits syncytialisation (2) and reduced TauT activity by nitration could contribute to abnormal syncytiotrophoblast renewal in PE.

(1) Hirst et al (2012) Reprod Sci 19:377A.
(2) Parsons et al (2009) Placenta 30:A83.

P1.52

GESTATIONAL DIABETES, TNF-α AND LEPTIN AFFECT FOLIC ACID UPTAKE BY THE HUMAN SYNCTYTIOTROPHOBLAST: EVIDENCE FOR JAK/STAT SIGNALLING INVOLVEMENT

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Objectives: The objectives of this work were to investigate the effect of gestational diabetes mellitus (GDM) upon folic acid (FA) placental transport and to identify specific GDM molecular hallmarks that may interfere with this process.
Methods: We compared ^{3}H-FA uptake by human cytotrophoblasts isolated from normal and GDM pregnancies (NTB and DTB cells, respectively) and investigated the effect of GDM-associated hallmarks upon ^{3}H-FA uptake by BeWo cells. For this, BeWo cells were exposed to different concentrations of D-glucose, insulin, lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α) or leptin for 1, 4, 24, 48, or 72 h. Radioactivity in the intracellular media was measured by liquid scintillation counting.
Results: ^{3}H-FA uptake by NTB and DTB cells was time-dependent, acidic pH-stimulated and inhibited by FA and its analogs. When compared to NTB, ^{3}H-FA uptake by DTB cells was more sensitive to acidic pH changes and to 5-methyltetrahydrofolate and pemetrexed inhibition, indicating a proportionally greater involvement of the proton-coupled folate transporter. A 4 h-exposure of BeWo cells to LPS (1-10 μg/ml) or to high levels of TNF-α (300 ng/l) significantly reduced ^{3}H-FA uptake. Moreover, hyperleptinemic conditions (100 ng/ml leptin) decreased ^{3}H-FA uptake by BeWo cells in a time-dependent manner, when compared to normoleptinemic conditions (1 ng/ml leptin). The inhibitory effect of leptin was partially reverted by inhibition of janus kinase 2 (JAK2).
Conclusion: In conclusion, GDM modulates ^{3}H-FA uptake by the syncytiotrophoblast, and leptin, via JAK/STAT (signal transducers and activators of transcription) signaling, as well as TNF-α downregulate ^{3}H-FA uptake by BeWo cells.
Supported by FCT, COMPETE, QREN and FEDER (PTDC/SAU-OSM/102239/2008, SFRH/BD/63086/2009 and SFRH/BPD/40170/2007).

P1.53

GESTATIONAL DIABETES MELLITUS REDUCES PLACENTAL UPTAKE OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS AND INTERFERES WITH TROPHOBLAST DEVELOPMENT

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Objectives: The objectives of this work were to investigate the effects of gestational diabetes mellitus (GDM) a) upon the placental transport of the long-chain polyunsaturated fatty acids (LC-PUFAs) arachidonic acid (AA) and docosahexaenoic acid (DHA), and b) upon viability, proliferation, differentiation and apoptosis of human primary cultured cytotrophoblasts.
Methods: We investigated ^{14}C-AA and ^{14}C-DHA uptake by human cytotrophoblasts isolated from normal and GDM pregnancies (NTB and DTB cells, respectively). Radioactivity in the intracellular media was measured by liquid scintillation counting. Additionally, we compared NTB and DTB cell viability (by lactate dehydrogenase (LDH) activity assay), proliferation (by ^{3}H-thymidine incorporation), differentiation (by alkaline phosphatase activity assay) and apoptosis (by TUNEL assay).
Results: Uptake of ^{14}C-AA and ^{14}C-DHA by NTB cells was: (1) mediated by both saturable (for lower substrate concentrations) and non-saturable (for higher substrate concentrations) mechanisms; (2) acidic pH-stimulated; (3) inhibited by long-chain fatty acids (with LC-PUFAs having a higher potency); and (4) strongly inhibited by the long-chain acyl-CoA synthetase (ACSL) inhibitor, triacsin C. When compared to NTB cells, DTB cells showed a markedly lower capacity for ^{14}C-AA and ^{14}C-DHA accumulation over time, through a decrease in both the saturable and the non-saturable components of uptake, and this was associated with a decrease in ACSL1 mRNA levels. Also, DTB cells showed higher turnover rates (higher proliferation and apoptosis rates) than NTB cells. Finally, uptake of LC-PUFAs in NTB cells was increased (by 20-25%) after short-term exposure to TNF-α (^{14}C-AA and ^{14}C-DHA) and insulin (^{14}C-DHA).
Conclusion: In conclusion, placental uptake of LC-PUFAs is markedly decreased in GDM. This change does not seem to be accounted for isolated hallmarks of GDM, such as increased inflammation or hyperinsulinemia.
Supported by FCT, COMPETE, QREN and FEDER (PTDC/SAU-OSM/102239/2008, SFRH/BD/63086/2009 and SFRH/BPD/40170/2007).

P1.54

INTERACTION OF THE PLACENTAL BCRP TRANSPORTER WITH ENVIRONMENTAL CHEMICALS

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Objectives: Over the last decade, the scientific community has voiced increasing concern regarding disruption of endocrine and reproductive systems as a consequence of *in utero* exposure to xenobiotics. These endocrine-disrupting chemicals (EDCs) are quite diverse and include plasticizers, mycotoxins, phytoestrogens, and pesticides. The breast cancer resistance protein (BCRP) is a chemical efflux transporter expressed in the human placenta that is responsible for fetal-to-maternal transport. Inhibition of the fetoprotective function of BCRP may be a mechanism involved in the susceptibility of the developing fetus to toxicities following EDC exposure. The purpose of this study was to characterize EDCs as potential substrates and inhibitors of the BCRP transporter.
Methods: Two *in vitro* screening assays, the BCRP ATPase assay and inverted membrane vesicle assay, were used to test interactions between BCRP and twelve chemicals with potential for endocrine disruption: bisphenol A, genistein, methoxychlor, prochloraz, zearalenone, tebuconazole, propiconazole, tributyltin, zeranol, myclobutanil, propargite and epoxiconazole.
Results: Based on the ATPase assay, it was confirmed that genistein is a substrate for BCRP, while slight increases in ATP hydrolysis were observed for tributyltin, zeranol, zearalenone, and bisphenol A, indicating they may be possible substrates. In both assays, all twelve EDCs inhibited BCRP transport of known substrates (sulfasalazine and lucifer yellow).
Conclusion: Genistein, tributyltin, zeranol, zearalenone and methoxychlor were some of the most potent inhibitors of BCRP transport. Cell-based studies are currently underway to further characterize the inhibitory kinetics of EDCs on BCRP-mediated transport and further characterize transport affinity. These findings may assist in risk assessment of perinatal exposure to these chemicals. Supported by ES-005022, DK-080774, ES-020522.

P1.55

PRE-GESTATIONAL DIABETES WITH CHORANGIOSIS AND A NODULAR CHORANGIOMA. A CASE REPORT

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Case Report: A 32-year-old known diabetic, G2P1L1, on insulin, metformin and glyburide prior to the pregnancy, was admitted in labour at 37 weeks. Her fasting blood and postprandial glucose values were 73mg% and 118mg % respectively. She underwent a caesarean section for transverse lie and delivered a full term, normal, live baby girl weighing 2.6kg with an Apgar score of 9 and 10.There were no congenital anomalies detected. The placenta weighed 540g, and was received intact. There were no gross anatomical or placental lesions such as calcification, thrombi or infarcts. The umbilical cord with the two arteries and a single vein was unremarkable. Histological examination of the placenta revealed pronounced diffuse increase in central vascularity of villi confirming a diagnosis of chorangiosis; more than 10 capillaries per 10 terminal villi in more than three areas viewed by a 10X objective with reticulin rich network). The capillaries were CD31 positive. Pericytes were smooth muscle actin positive. Occasional endothelial cells were Ki67 positive. Villi were lined by cytokeratin positive trophoblasts. Normally, chorionic villi contain five or less vascular channels, which extends even to the same vessel present in more than one plane of section examined. An incidental microscopic single well-circumscribed lesion was also seen comprising conspicuous positive capillary sized vessels highlighted by the reticulin stain embedded in a collagenous stroma, resembling a hemangioma. CD31 positive capillaries with surrounding perivascular **s**mooth muscle actin positive pericytes were present. Occasional trophoblastic cells were Ki67 positive. 1% of endothelial cells were Ki67 positive. The nodule was lined by cytokeratin positive trophoblasts. There was no evidence of abnormal trophoblastic proliferation. A diagnosis of nodular chorangioma was made.
Conclusion: We report chorangiosis and microscopic nodular chorangioma coexisting in a pre-gestational diabetic term placenta. Chorangiosis and Chorangioma are examples of villous capillary lesions of the placenta.

P1.56

INVESTIGATION OF 4 CASES RAISED D DIMER(DD) AFTER INJECT SUBCUTANEOUSLY LOW DOSE UNFRACTIONATED HEPARIN(LDUFH) TO PREVENT VENOUS THROMBOEMBOLISM(VTE) AFTER CAESAREAN SECTION

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Objects: To investigate cause of rise DD on postoperative days 7th and the efficacy of LDUFH for prevent VTE in high-risk group.
Methods: To make a comparison between low-risk group and high-risk group about influencing factors of DD on preoperative day, postoperative day 1st, 4th 7th, patient background and clinical course in 116 cases of Caesarean section injected LDUFH (total delivery 609 cases) from October 2010 to February 2012.
Results: The difference of variance of DD was great high-risk group than low-risk group on postoperative days 7th. There were significantly different between high-risk group and low-risk group about placental weight and birth weight. There were uncorrelation between placental weight and time-dependent change of DD in high-risk group.
Conclusion: We could not figure out cause of rise DD on postoperative days 7th and could not obviously show the usefulness of LDUFH. We might be thought to be causally related to placental weight about difference of variance of DD in high-risk group.

P1.57

TUMOUR HOMING PEPTIDES AS TOOLS FOR TARGETED DELIVERY TO THE PLACENTA

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Objectives: Tumour-specific "homing peptides" are short peptide sequences that bind to epitopes expressed on the endothelium of tumour vasculature, but do not bind to the vascular beds of other organs. Tumour homing peptides have been used to target intravenously administered drugs directly to their site of action. As the placenta behaves like a slow growing tumour, and remodelling uterine spiral arteries exhibit a unique vascular phenotype, we examined whether tumour homing peptides can be exploited to target the materno-fetal interface.
Methods: A panel of T7 bacteriophage, each displaying a different tumour homing peptide on its surface, were pooled and intravenously injected into a pregnant mouse. After 30 minutes, and following cardiac perfusion to remove unbound phage, the uterus and placentas were collected and homogenized. Bound phage were titered and sequenced, to determine whether phage bearing particular surface peptides were preferentially enriched in these tissues. Individual synthetic peptides labelled with 5(6)-carboxyfluorescein (FAM), and peptide-coated iron oxide nanoworms were utilised to validate utero-placental homing: peptides or nanoworms were intravenously injected, and after 3 hours, multiple organs were collected for analysis by fluorescence microscopy.
Results: Phage bearing two tumour homing sequences, designated KRK and iRGD, were enriched in uterine and placental tissue. Intravenous injection of the synthetic peptides FAM-KRK or FAM-iRGD confirmed placental targeting; both peptides bound to the placental labyrinth and to the endothelium of decidual spiral arteries at multiple time points in gestation, but were not observed in other organs. Iron oxide nanoworms coated with FAM-KRK or FAM-iRGD accumulated at the materno-fetal interface, whereas nanoworms coated with control peptides did not. FAM-KRK and FAM-iRGD also bound to the syncytium of human first trimester and term placental explants.
Conclusion: These data provide proof of principle for the use of selected tumour homing peptides to mediate targeted delivery to the placenta.

P1.58

IDENTIFICATION OF NOVEL PLACENTAL HOMING PEPTIDES

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Objectives: Phage display has previously been used to identify novel peptides that bind to the vasculature of specific organs, providing the basis for targeted drug delivery systems. To this end, we have screened a phage library to identify novel peptide sequences that bind exclusively to the mouse placenta.
Methods: Homogenized murine uteroplacental tissue was incubated with a T7 bacteriophage library, which had been engineered so that each virus particle displayed one of 10^{10} random peptide sequences on its surface. After extensive washing, bound phage were titered, amplified and purified. Following three rounds of ex vivo screening, the resulting phage pool was intravenously injected into a pregnant mouse. Cardiac perfusion was performed after 30 minutes to remove unbound phage, and the uterus and placentas were collected. Bound phage were titered, amplified and purified; four rounds of in vivo screening were performed in total. Individual clones from the second, third and fourth rounds were sequenced to determine the identity of their surface peptides. Corresponding synthetic peptides labelled with 5(6)-carboxyfluorescein (FAM) were used to validate homing; peptides were intravenously injected into pregnant mice and after three hours, organs were collected for analysis by fluorescence microscopy.
Results: Phage screening identified three peptide sequences that were enriched in uterus and placenta, designated NKG, RSG and RGR. Injection of the synthetic peptides FAM-NKG, FAM-RSG or FAM-RGR confirmed placental targeting; all peptides bound to the placental labyrinth and endothelium of decidual spiral arteries. FAM-NKG did not bind to the vasculature of any other organ screened; however, FAM-RSG and FAM-RGR occasionally bound to cells in the spleen. All peptides bound to the syncytium of human placental explants cultured *in vitro*.
Conclusion: We have identified novel peptides that bind to the surface of the placenta, which will form the basis for a targeted drug delivery system.

P1.59

DOWNREGULATION OF LUTEINIZING HORMONE RECEPTOR (LHR) MRNA IN THE RAT UTERUS

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Objectives: The luteinizing hormone receptor (LHR) belongs to the family of G protein-coupled receptors that mediate biological effects through cAMP. The expression of this receptor was previously thought to be restricted to gonadal tissue. However, recent studies have shown its presence in many other tissues throughout the reproductive and non-reproductive organs. In this paper, we have investigated the regulation of LHR expression in rat uterus.
Methods: We have detected LHR mRNA in the immature rat uterus by Northern blot and down-regulation of this receptor mRNA in the pregnant mare serum gonadotropin (PMSG) - human chronic gonadotropin (hCG) treated immature rats.
Results: After administration of hCG, the mRNA levels in the rat uterus declined to a very low level from day 1 to 3 and then rebounded and reached higher than pretreatment values at day 4.
The cultured uterus displayed an hCG concentration-dependent increase in cAMP production in medium, and the immunohistochemical experiment showed that these receptor proteins are expressed in the epithelial cells of endometrium.
Conclusion: These results suggest that functional LHR are present in the immature rat uterus and are down-regulated and up-regulated by signals resulting from hCG treatment.
We think that the uterus model of LH receptor expression might contribute to the investigation of mechanism of LH action in reproductive organs.

P1.60

MATERNAL ENDOTHELIAL FUNCTION GRADUALLY DETERIORATE IN NORMAL PREGNANCY

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Objective: Elevated circulating sFlt-1 levels are observed in preeclamptic patients, and are closely associated with endothelial dysfunction. However, the relationship between circulating sFlt-1 levels and the endothelial function in normal pregnancy remains unclear. The aim of this study was to elucidate the alteration of endothelial function and its correlation to plasma sFlt-1 levels using peripheral arterial tonometry (PAT) throughout normal gestational period as well as the process of preeclampsia (PE).
Methods: We measured reactive hyperemia index (RHI) to evaluate the endothelial function using Endo-PAT2000 system during normal pregnancy and simultaneously measured plasma sFlt-1 levels by ELISA. We also measured endothelial function and circulating sFlt-1 levels in non-pregnant women, preeclamptic patients and pregnant women who develop PE later.
Results: RHI gradually deteriorated along with the progression of gestational age in normal pregnant women. Plasma sFlt-1 levels exhibited a gradual increase at the late pregnancy and were inversely correlated with RHI. In the PE patients, RHI showed a higher value in the presence of higher plasma sFlt-1 levels compared with normal pregnant women at similar gestational ages. The number of patients who developed PE at the later gestational stage was too small to assess another topic whether endothelial function at the earlier stage of gestation is able to predict the onset of PE.
Conclusion: Our study using PAT showed maternal endothelial function gradually deteriorated along with the gestational age and endothelial function and plasma sFlt-1 levels was inversely correlated in the normal pregnancy. PAT system may be of use to identify novel biomarkers for PE.

P1.61

SPHINGOSINE 1-PHOSPHATE REGULATES ENDOTHELIAL PERMEABILITY AND ACCESS OF CIRCULATING VASOCONSTRICTORS TO SMOOTH MUSCLE CELLS IN INTACT PRESSURIZED UTERINE ARTERIES

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Objectives: Increased endothelial permeability occurs in vascular-related diseases such as preeclampsia. The relationship among arterial endothelial barrier function, access of circulating vasoconstrictors to underlying smooth muscle and vascular tone has not been explored in intact uterine arteries. Increased uterine vascular tone reduces nutrient transfer to the placenta and fetus. Sphingosine 1-phosphate (S1P), a bioactive lipid, regulates endothelial permeability. We hypothesized that infusion of high S1P concentrations inside isolated pressurized uterine arteries would increase endothelial permeability, access of a co-infused vasoconstrictor to smooth muscle and vascular tone. This would be exacerbated in pregnancy.
Methods: S1P (10 nM, 1μM), U46619 (5nM), a vasoconstrictor, or the combination was infused inside pressurized uterine arteries isolated from non-pregnant (NP) or late pregnant (LP) mice. Vasoconstriction was compared to that achieved by adding these agents to the outside of arteries (extraluminal addition). Endothelial permeability to S1P was also assessed using Evans Blue Dye (EBD) leakage.
Results: In uterine arteries from NP mice intraluminal infusion of U46619 alone did not induce vasoconstriction (3.78±4.13%) whereas extraluminal addition did (56.9±6.75%). Co-infusion of U46619 with 10nM S1P did not affect vasoconstriction (6.25±5.57%); however, co-infusion with 1μM S1P did (33.2±8.58%). Infusion of 10nM or 1μM S1P alone did not induce vasoconstriction. Unlike the lack of response in uterine arteries from NP mice, infusion of U46619 alone induced vasoconstriction in arteries from LP mice, which was prevented by co-infusion with 10nM S1P but not 1μM S1P. Permeability measured by EBD leakage was increased by infusion of 1μM but not 10nM S1P.
Conclusion: S1P at low concentration maintains endothelial barrier function to prevent access of circulating vasoconstrictors to the smooth muscle in uterine arteries, which is particularly important in pregnancy. Pathophysiological changes in S1P concentrations and/or S1P receptor expression could contribute to vascular complications in pregnancy.

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P1.62

INVOLVEMENT OF A_{2A} ADENOSINE RECEPTORS IN INSULIN-INCREASED L-ARGININE TRANSPORT IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Objectives: Adenosine causes vasodilatation of human placenta vasculature by increasing L-arginine transport via the cationic amino acid transporters 1 (hCAT-1) involving A_{2A} adenosine receptors (A_{2A}AR) activation in human umbilical vein endothelial cells (HUVEC). hCAT-1 activity and expression is increased by insulin in HUVEC and A_{2A}AR stimulation increases insulin sensitivity in subjects with insulin resistance; however, a potential A_{2A}AR involvement in L-arginine transport modulation by insulin in HUVEC is unknown. Our aim was to characterize whether insulin-stimulation of hCAT-1 transport activity involves A_{2A}AR in HUVEC.
Methods: Primary cultured HUVEC (passage 2) from full-term normal pregnancies were used. Insulin (1 nM, 8 hours) was assayed on hCAT1 expression (*SLC7A1* promoter activity (firefly/renilla luciferase for pGL3-hCAT1^{-1606} and pGL3-hCAT1^{-650}), mRNA expression (quantitative real time PCR), protein abundance (Western blot)) and L-arginine transport (3 μCi/ml, 1 minute, 37oC). Moreover, we analyzed total (Akt) and phosphorylated Akt (P∼Akt) levels. Assays were done in absence or presence of ZM-241385 (10 nM, A_{2A}AR antagonist), CGS-21680 (30 nM, A_{2A}AR agonist) and/or NBTI (10 μM, adenosine transport inhibitor).
Results: Insulin and NBTI increased the maximal velocity without altering the apparent K_m for L-arginine transport, and hCAT-1 expression (protein and mRNA). These effects were blocked by 10 nM ZM-241385 (A_{2A}AR antagonist). ZM-241385–inhibited *SLC7A1* reporter transcriptional activity was similar in cells transfected with pGL3-hCAT-1^{-1606} or pGL3-hCAT-1^{-650} constructs, and comparable to the activity determined for pGL3-hCAT-1^{-650} construct in presence of NBTI + insulin. However, reporter activity was increased by NBTI only in cells transfected with pGL3-hCAT-1^{-1606} and the ZM-241385 sensitive fraction of NBTI response was similar in absence or presence of insulin. Insulin increase P∼Akt/Akt and this effect was unaltered by agonists/antagonists of A_{2A}AR.
Conclusion: hCAT-1 expression and activity is under regulation by insulin via a mechanism requiring functional A_{2A}AR in HUVEC. This mechanism does not involve Akt signaling pathway. These effects could be determinant in diseases associated with fetal insulin resistance.

P1.63

THE DIFFERENTIAL EXPRESSION OF KISS 1, MMP9 AND ANGIOGENIC REGULATORS IN DIFFERENT FETO-MATERNAL COMPARTMENTS OF HEALTHY PREGNANCIES: IMPLICATIONS FOR TROPHOBLAST INVASION AND VESSEL DEVELOPMENT

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Objectives: To investigate the transcript and protein expression profiles of genes involved in trophoblast invasion and angiogenesis across the fetomaternal compartments of healthy human pregnancies.
Methods: Three feto-maternal tissue compartments, namely the placenta, placental bed and decidua parietalis were sampled at elective caesarean delivery of healthy term pregnancies. Real-time RT PCR was employed to investigate gene expression while Immunohistochemistry and Western Blot analyses were utilised to study protein expression in these tissues. The expression of genes involved in trophoblast invasion namely *Kiss1, Kiss1 Receptor (Kiss1R)* and *MMP9 were* investigated. In addition, we examined the expression of angiogenic ligands *VEGF A* and *PROK1* as well as their respective receptors *(VEGFR1, VEGFR2 and PROK1R*) at the three feto-maternal sites.
Results: We found that the expression of *Kiss1 ($p<0.001$), Kiss1R ($p< 0.05$)* and *MMP9 ($p<0.01$)* were higher in the placenta in comparison to the placental bed and decidua parietalis. The expression of *VEGF A* was highest in the placental bed (*$p<0.001$*) whereas that of its receptors differed across the feto-maternal interphase. While *VEGFR1* expression was highest in the placenta *($p<0.01$),* the expression of *VEGFR2 was* highest in the placental bed *($p<0.001$).* In contrast, both *PROK1 ($p<0.001$)* and its receptor *PROK1R ($p<0.001$)* had highest expression in the placenta.
Conclusion: Genes involved in invasion were highly expressed in the fetal compartment which suggests that the influence on invasion capacity may largely be exercised at the fetal level. Furthermore, our findings on angiogenic gene expression profiles suggest that angiogenesis may be regulated by two distinct pathways with the *PROK1/PROK1R* system specifically mediating angiogenesis in the fetus and *VEGFA/VEGFR2* ligand-receptor pair predominantly but not exclusively mediating maternal angiogenesis.

P1.64

THE EFFECTS OF PLACENTAL PERICTYES ON THE ANGIOGENESIS OF MICROVASCULAR ENDOTHELIAL CELLS

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Objectives: Angiogenesis plays a key role in placental development, remodeling and fetal growth. Impaired placental angiogenesis is associated with fetal growth restriction and preeclampsia. The placental microvasculature is very important to placental angiogenesis. The perivascular cells (pericytes) are also associated with stabilization and remodeling of the placental vasculature. We hypothesized that isolated human placental pericytes can increase and stabilize placental capillary formation when grown in co-culture.
Methods: Human placental microvascular endothelial cells (HPMVECs) and human placental pericytes (PC) were isolated from microvillus of term placenta and cell types were confirmed using immunocytochemistry (anti-CD31 and vWF for HPMVECs and anti-NG2 for PCs). The capillary formation with HPMVECs grown on nutrient-depleted matrigel in EGM2 and measured the number of branching points formed at 3 and 5 hours. RNA expression of angiogenic factors and their receptors (PDGFb, PDFGRb, VEGFa, VEGFR1, VEGFR2, Angiopoietin 1 (Ang 1) and TEK) were quantified by real-time PCR. We compared the results of HPMEVCs alone and HPVMECs co-cultured with PCs. Statistical analyses were performed with student's *t*-test. P values < 0.05 were considered statistically significant.
Results: HPMEVCs stained positively for anti-CD 31, vWF and NG2. In contrast, PCs only stained positively for anti-NG2. The capillary tube formation of HPMVECs co-cultured with PCs was accelerated compared to HPMVECs alone. The number of branching points were significantly increased in HPMVECs co-cultured with PCs at 3 hours ($p<0.01$) and even at 5 hours ($p<0.01$). PC increases VEGFR1 and VEGFa, but reduces VEGFR2 and slightly reduces PDGFb expression in HPMVECs. In contrast, there is no change in PDGFRb, TEK or Ang1 expression.
Conclusion: Human placental pericytes accelerate and stabilize capillary formation from HPMVECs and the underlying mechanisms may include increased VEGFR1 and VEGFa and decreased VEGFR2 and PDGFb expression.

P1.65

A RETROSPECTIVE STUDY ON NEONATAL OUTCOME RELATION WITH CHORIOAMNIONITIS DELIVERED FROM 26 TO 32 WEEKS OF GESTATION

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Objectives: Chorioamnionitis is a main cause of preterm delivery. Severe chorioamnionitis may have a significant impact on neonatal outcome. We conducted a study on pathological findings of chorioamnionitis and its association with neonatal outcome.
Methods: This was a retrospective study of 138 patients who delivered from 26 weeks 0 day to 32 weeks 6 days, between January 2005 and December 2010 in our hospital. The cases of chromosomal abnormality or multiple gestations were excluded. The patients were classified as [Group with CAM; n=95) (CAM is grade 1 or more by Blanc classification) or [Group without CAM; n=43] (No pathological CAM). [Group with CAM] was subdivided into the following groups; [Mild CAM; n=70] (no umbilical cord inflammation in the CAM of grade 1 or 2) or [Severe CAM; n=25] (CAM of grade 3 and / or umbilical cord inflammation). We evaluated maternal factors and neonatal outcomes.
Results: There were no significant differences except maternal age and the primiparous rate between [Group with CAM] and [Group without CAM]. Comparing [Mild CAM] with [Severe CAM], there were statistically significant differences in gestational age at delivery (30.0±2.0 weeks vs. 28.6±1.9 weeks, p=0.003), maternal C-reactive protein (CRP) before delivery (0.3 mg/dL [0.0-5.5] vs. 1.1 mg/dL[0.2-8.2], $p<0.001$), rate of maternal CRP≧2 mg/dL (9.5% vs. 34.8%, p=0.013), rate of chronic lung disorders (CLD) infant in need of home oxygen therapy (HOT) (0/10 vs. 3/6 , p=0.035).
Conclusion: The severe CAM group showed earlier gestational age at delivery and had an impact on infant's necessity of HOT. To evaluate severe CAM and umbilical cord inflammation with a proper assessment of maternal CRP was suggested to be important in determining the appropriate time of termination.

P1.66

REGULATION OF TROPHOBLASTIC CELLS INVASION BY ONCOSTATIN M AND LEUKEMIA INHIBITORY FACTOR (LIF)

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Objectives: Trophoblast cells perform invasion similar to tumors, but in a well regulated physiological manner and dysregulation may lead to severe pathologies. Numerous factors regulate trophoblast invasion for successful pregnancy including cytokines. Leukemia inhibitory factor (LIF) and Oncostatin M (OSM) are members of the interleukin-6 superfamily. LIF induces trophoblast invasiveness via signal transducers and activators of transcription 3(STAT3), but activation mechanisms seem to differ in different cell lines and are not yet completely investigated. Therefore, the aim of our study is to analyze and compare the role of Extracellular Regulated Kinase (ERK)/STAT in trophoblast and choriocarcinoma cells with different invasive capacities.

Methods: The immortalized human trophoblast cell line HTR-8/svneo, the choriocarcinoma cell line JEG-3, and the hybrids of JEG-3 derivates and 1st and 3rd trimester trophoblast cells ACH-3P and AC1-M59, were incubated in presence or absence of LIF or OSM. The activation and expression of STAT3 and extracellular regulated kinase 1/2 (ERK1/2) were measured by gel electrophoresis and Western blotting and quantified by use of a chemiluminescence gel documentation system. DNA binding assay was analysed using TransAm STAT family kit. The effect on cell proliferation and invasiveness were determined by a MTS colorimetric assay and a Matrigel invasion assay. Gelatin zymography was performed on conditioned medium of choriocarcinoma cells. For determined the role of STAT3, it was blocked with a chemical inhibitor.
Results: LIF and OSM stimulated the phosphorylation of STAT3 and ERK1/2 in all analyzed cell lines, but at different intensities. LIF also enhanced proliferation of ACH-3P cells, AC1-M59 cells and JEG-3 cells but stimulated invasiveness only of ACH-3P cells and JEG-3 cells. In contrast, proliferation and invasion were not affected by OSM. Activity of MMP-2 and MMP-9 decreased in choriocarcinoma cells treated with STAT3 inhibitor.
Conclusion: These findings demonstrate that the LIF-ERK1/2-STAT3 axis exists in different trophoblastic cell lines, but its activation may lead to different functions, which may be due to specific properties of each cell line.

P1.67

COMPARATIVE ANALYSIS ON THE INVASION POTENTIALS OF TRANSFORMED HUMAN FIRST TRIMESTER TROPHOBLAST AND CHORIOCARCINOMA CELL LINES

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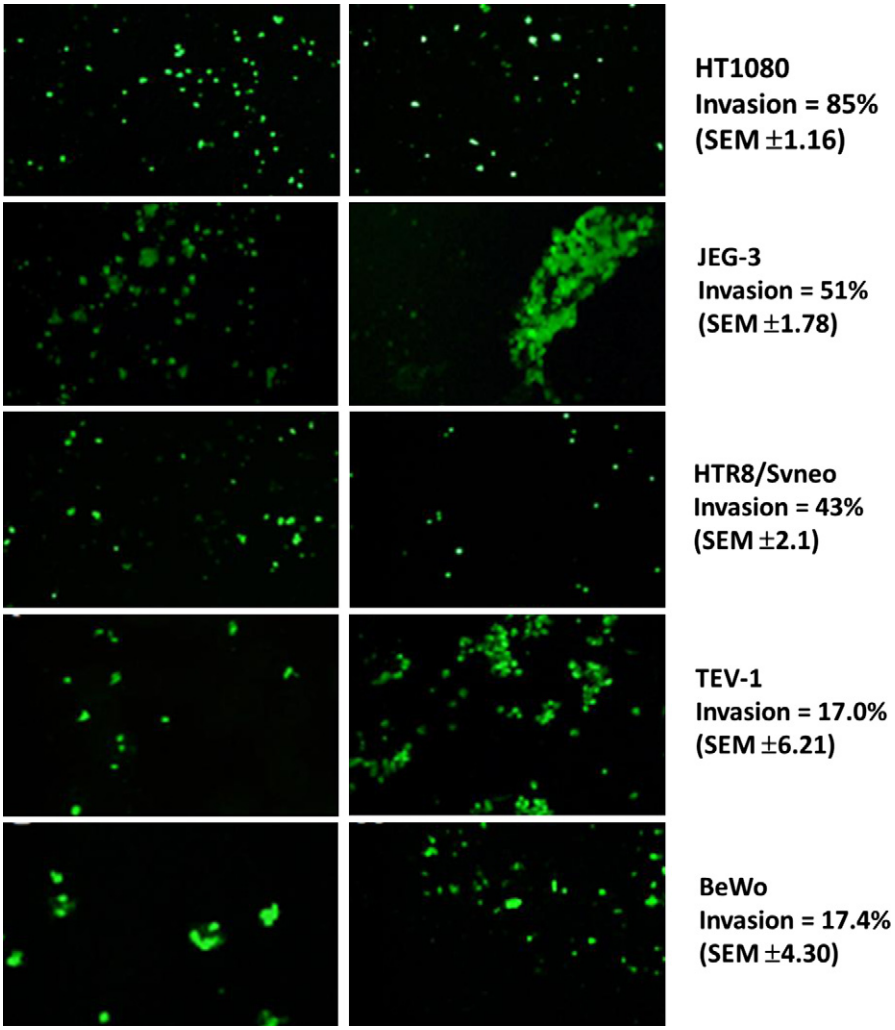
Placental derived cell lines have been used as an in vitro model to study the pathophysiology of many pregnancy-related disorders. To satisfy the need of long term culture these cell lines were developed either from choriocarcinoma (JEG-3 and BeWo) or by transformation of first trimester extravillous trophoblast cells (TEV-1 and HTR-8/SVneo). However these cell lines show different morphological and physiological characteristics; therefore they "behave" differently from one another. This study aims to compare the invasion potentials of human first trimester trophoblast cell lines, TEV-1 and HTR-8/SV-neo, with choriocarcinoma cell lines, JEG-3 and BeWo in relation invasiveness of human fibrosarcoma cells, HT1080 (positive control).

Cell invasion assays were carried out in the 24 well-plate BD BioCoat™ Tumour Invasion System. Cell suspensions in serum free media were added into the apical inserts of test/control plates and invasion was triggered by adding the chemo attractant (5% foetal bovine serum) to the basal chambers. The setup was incubated for 22 hours at 37 ◦ C with 5% CO2 and the percentage of invading cells was calculated.

Percentage invasion of HT-1080 was approximately 80%, which agreed with the predicted results by the assay manufacturer. Of the first trimester extravillous trophoblast cell lines, HTR-8/SVneo showed 43% (SEM±2.1) invasion whereas TEV-1 cell line was lower at 17% (SEM±6.21). Likewise the choriocarcinoma cell lines JEG-3 showed a higher percentage invasion at 51% (SEM±1.78) than BeWo (17.4% SEM±4.3) (see figure). This shows JEG-3 versus HTR8/SVneo or BeWo versus TEV-1 are suitable cell lines for comparative invasive assay.



These results suggest that invasion properties are different in different placental and choriocarcinoma cell lines. Therefore care should be taken in selecting a comparative pair of choriocarcinoma and first trimester trophoblast cell lines as models for trophoblastic tumour cells versus normal trophoblast cells.

P1.68

ROLE OF N-ACETYLGLUCOSAMINYLTRANSFERASE IVA IN CHORIOCARCINOMA

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Objectives: Gestational trophoblastic diseases (GTDs) are related to trophoblasts and the glycoprotein hormone human chorionic gonadotropin (hCG) is a useful diagnostic and follow-up marker for GTDs, although it is secreted by trophoblasts of all kinds of pregnancies as well as GTDs. However, the asparagine-linked sugar chains on hCG in the serum and urine from patients with invasive mole and choriocarcinoma contain abnormal biantennary structures, which are not detected in normal pregnant women and hydatidiform molar patients. N-acetylglucosaminyltransferase IV (GnT-IV) catalyzes β1, 4-N-acetylglucosamine (β1-4GlcNAc) branching on asparagine-linked oligosaccharides and is required to synthesize the abnormal biantennary sugar chain structures on hCG. The aim of this study was to clarify GnT-IVa expression in GTDs and human placentas and determine the role of GnT-IVa in choriocarcinoma.
Methods and Results: Immunohistochemistry showed that GnT-IVa was not detected in hydatidiform mole but highly expressed in trophoblastic cells of choriocarcinoma and invasive mole, which are malignant subtypes of GTDs. These results were the same as those of RT-PCR and Western blot analyses. Small interfering RNA (siRNA)–mediated knockdown of GnT-IVa expression in Jar cells, a choriocarcinoma cell line, significantly reduced their migration and invasive capacities, although MTS assay showed that there were no effects on cell proliferation. *In vivo* studies using athymic nude mice bearing Jar cell tumors further demonstrated that knockdown of GnT-IVa significantly suppressed tumor engraftment and growth.
Conclusion: These findings suggest that GnT-IVa might be involved in tumorigenic activity of choriocarcinoma.

P1.69

THE INFLUENCE OF ONCOSTATIN M ON THE EPITHELIAL-MESENCHYMAL TRANSITION AND TROPHOBLAST INVASION

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Objectives: Oncostatin M (OSM), a cytokine of the interleukin-6 (IL-6) family, can either promote or inhibit cell growth in various normal and tumor cells and is expressed in rheumatoid arthritis, multiple sclerosis, multiple myeloma, and other inflammatory and neoplastic conditions. Our objective was to evaluate the effects of exogenous OSM on the invasion of trophoblasts and modulation of epithelial-mesenchymal transition (EMT).
Methods: We investigated OSM effects on the expression of both cell-cell contact proteins and mesenchymal marker in the human placental cell line derived from first trimester extravillous trophoblasts (HTR8SVneo). We studied also OSM effects on the in vitro invasion of HTR8SVneo cell line.
Results: We found the differential regulation of E-cadherin, vascular endothelial (VE)-cadherin, and vimentin by OSM. OSM attenuated the constitutive RNA and protein expressions of E-Cadherin, but enhanced expressions of vimentin. There was no significant change in the expressions of VE-Cadherin. We also found that OSM increased invasion activities of HTR8SVneo cells in time-dependent and dose-dependent manners.
Conclusion: This study suggests that OSM enhances EMT of trophoblasts during the first trimester and it might be related with the increased invasion of extravillous trophoblasts.

P1.70

INTERVENTIONAL RADIOLOGY FOR PLACENTA PREVIA ACCRETA AT A TERTIARY CARE UNIT IN JAPAN

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Objectives: It is apparent that high risk pregnancy has been increased these days, especially abnormal placentation such as placenta previa, placenta accreta. This is related to the rate of caesarean section due to the maternal age and/or pregnancy by assisted reproductive technology. Although uterine artery embolization (UAE) is a well-recognized treatment for postpartum hemorrhage with a high clinical efficacy, the reported success rate of UAE for postpartum hemorrhage associated with placenta accreta is not high. We reviewed the management of placenta previa accreta at our hospital and highlights recent advances and developments.
Methods: Eleven consecutive patients between 1999 and 2012 who underwent UAE for the management of placenta previa accreta were included in this retrospective study. The entire patient had hysterectomy after caesarean section. Four patients underwent UAE during the operation (UAE group) and 7 did not prepare for UAE during the operation (non-UAE group). Medical records were reviewed regarding the delivery and UAE procedure.
Results: The amount of blood loss during the operation was 1901±1057.0 ml (mean ± SD) in the UAE group and 3486.1±1743.0 ml in the non-UAE group. The duration of operation was 213.5±44.5 min in the UAE group and 215.6±61.8 min in the non-UAE group respectively. The UAE group used only autologous blood transfusion (800±282 ml) and was able to avoid homologous blood transfusion whereas the non-UAE group needed additional homologous blood transfusion 1600±1222 ml in average.
Conclusion: Appropriate surgical and pharmacological treatments should be combined for each hemorrhagic condition. Interventional radiology (IVR) is the highly effective option to reduce blood loss. Although there are no randomized controlled trials, our analysis showed that IVR is one of the effective techniques in hemostatic control in placenta accreta previa. Further analysis is required to clarify the role of IVR in the management of obstetric hemorrhage.

P1.71

CD44 ACCELERATES TROPHOBLASTIC INVASION: *IN VITRO* INVASION ANALYSIS USING HTR8/SVNEO CELLS

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Objectives: Although extravillous trophoblast (EVT) invasion into the decidua is essential for successful pregnancy, its detailed mechanism remains unclear. In cancer cells, binding of HA to CD44 induces cell migration and invasion. It was previously reported that EVT expresses an adhesion molecule, CD44 on their cell surfaces (Goshen et al. Mol Hum Reprod 2: 685-691, 1996). Interestingly, a ligand of CD44, hyaluronic acid (HA) is located in the decidua. These facts led us to speculate that interaction of CD44 and HA may be involved in mechanisms of human extravillous trophoblast invasion into the decidua. The purpose of this study was to test this hypothesis using HTR8/SVneo cells as an *in vitro* model of EVT.
Methods: The mRNA and protein expressions of CD44 were measured by real-time PCR and Western blot analyses, respectively. HA-induced invasion of HTR8/SVneo cells was evaluated by matrigel-coated transwell assay using Boyden chamber. To examine an effect of CD44 on the invasion, small interfering RNAs (siRNAs) against *CD44* mRNA were generated and used for *CD44* knockdown. The methylation of CpG sites within CD44 promoter region was examined by a bisulfite DNA sequencing method.
Results: HTR8/SVneo cells expressed CD44 as both mRNA and protein levels. HA significantly enhanced invasion of HTR8/SVneo cells. The HA-induced invasion was inhibited with *CD44* siRNA transfected HTR8/SVneo cells. The CpG sites within CD44 promoter region were unmethylated in HTR8/SVneo. However, other *in vitro* models of villous trophoblast, BeWo and JEG3 cells did not express CD44, and their CpG sites within CD44 promoter region were hypermethylated. In addition, HA did not affect the invasion of BeWo or JEG3 cell.
Conclusion: Our results imply that the CD44-HA interaction plays a role in EVT invasion. Furthermore, the hypomethylation of CD44 promoter region may be associated with the upregulation of CD44 expression in EVT.

P1.72

DNA MICROARRAY ANALYSIS OF INVASIVE TROPHOBLAST IN PLACENTA ACCRETA

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Placenta accreta is an abnormal condition of invasive placental implantation that causes massive hemorrhage at delivery and threatens maternal life. Abnormal villous invasion is found when decidua is defective and this condition is associated to previous uterine operations including caesarean sections, however, its mechanisms are still unclear. The objective of the current study is to examine molecular biological change of trophoblast invasion in placenta accreta by DNA microarray analysis. We isolated villous trophoblast from invasive site and control from non-invasive site in a case of placenta accreta and extracted complementary DNA by RT-PCR. A DNA microarray analysis was performed and 104 transcripts were identified as significantly overexpressed gene in invasion site. Gene ontology analysis revealed enhancement of gene known to play a role in peptidase activity. The 3 most significantly overexpressed genes were matrix metalloproteinase-19 (MMP-19), a disintegrin and metalloproteinase-28 (ADAM-28) and cathepsin L2. To ascertain the micoarray results we performed immunohistochemistry in several accreta cases and revealed poor expression of MMP-19 and no difference between invasion and non-invasion site in ADAM-28, however a abundant expression of cathepsin L2 in especially in extra-villous trophoblast in invasion site. Thus, our data suggests a role for cathepsin L2 in the regulation of abnormal invasion of trophoblast in case of placenta accreta.

P1.73

REGULATORY ROLE OF SPHINGOSINE-1-PHOSPHATE IN MIGRATION OF HUMAN TROPHOBLAST

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Objectives: In the first-trimester of pregnancy, cytotrophoblast differentiate into extravillous trophoblast (EVT) at villous-anchoring sites. EVT invades and remodels maternal artery to secure the adequate placental blood flow to the fetus. We previously reported that maternal platelets are attached to the endovascular trophoblast and activated inside the spiral arteries. We also demonstrated that platelet-derived soluble factors as a whole stimulate the migration of EVT cells isolated from the first-trimester chorionic villous explant cultures, suggesting that platelets play an important role in maternal spiral artery remodeling by EVT cells. In this study, we focused on another platelet-derived soluble factor, sphingosine-1- phosphate (S1P) and examine its physiological roles in EVT function.
Methods: Expression of S1P receptors was examined using the isolated EVT cells derived from the first-trimester chorionic villous explant cultures, Swan 71 cells (trophoblastic cell line) and BeWo cells (choriocarcinoma cell line) by RT-PCR, immunocytochemical staining, and Western blotting. Effects of S1P on proliferation and invasion of the isolated EVT cells, Swan71 cells, and BeWo cells were examined by WST assay and Matrigel invasion assay, respectively.
Results: Among five SIP receptors (S1P1-5), S1P2 and S1P3 were expressed in the isolated EVT cells and Swan71 cells. S1P promoted the invasion of these cells without affecting their proliferation. By contrast, S1P promotes proliferation of BeWo cells, which express only S1P2, without affecting their invasion.
Conclusion: S1P may be one of platelet-derived soluble factors that drives EVT invasion towards maternal artery.

P1.74

IL-33, A NOVEL IL-1 FAMILY MEMBER, REGULATES FUNCTION OF PRIMARY FIRST TRIMESTER TROPHOBLASTS

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Objectives: Our aim was to firstly characterize expression pattern of IL-33 and its receptor IL1RL1 (ST2L) in the placenta and the fetal-maternal interface. In a second step, we evaluated its potential in regulating proliferation and invasion of first trimester trophoblasts.
Methods: In order to identify cellular sources of IL-33 we performed Western Blot analyses of FACS-sorted CD45+ and CD45- cells, isolated from placental villi and decidua. Moreover, we performed immunohistochemical (IHC) stainings of first trimester placental and decidual sections to further characterize IL-33-expressing cells. IL1RL1-positive cells were identified by IHC stainings and FACS analyses. We further stimulated first trimester floating explants with rhu IL-33 and/or its natural inhibitor sST2 and determined BrdU incorporation in villous CTBs and proximal CC trophoblasts. Migration was evaluated by studying differentiating villous explant cultures on collagen-I or invading isolated trophoblasts in the absence or presence of IL-33 and/or sST2. Protease activity was measured using supernatants of IL-33-stimulated first trimester villous explants and primary EVTs.
Results: Cytoplasmatic IL-33 is found in villous and decidual macrophages and its receptor IL1RL1 is strongly expressed in villous CTBs, CC trophoblasts and invasive decidual iCTBs. Interestingly, macrophages expressed full length IL-33 and a 20 kDa form similar in size to a recently published secreted IL-33 fragment. Moreover, IL-33 induced proliferation of villous and CC trophoblasts and enhanced invasion of primary EVTs in vitro. Both effects were reversible by the addition of sST2. Finally, IL-33 induced secretion of uPA, PAI-1/2 and MMP9 in villous explant cultures and primary EVTs.
Conclusion: Placental and decidual macrophages constitute putative sources for extracellular IL-33 to activate ST2L-expressing CTBs and EVTs. Functional in vitro assays further suggest that IL-33 influences trophoblast invasion and proliferation. Taken together, we propose that macrophage-derived IL-33 may act as a novel key regulator of trophoblast function.
Funding: Supported by grant P-22687-B13 of the Austrian Science Funds (FWF), and by grant 13955 of the Austrian National Bank.

P1.75

PATHOLOGIC DIFFERENTIATION BETWEEN PLACENTA ACCRETA AND ADHERENT PLACENTA

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Purpose: When placenta accreta occurs without the placenta being delivered we must be careful about obstetric bleedings, hemorrhagic shock, sepsis, and DIC. The removal of the placenta may have to be performed by manual abrasion, or else a hysterectomy may become necessary. The outcome depends on the degree of villous invasion and range of myometrium. Adherent placenta is different to placenta accreta in that adherent placenta is placenta attached to uterus smooth muscle. Placenta accreta is placenta which invades uterus smooth muscle by the pathologic examination. When the placenta and uterus are submitted to pathologic examination, there is a difference between adherent placenta and placenta accreta. I determined the differentiation of these pathologic findings and the clinical conditions.
Method: I examined one percreta, four increta, two accrete and five adherent placenta, pathologically.
Results: The five cases of percreta and increta led to hysterectomy. The two accrete and five adherent placentas had damage, but avoided hysterectomy. The five cases that resulted in hysterectomy showed villous invasion into uterus smooth muscle pathologically. As for the seven cases that were treated manually, two placentas accrete had damage to the placenta and come with attached uterus smooth muscle. Five adherent placentas did not have uterus smooth muscle with placenta. However, there were decidual inflammation and a very thin decidua.
Discussion: In Japan a definition of the adherent placentas already exists, I have added a newly pathologic characteristic; decidual inflammation and a very thin decidua. I hope it will be possible to make use of these pathologic findings.

P1.76

NUCLEAR MATRIX ASSOCIATED GENES, SATB1 AND SATB2 REGULATE TROPHOBLAST DIFFERENTIATION

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Objectives: There is still much to be elucidated in the molecular system of trophoblast differentiation. To identify a novel pathway in trophoblast differentiation, we focused on two nuclear matrix associated genes, Satb1 and Satb2 (Satbs), which are highly expressed in murine trophoblast stem (TS) cells.
Methods: Satbs mRNA and protein expression were examined in placentas of rat early embryos and in several trophoblast cell lines: mouse, rat TS cell lines and Rcho-1 cells. Effects of knockdown or overexpression of Satbs were examined in the trophoblast cells.
Results: Satbs were highly expressed in the trophoblast cells maintained in the stem cell state and rapidly declined after induction of differentiation. Satbs proteins were also expressed in the rat placenta at early gestational stages and disappeared as gestation went on. Knockdown of Satbs expression induced trophoblast differentiation, whereas forced expression of Satbs promoted TS cell proliferation and inhibited its differentiation. We identified a well-known regulator of TS cells, Eomes as a direct target for Satbs. Satbs knockdown decreased Eomes mRNA levels and its promoter activity. On the other hand, forced Satbs expression increased Eomes mRNA levels and its promoter activity. Our electrophoretic mobility shift assay and chromatin immunoprecipitation analyses demonstrated that Satbs proteins physically associate with a regulatory site within the Eomes promoter.
Conclusion: Satbs promote TS cell maintenance and inhibit its differentiation. This function is mediated in part by regulating the expression of Eomes, which is a key regulator of trophoblast differentiation.

P1.77

ISOLATION OF TROPHOBLAST HOESCHT SIDE POPULATIONS FROM HUMAN FIRST TRIMESTER VILLI

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Objectives: We currently understand very little about how human trophoblast lineages develop and what regulates their cell fate decisions. Whether placental villi contain trophoblast stem cells is not yet established. When stained with Hoescht and analysed by flow cytometry, many stem cell populations form a 'side-population' as a result of their ability to rapidly efflux the stain. We used this trait to examine whether trophoblast stem cells are resident in first trimester villous tissue and to isolate these cells to use as a functional model of human trophoblast differentiation.
Methods: Villi from placentae of 6-9 weeks of gestation were dissected from the membranes, digested overnight in trypsin, and harvested by repeated washing with PBS. Cells were stained with Hoescht 33342 and Propidium Iodide. Live Hoescht side populations were identified and isolated using a Fluorescence Activated Cell Sorter. Purity was determined by immunofluorescent double labelling for cytokeratin 7 and vimentin.
Results: 94.7% ($\pm$2.3% SE, n=3) of live cells obtained from villi were cytokeratin positive. 0.48% ($\pm$0.13 SE, range 0.1-1.28%, n=9) of these cells formed a Hoescht side-population. Immunohistochemical analysis revealed that 98.4% ($\pm$0.2% SE, n=3) of side-population cells were cytokeratin positive and vimentin negative.
Conclusion: We have isolated a population of trophoblasts from first trimester placental villi that form a Hoescht side population, which is characteristic of stem cells. Future characterisation of this population will determine whether these stem-cell-like trophoblasts express key stem cell markers and are able to differentiate into syncytiotrophoblast and extravillous trophoblast.

P1.78

IDENTIFICATION OF HUMAN TROPHOBLAST STEM CELLS

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Objective: Although insufficient formation of placenta cause diseases, such as the fetal growth restriction, the mechanism of placental formation remains undetermined. It has been suggested that trophoblast stem cells (TSCs) exist in placenta. Stem cell enriched subpopulations, side-population (SP) cells have been identified in several tissues. In this study, we isolated SP cells from HTR-8 SVneo cells originated from the extravillous cytotrophoblast in the first trimester and JAR cells, which are a choriocarcinoma cell line after normal pregnancy and investigated the characteristics of these cells.
Methods:

1) SP cells were isolated from HTR-8 SVneo cells and JAR cells.
2) We performed microarray expression analysis for screening up-regulated genes in TSCs on a set of HTR-8SVneo-SP cells and -NSP cells.
3) Time-dependent transition of supposed gene expression in HTR-8/SVneo SP cells cultured with the differentiation condition was confirmed by real time PCR.
4) We investigated the SP specific gene expression in choriocarcinoma cell line JAR.

Result:

1) SP cells exist in both HTR-8/SVneo cells and JAR cells. They have long-term proliferative capacity of the cell cultures.
2) In microarray analysis on a set of HTR-8SVneo-SP cells and -NSP cells,

the TSC markers in mice such as CDX2, SOX2 and BMP7 are significantly up-regulated in SP cells compared with NSP cells. We also identified two up-regulated genes (geneA and geneB).

3) After HTR-8/SVneo SP cells were cultured in the differentiation condition medium for 7days, the expression of these TSC markers and gene A were gradually down-regulated.
4) The expression of gene B, but not gene A, was also up regulated in JAR-SP cells compared with JAR-NSP cells.

Conclusion:

1) HTR-8/SV neo-SP cells are stem cell enriched subpopulations.
2) Gene A is a candidate of the TSC marker. 3) Gene B is a candidate of stem cell maker of the choriocarcinoma.

P1.79

THE INTERACTION OF NEUTROPHILS AND HUMAN PLACENTAL MULTIPOTENT MESENCHYMAL STROMAL CELLS DURING PLACENTAL INFECTION

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Objective: The role of human placental multipotent mesenchymal stromal cells (hPMSCs) in the placental inflammation has not been explored. We hypothesize that placenta-specific hPMSCs involve in early phases of bacterial inflection.
Methods: hPMSCs were isolated from term placentas and neutrophils were from peripheral blood of healthy volunteers. Toll-like receptors (TLR) and cytokine expression by hPMSCs and the influence of TLR4 ligand, lipopolysaccharide (LPS), on the alteration of TLR4 and cytokine expression in hPMSCs was studied by RT-PCR and FlowMultiplex kit. hPMSC-conditioned medium for stimulation experiments was collected with or without 1 μg/ml LPS stimulation after confluent culture. Fresh RPMI1640 culture medium and LPS were used as controls. The neutrophil activation, migration, production of reactive oxygen species, apoptosis and STAT3 phosphorylation were assessed by flow cytometry, transwell.and Western blot.
Results: hPMSCs expressed TLR1, TLR3, TLR4, TLR5, TLR7 and TLR9. LPS stimulation increased the expression of TLR4 and the production of IL6 and IL8 by hPMSCs. Neutrophil CD11b expression was activated and cell migration was promoted by hPMSC-conditioned medium, which was further enhanced by hPMSC-conditioned medium previously challenged with LPS. These effects were blocked by anti-IL8 neutralizing antibody. LPS stimulation increased the reactive oxygen species production by neutrophils. hPMSC-conditioned medium induced STAT3 activation in neutrophils, which was inhibited by neutralizing antibody to IL6. hPMSC-conditioned medium rescued neutrophils from apoptosis. The conditioned medium pretreated by LPS significantly decreased the anti-apoptotic effect than that of conditioned medium without LPS pretreatment. The depletion of IL-6 from the conditioned medium further inhibited the anti-apoptotic effect on neutrophils.
Conclusion: Our results demonstrate the functional interaction between hPMSCs and peripheral blood neutrophils upon bacterial challenge and suggest IL-8 produced by hPMSC recruits neutrophils and IL-6 expression by hPMSCs reduces the neutrophil apoptosis.

P1.80

CYTOGENETIC AND STEM CELL MARKER CHARACTERIZATION OF HTR8/SVNEO AND JEG-3 CELLS

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The genetically anonymous HTR8/SVneo cell line has become a popular model of trophoblast cells because of its purported proximity to the physiological situation. HTR8/SVneo cells are simian-virus-transfected trophoblast cells in contrast to other trophoblastic cell lines, such as JEG-3, that are derived from choriocarcinoma tumors or metastases.
It has been suggested that HTR8/SVneo possess progenitor characteristics, while JEG-3 has been described as a candidate cancer stem cell (CSC). Stem cell transcription markers propagate stem cell characteristics such as self-renewal in both physiological, as well as cancer stem cells. Our aim was to **characterise the expression of trophectoderm, embryonic and cancer stem cell markers in HTR8/SVneo in comparison to JEG-3.** Furthermore, we **characterize HTR8/SVneo cytogenetically** to distinguish these cells from others.
Methods: The expression of embryonic stem cell (ESC) transcription markers, Notch1, Sox2, Oct4, Nanog and STAT3, and the trophoblast stem cell (TSC) marker Cdx2 were identified per Western blot, immunocytochemistry and immunofluorescence. Flow cytometry revealed expression of CD24, CD34, CD44 and CD133, which are surface markers for progenitor and cancer stem cells. LIF stimulation of stem cells maintains stemness, and is used here to follow STAT3 activation.
Results: STAT3 is intensively, constitutively expressed in both cell lines. Upon LIF stimulation, this molecule is found both in tyrosine (strong) and serine (weak) phosphorylated forms. We detected all stem cell markers in both cell lines. The surface marker expression profile for HTR8/SVneo is $CD24^{low}/CD34^{low}/CD44^{high}/CD133^{-}$, while JEG-3 cells are $CD24^{low}/CD34^{-}/CD44^{-}/CD133^{-}$.
Conclusion: The constitutive expression of STAT3 in HTR8/SVneo is probably SV-mediated, but the expression ESC and TSC markers suggest that HTR8/SVneo has acquired progenitor characteristics through an SVneo-transfection mediated back-differentiation. The surface marker expression profile of HTR8/SVneo is similar to that of several cancer stem cells (e.g. breast, prostate), while that of JEG-3 rather indicates its metastatic derivation.
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P1.81

TROPHOBLAST AND EMBRYONIC STEM CELL MARKERS ARE EXPRESSED IN VILLOUS TROPHOBLAST OF HEALTHY, HUMAN 1^{ST} , BUT NOT 3^{RD} TRIMESTER, PLACENTAE

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Objectives: Stem cells are highly proliferative, undifferentiated cells and stem cell markers are involved in maintaining these characteristics. In the blastocyst, differential expression between embryonic and trophoblast stem cell transcription factors is thought to discriminate cell fate. Although villous cytotrophoblasts are functionally considered a trophoblast stem cell or at least a progenitor cell, which replenishes the outer syncytiotrophoblast layer when necessary, little data is available characterizing the expression of stem cell transcription factors beyond the blastocyst stage. We aimed to describe the expression of trophoblast and embryonal stem cell factors in the placenta between 1^{st} and 3^{rd} trimester in order to discriminate if these markers might be involved in progenitor cell functions.
Methods: We analyzed 8 each of samples derived from 1^{st} trimester (elective abortions) and control (normal term pregnancy placentae). We accomplished immunoperoxidase staining to detect the stem cell markers: Cdx2 (trophectoderm), Sox2, Notch1, Nanog and Oct4A (embryonal).
Results: We detected all stem cell markers in all samples of 1^{st} trimester placentae. The expression pattern is homogenous in syncytio- and cytotrophoblast in early pregnancy and grows increasingly mosaic-like towards the end of the 1^{st} trimester. It appears that the syncytiotrophoblast loses the signal first. The signals are lost or starkly diminished in the 3^{rd} trimester. Here only singular, apparently cytotrophoblast, cells express these markers.
Conclusion: Unexpectedly, both embryonic, as well as trophoblast stem cell markers are expressed in the first trimester trophoblast and appears most vivid among the villous trophoblast of very early pregnancy. Loss of stem cell transcription factor expression in term placentae indicates temporal regulation, and probably a specific function which is yet to be elucidated. Association of stem cell factor expression with reproductive pathologies should constitute the aim of further projects.

P1.82

CHARACTERIZATION OF MESENCHYMAL STEM CELL LINES FROM THE HUMAN PLACENTA AND DECIDUA BY MEASUREMENT OF THE NOVEL STEMNESS MARKER, ALDEHYDE DEHYDROGENASE (ALDH)

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Objectives: During pregnancy there is a high state of oxidative stress in the placenta and decidua. Placenta-derived mesenchymal stem cells (MSCs) reside in vascular niches and are exposed to circulating oxidative stress response products. ALDH activity is a widely used "universal" marker for stem cells. ALDH efficiently detoxifies aldehyde products generated by reactive oxygen species, which is necessary for stem cell survival. Our aim was to isolate placental and decidual MSC lines, and characterise them with respect to their ALDH activity.
Method: Primary chorionic MSCs (CMSC) and decidual MSCs (DMSC) were transformed with the human telomerase reverse transcriptase gene (hTERT) to generate CMSC29 and DMSC23 lines. In vitro differentiation into mesenchymal lineages was performed with commercial kits. Flow cytometry was performed with well-established MSC markers. Cell lines were tested for decidualization by ELISA. The commercial "Aldefluor" flow cytometry assay was used to detect cells with high ALDH activity ($ALDH^{br}$ cells).
Results: Both cell lines showed >95% expression of MSC markers CD73, CD105, CD90, CD44, CD146, CD166 and lacked expression of (<2% positive) CD45, CD19, HLA-DR as expected. Both cell lines could be differentiated into osteocytes, adipocytes and chondrocytes. Only DMSC23 showed increased prolactin (decidual marker) levels in the decidualization assay. The proportion of $ALDH^{br}$ was significantly lower in CMSC29 compared with DMSC23 (CMSC29 0.17%±0.07%; DMSC23 5.20%±1.14%, unpaired t-test, $P<0.01$, $n=3$). A similar result was obtained in primary CMSC and DMSC populations CMSCs 0.03%±0.02%; DMSCs 5.67%±1.72%, unpaired t-test, $P<0.05$, $n=6$)
Conclusion: The establishment of CMSC29 and DMSC23 will assist stem cell culture studies by providing uniform populations of chorionic and decidual MSCs respectively. The significantly lower proportion of $ALDH^{br}$ cells in CMSC29 compared with DMSC23, which was also found in primary CMSCs and DMSCs respectively, may reflect the very different oxidative stress environments of the two stem cell types.

P1.83

ISOLATION AND CHARACTERIZATION OF CELLS OF THE YOLK SAC AND EQUINE AMNIOTIC MEMBRANE AS A SOURCE FOR CELLULAR THERAPY

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Stem cells derived from extra-fetal sources may represent attractive alternative candidates, with potential use cellular therapy, giving new perspectives for developmental biology and regenerative medicine.
Objectives: The aim of this work was isolate and characterize horse stem cells from the yolk sac (YS) and amniotic membrane (AM), and to define the biological properties of these cells.
Methods: The vitelline and amniotic fragments were cultured in α-MEM, supplemented with 20% fetal bovine serum, 1% nonessential amino acids, 1% glutamine and 1% antibiotic, incubated at 37 ° C and 5% CO2.
Results: The stem cells isolated from the YS and the AM, in the fifth passage (P5), showed normal karyotype ($2n = 64$). The cells from YS membrane showed a progressive growth, not constant until the P5, and start decline in P10. For the AM, the cell population showed progressive growth with peaks, until the P8, and a marked decline in P10. In immunocytochemistry, the yolk sac cells and amniotic membranes showed positive reaction for: Oct3/4, Nanog, SSEA-3, vimentin, cytokeratin 18 (CK18) and PCNA3. Either the YS as the AM expressed the markers CD45, CD105, Oct3/4, Nanog, CD34 and Stro-1. Both cell lines demonstrated the capacity to differentiate into adipocytes, osteocytes and chondrocytes and had no carcinogenic potential in NUDE mice.
Conclusion: Based on the results above, the cells of the yolk sac and amniotic membrane, showed plasticity and potential to cell differentiation. In addition, these cells could be an interesting source and a valuable for future assays involving cellular therapy in Regenerative Veterinary Medicine.

P1.84

DIFFERENTIAL EFFECTS OF PROGESTERONE ON COX-2 AND MN-SOD EXPRESSIONS ARE ASSOCIATED WITH HISTONE ACETYLATION STATUS OF THE PROMOTER REGION IN HUMAN ENDOMETRIAL STROMAL CELLS

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Objective: In the human endometrium, endometrial stromal cells (ESC) are exposed to cytokines. Cyclooxygenase-2 (COX-2) is a cytokine-inducible enzyme controlling synthesis of prostaglandins, which are responsible for endometrial shedding. Manganese superoxide dismutase (Mn-SOD) is another cytokine-inducible gene, and protects cells by scavenging superoxide radicals. ESC are also regulated by progesterone during the secretory phase. The present study investigated the mechanism by which TNFα and progesterone affect the expression of COX-2 and Mn-SOD in ESC.
Methods: ESC were incubated with TNFα and progesterone. COX-2 and Mn-SOD mRNA expressions were determined by real-time RT-PCR. NF-κB binding to the promoter region or histone acetylation status of the NF-κB response element was analyzed by a chromatin immunoprecipitation (ChIP) assay.
Results: TNFα increased COX-2 and Mn-SOD mRNA levels. Progesterone (10^{-6} M) suppressed TNFα-induced COX-2 mRNA expression whereas TNFα-induced Mn-SOD expression was not inhibited by progesterone. The inhibitory effect of progesterone was abolished by knock-down of progesterone receptors by siRNA. ChIP assay revealed that TNFα increased NF-κB binding at both the COX-2 promoter and the Mn-SOD enhancer, and that progesterone only inhibited the NF-κB binding at the COX-2 promoter. The histone acetylation level of the NF-κB response element of the Mn-SOD enhancer was lower than that of the COX-2 promoter. However, when histone acetylation was induced by histone deacetylase inhibitors, progesterone inhibited the TNFα-induced NF-κB binding to the Mn-SOD enhancer.
Conclusions: TNFα increased COX-2 and Mn-SOD expression via NF-κB activation. Progesterone inhibited COX-2 expression by inhibiting the binding of NF-κB to its response element, but did not inhibit TNFα-induced Mn-SOD expression. The gene-specific action of progesterone may be due to the difference in chromatin structure at the NF-κB response elements in the COX-2 promoter and Mn-SOD enhancer.

P1.85

BIRTH WEIGHT AND DIFFERENCES OF 11β-HYDROXYSTEROID DEHYDROGENASE TYPE 1 AND 2 GENE EXPRESSION IN PLACENTAS OF JAPANESE WOMEN ENROLLED IN HAMAMATSU BIRTH COHORT FOR MOTHER AND CHILDREN STUDY (HBC)

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Objectives: Fetal exposure to maternal glucocorticoids is hypothesized to affect various aspects of fetal growth and development; however, the long-term effect is yet to be fully clarified. The 11β- hydroxysteroid dehydrogenase (HSD)s enzymes regulate maternal as well as fetal glucocorticoids activity in the placenta and play a pivotal role in the determination of glucocorticoids activities in fetal circulation all though the course of fetal development. The 11β-HSDs have two isoenzymes; 11β-HSD1 forms active cortisol from inactive cortisone, while 11β-HSD2 works inversely. The purpose of the present study is to investigate the association between the gene expression of 11β-HSD1 and 11β-HSD2 in placentas and birth weight, infantile growth and development.
Methods: Forty-two placentas were collected from Japanese pregnant women during cesarean section due to obstetrical indication at 31-40 week of gestation. They were enrolled in Hamamatsu Birth Cohort for Mother and Children Study (HBC) and growth and development of their children are going to be followed until 4 years old under informed consent. Each birth weight was assessed by the formula of [(birth weight)-(mean birth weight)] / standard deviation (SD) of body weight = Z-score at birth by using centile charts for birth weight for gestational age in Japanese singleton births. 11β-HSD1 and 2 gene expression was measured by quantitative RT-PCR
Results: The 11β-HSD1 mRNA levels were positively correlated with Z-scores of birth weight ($r=0.30$, $p<0.05$), but not placental weight. By contrast, 11β-HSD2 mRNA levels correlated with Z-scores of neither birth nor placental weight.
Conclusion: Positive correlation was observed between birth weight and gene expression of 11β-HSD1, suggesting a possibility that placental metabolism of glucocorticoids might take part in the regulation of fetal, not placental, growth. The association of placental expression of 11β-HSD1 gene and infantile growth and development is now under investigation in HBC study team.

P1.86

THROMBIN ENHANCES SOLUBLE FMS-LIKE TYROSINE KINASE 1 EXPRESSION IN TROPHOBLASTS — POSSIBLE INVOLVEMENT IN THE PATHOGENESIS OF PREECLAMPSIA

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Objectives: To investigate the possible impact of thrombin on soluble fms-like tyrosine kinase 1 (sFlt-1) expression in trophoblasts.
Methods: **T**rophoblast cell line (HRT-8/SVneo, H8) was treated with thrombin, protease-activated receptor 1 (PAR-1) specific agonist SFLLERN and thrombin antagonist PPACK. mRNA expression of slft-1, Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PlGF) in trophoblasts using real-time PCR. The secretion of sFlt-1, VEGF and PlGF protein from trophoblasts, using Enzyme-Linked Immuno Sorbent Assay (ELISA).
Results: Administration of thrombin (10U/ml) and PAR-1 specific agonist SFLLRN (300 μM) increased sFlt-1 mRNA expression (4.24 ± 0.74 and 4.21 ± 0.79 fold, respectively, $P < 0.05$ for both) and protein secretion (5.08 ± 0.42 fold, $P < 0.001$ and 1.89 ± 0.16 fold, $P < 0.05$, respectively) in H8. The induction of sFlt-1 protein secretion by thrombin was dose dependent. The effect of thrombin was completely reduced by thrombin inhibitor PPACK. Thrombin increased mRNA expression of VEGF ($P < 0.05$) but did not change VEGF secretion and PlGF mRNA expression and secretion.
Conclusion: During placental development, thrombin, generated in the local hemorrhage in the utero-placenta increase trophoblasts expression of sFlt-1. Consequently, thrombin may contribute to the pathogenesis of preeclampsia.

P1.87

EFFECT ON THE PRODUCTION OF PLGF AND SVGFR1 FROM PRIMARY TROPHOBLAST BY CYTOKINE AND TLR LIGAND

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Objectives: The TNF-α and IFN-gamma such cytokines play an important role in maintenance of the pregnancy and the abnormal pregnancy. Tool like receptors also innate immune response during pregnancy. PlGF is a vascular endothelial growth factor derived from placenta. sVEGFR1 is its soluble receptor and regulate the PlGF function. To elucidate action of cytokine and TLR ligand to angiogenic and antiangiogenic factor in trophoblast, we examined the effect on the production of PlGF and sVGFR1 from primary trophoblast by cytokine and TLR ligand
Methods: Villous tissues were obtained from healthy pregnant women aged 16 to 36 years who asked artificial abortion at 7 to 11 weeks' gestation with the informed consent. The trophoblasts were isolated from early pregnant villous tissues and cultured with serum-free medium. Subsequently, trophoblasts were treated with TNF-α and IFN-gamma and TLR ligand (TLR 1-9) for 24 hours. The levels of PlGF and sVEGFR1 were measured by ELISA.
Results: The production of PlGF in the primary trophoblast increased by adding TNF-alpha and INF-gamma. The sVEGFR1 production increased by adding INF-gamma. Increased sVEGFR1 was found by adding TLR-9 ligand.
Concluson: The TNF-alpha and INF-gamma may promote PlGF and sVEGFR1 production. Moreover TLR ligand also may relate the production of sVEGFR1. Cytokines such as TNF-alpha and INF-gamma and TLR ligand may work for placentation and maintenance of pregnancy.

P1.88

EFFECT ON THE PRODUCTION OF SOLUBLE ENDOGLIN FROM TROPHOBLAST AND CHORIOCARCINOMA CELLS BY IGG TAKEN FROM PREECLAMPSIA SERA

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Objectives: The soluble endoglin (sEng) is an antiangiogenic protein that may inhibit TGF-β1 signaling and endothelial nitric oxide synthase activation in endothelial cells. The levels of sEng increased in sera obtained from preeclampsia. The factors that increase the sEng in preeclampsia have not been known well. In preeclampsia many autoantibodies such as anti phospholipid, anti endothelial antibody, anti angiotensin receptor agonistic antibody and others were reported. To investigate the factors that may increase sEng in preeclampsia, we examined the effect of IgG taken from preeclampsia sera on the production of sEng and expression of sEng mRNA from trophoblast and choriocarcinoma cells.
Methods: Serum samples were taken from women with normal pregnancy and with preeclampsia. IgGs were elluted by Protein G affinity chromatography. Villous tissues were obtained from healthy pregnant women who had artificial abortion from 7 to 11 weeks' gestation with the informed consent. The primary first trimester trophoblast and choriocarcinoma (JEG-3) cells were cultured with IgG fraction for 24 hrs, and the sEng levels in supernatants and expression of sEng mRNA in those cells were evaluated.
Results: The addition of preeclampsia IgG into primary trophoblast and JEG-3 cells led to increased release of sEng. IgG fraction obtained from preeclampsia sera increased the expression of sEng mRNA in JEG-3 cells.
Conclusion: The results suggest that the IgG taken from preeclampsia sera may increase the protein production of sEng and mRNA expression of sEng from trophoblast and JEG-3 cells without hypoxia. IgG fraction in preeclampsia sera may play a role of high level of serum sEng in preeclampsia patients.

P1.89

INTERPLAY BETWEEN IGF AND EGF SIGNALING DURING PROLIFERATION AND MIGRATION OF BOVINE PLACENTAL CELLS IN VITRO

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Objectives: Growth, invasion and angiogenesis are important processes in placentae of different species which are regulated by growth factors such as EGF (epidermal growth factor) and IGFI/II (insulin-like growth factor I/II). Like cancer cells, bovine trophoblast cells undergo epithelial-mesenchymal-transition thereby differentiating into migrating trophoblast giant cells which invade the caruncular epithelium. As motility and proliferation are basic mechanisms for the restricted invasion we have investigated the influence of IGFI, IGFII and EGF (alone and in combination) on these events.
Methods: Bovine caruncular epithelial cells (BCEC-12), bovine placental fibroblasts and bovine trophoblast cells (F3) were stimulated with IGFI (50/100ng/ml), IGFI+EGF (50/100+20ng/ml), EGF (20ng/ml), IGFII (50/100ng/ml) and IGFII+EGF (50/100+20ng/ml). Cell proliferation was examined by MTT-Assay, motility by live-cell-imaging and signal transduction by phosphorylation-analysis of MAPK (mitogen-activated protein kinase) 42/44. Statistical significance was confirmed using Turkey's difference test ($p<0.05$).
Results: We observed a significant increase in proliferation after stimulation with IGFI, IGFI+EGF and IGFII+EGF in all cell lines. Incubation with IGFII elevated proliferation of F3 and fibroblasts, whereas EGF alone only had a significant effect on F3. A significantly enhanced motility occurred after stimulation with IGFI or IGFII+EGF in all three cell lines; after treatment with EGF or IGFI+EGF in BCEC-12 and F3 and due to IGFII treatment in BCEC-12 and fibroblasts. Western Blot revealed that stimulation with EGF, IGFI+EGF and IGFII+EGF increased MAPK phosphorylation in all cell lines, though to a highly varying degree. IGFI showed an activating effect in F3 and fibroblasts. No increase in phosphorylation was observed for IGFII.
Conclusion: Our in vitro study provides evidence that IGFI/II and EGF play an important role in proliferation and motility in bovine placental cells suggesting a similar action in vivo. Further analysis of the interplay between the IGF- and EGF-systems will help to understand the delicate regulation of placenta development and function.

P1.90

CHARACTERIZATION OF THE INSULIN-LIKE GROWTH FACTOR (IGF) SYSTEM IN THE UTEROPLACENTAL UNIT OF COWS NEAR TERM

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Objectives: Fertility of high producing dairy cows is frequently compromised by post partum metritis since uterine and ovarian function are affected by the ongoing infection. Despite a common bacterial contamination of the uterus after parturition, only a subgroup of the cows develops clinical signs of a metritis. This is regularly followed by longer calving intervals, which have been associated with a negative energy balance and altered blood insulin-like growth factor-I (IGF-I) concentrations. Consequently, we aimed to examine whether IGF-I could be involved in the development of metritis, and if there is a correlation between blood IGF-I level and local uteroplacental expression of IGF system members.
Methods: Placentomal (P) and interplacentomal (IP) tissue samples were taken at caesarean sections on day 275 p.i. from 17 healthy, pregnant Holstein Friesian cows, which had a similar body condition score. Groups were formed according to the prepartum blood IGF-I levels (IGF-I high [n=10] and IGF-I low [n=7]). IGF-I, IGF-II, IGF1R, IGF2R, and IGF binding proteins (BP) -2, -3, and -7 were localized via immunohistochemistry in P and IP regions.
Results: In P areas IGF-II, IGF2R, IGFBP-2, and IGFBP-7 were mostly localized in the maternal epithelium, whereas IGF-I occurred mainly in the maternal stroma. The expression of IGFBP-3 was ubiquitous in this region, while IGF1R was primarily found in fetal and maternal blood vessels. In IP areas all analyzed proteins predominantly immunostained the maternal luminal and glandular epithelia. IGF-I alone was also found in maternal stroma. No differences in expression were observed between the IGF-I high and IGF-I low groups.
Conclusion: In conclusion, IGF-I blood levels were not related to the local expression patterns of the IGF system members. Specific functions in the uteroplacental unit are supported by the distinct localization of IGF system components in particular cell types. *Funded by Pfizer Inc.*

P1.91

STRUCTURAL AND FUNCTIONAL ANALYSIS OF RARE MISSENSE MUTATIONS IN HUMAN CHORIONIC GONADOTROPIN β-SUBUNIT

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Objectives: Heterodimeric human chorionic gonadotropin (hCG) is one of the key hormones determining early pregnancy success. We have previously identified rare missense mutations in hCGβ genes (*CGB5* p.Val56Leu, *CGB8* p.Arg8Trp and p.Pro73Arg) with potential pathophysiological importance. Current study aimed to determine the prevalence of these mutations in Northern Europe and assess their structural and functional consequences by applying a combination of *in silico* and *in vitro* approaches.
Methods: Carrier status of each mutation was determined for 655 recurrent miscarriage (RM) patients and 431 healthy controls from Northern Europe using PCR-RFLP. The impact of the mutations on the protein dynamics and assembly of hCG was predicted with *in silico* structural and molecular dynamics analysis. The dimerization quality of mutated and wild-type hCGβ was assessed *in vitro* using co-immunoprecipitation and immunoassays. Bioactivity of the hCG variants was estimated by measuring hLH/CG receptor mediated cAMP signaling.
Results: Mutation *CGB5* p.Val56Leu was identified in a single heterozygous RM patient and it caused a structural hindrance in the hCGα/β dimerization. Although only 10% of the mutant hCGβ was assembled into intact hCG compared to the wild-type, a stronger signaling response was triggered upon binding to its receptor, thus compensating the effect of poor dimerization. Mutation *CGB8* p.Pro73Arg was found with equal frequency (0.46%) among RM patients and controls. Approximately 50% of secreted p.Pro73Arg β-subunits acquired an alternative conformation but biological activity of assembled hCG remained unaffected. For the *CGB8* p.Arg8Trp substitution, the applied methods revealed no structural or functional alterations.
Conclusions: Although two studied mutations alter the conformation or assembly of hCG, the overall functional characteristics of the hormone remain unaffected. Considering our and previously published data on missense mutations in *CGB* genes, we propose that only mutations with neutral or mild functional consequences might be tolerated in the major *hCGβ* genes *CGB5* and *CGB8*.

P1.92

FETAL GROWTH RESTRICTING EFFECTS OF A SINGLE COURSE OF ANTENATAL BETAMETHASONE IN WOMEN: ROLE OF HUMAN PLACENTAL LACTOGEN

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Objectives: Betamethasone (BET) is widely used as a treatment of women who are at high risk of preterm birth. In sheep BET-induced growth restriction was associated with reduced placenta lactogen (PL), a key regulator of fetal growth. We therefore investigated antenatal BET treatment effects in humans on birth weight and PL.
Methods: BET (single course of 2x12mg, normally grown fetuses between 23+5-34+0wks) exposed women (n=46) who delivered between 23+5-42+0wks were compared to gestational age-matched controls (n=50). Maternal blood samples were obtained before, during and after BET treatment and at the time of birth. BET effects on fetal anthropometrics, placental morphometry and placental PL protein levels were analysed by univariate analyses of variance with gestational age and treatment as cofactors.
Results: BET significantly decreased birth weight -16.8%, head circumference -8.6% and body length -6.0% compared to controls. Placental basal plate size -18.2% and placenta thickness -11.1% were significantly reduced after BET treatment in fetuses born $\leq$37+0wks. Placenta weight was unaffected. BET treatment significantly reduced PL-positive syncytiotrophoblast nucleus number but increased nucleus circumference +6.2% and nucleus surface area +12.3% in central regions of the placenta compared to controls. These changes were associated with BET induced increases in placental PL protein levels +11.1%. Maternal plasma hPL levels were not affected. Birth weight only weakly correlated with maternal plasma PL levels.
Conclusion: A single course of BET treatment reduced birth weight and affected placental size and thickness. Similar to our sheep model, reduced PL syncytiotrophoblast nucleus number was associated with an increase in cell size, and increased PL protein levels in the placenta but did not result in increased placental hPL output into the maternal circulation. PL is a key regulator in both placental and fetal development late in gestation and appears to be vulnerable to GC exposure at this time.

P1.93

PROTEOMICS ANALYSIS OF ESTROGEN RESPONSIVE MITOCHONDRIAL PROTEIN *S*-NITROSYLATION VIA MITOCHONDRIAL ENOS IN HUMAN UMBILICAL CORD VEIN ENDOTHELIAL CELLS

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Objectives: Mitochondria are known to be the primary subcellular organelle that nitric oxide (NO) targets in many cells. We have recently shown that estrogens stimulate dynamic protein S-nitrosylation (SNO) in endothelial cells. However, the effects of estrogens on global mitochondrial protein SNO are unknown. In this study, we determined if estrogen stimulates mitochondrial protein SNO and if yes, to identify the estrogen responsive mitochondrial *nitroso*-proteins and to test if it occurs via mitochondrial endothelial NO synthase (eNOS).
Methods: Human umbilical cord vein endothelial cells (HUVEC) were treated with estradiol-17β (E2, 10 nM) or nitrosoglutathione (GSNO, 1mM) for 20 min. The cells were fixed and SNO-proteins were labeled by biotin switch (BST) and visualized with fluorescently labeled subcellular organelle trackers by fluorescence microscopy. Mitochondrial proteins were purified using the magnetic microbeads and subjected to BST. Levels of SNO proteins were determined by immunoblotting with anti-biotin antibody. The biotin-labeled mitochondrial SNO proteins were tryptically digested for capturing the biotin-labeled SNO-peptides using avidin-coated beads and identified by Mass Spectrometry. Mitochondrial, membrane, or Golgi targeted eNOS was overexpressed in HUVEC for investigating the effects of subcellular NO production on E2-stimulated SNO.
Results: E2 and GSNO significantly stimulated mitochondrial protein SNO. Mitochondria were mainly labeled *in situ* with the greatest protein SNO response to estrogen and GSNO. Proteomics analysis of the SNO-peptides identified 12, 33 and 57 SNO-proteins in the mitochondrial proteomes in control, E2- and GSNO- treated HUVEC, respectively. Function analysis suggested that SNO-proteins are associated with various mitochondrial functions, including apoptosis, energy and redox regulation, iron homeostasis, *etc*. E2 stimulated protein SNO was enhanced by overexpression of mitochondrial or Golgi, but not membrane, targeted eNOS in HUVEC.
Conclusion: Estrogen rapidly stimulates protein SNO in endothelial mitochondria via mitochondrial eNOS, implicating mitochondrial protein SNO to be crucial for mediating the vasoprotective effects of estrogens.

P1.94

CHANGES IN BLOOD MACROPHAGE COLONY-STIMULATING FACTOR CONCENTRATION AFTER ARTIFICIAL INDUCTION OF ABORTION IN JAPANESE BLACK COWS

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Objectives: Macrophage colony-stimulating factor (M-CSF) appears to play an important role in the maintenance of pregnancy in cows. Blood M-CSF concentration varies with the day of pregnancy in normal pregnant cows. The objective of this study was to investigate the changes in blood M-CSF concentration and the relationship between M-CSF and sex steroid hormone concentrations after artificial induction of abortion during the middle period of pregnancy in cows.
Methods: Five pregnant Japanese Black cows were used in this study. They were administered cloprostenol (PGA) to induce abortion between 91–103 days of pregnancy. The fetal status was monitored by ultrasonography, and blood was collected for estimating M-CSF, estrogen (E2) and progesterone (P4) concentration. M-CSF concentration was measured by the enzyme-linked immunosorbent assay and E2 and P4 concentrations were measured by time-resolved fluorometry. M-CSF concentration was taken as a response variable, while the number of days after initial PGA administration, E2 and P4 concentrations, and ovarian dynamics (the growth and regression of corpus luteum and dominant follicle) were taken as explanatory variables. The relationship between M-CSF concentration and explanatory variables was analyzed using a generalized linear model assuming that the response variable was followed by gamma distribution and a logarithmic link function.
Results: The pattern of the changes in M-CSF concentration varied in the cows. The relationship between explanatory variables and M-CSF concentration was different for each cow.
Conclusion: Blood M-CSF concentration may not change with a certain pattern after artificial induction of abortion. Furthermore, P4 and E2 concentrations and ovarian dynamics may not play a central role in changing blood M-CSF concentration after artificial abortion induction.

P1.95

RAP1, A SMALL GTP BINDING PROTEIN, MEDIATES EGF AND HB-EGF SIGNALING AND REGULATES EGF RECEPTOR IN AN EXTRAVILLOUS TROPHOBLAST CELL LINE

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Extravillous trophoblasts (EVT) invade into endometrium to establish the feto-maternal interaction which is essential for maintenance of pregnancy and fetal development. Epidermal growth factor (EGF) and heparin-binding EGF-like growth factor (HB-EGF) bind to their receptors (EGFR) and stimulate EVT proliferation and invasion through activation of phosphoinositol-3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK) signaling pathways.
Objective: We examined possible roles of small GTP-binding protein Rap1 in EGF or HB-EGF-mediated EVT proliferation and invasion.
Methods: EVT cell line (HTR8/SVneo) was stimulated with EGF or HB-EGF and Rap1 activation (GTP-Rap1) was examined by pull-down assay. Effect of siRNA mediated Rap1 knockdown on serum-, EGF- or HB-EGF-stimulate EVT proliferation and invasion was assessed by the WST-8 assay and the transwell assay, respectively. In addition, effect of Rap1 knockdown on the activation of EGF signaling pathways and the expression of EGFR were examined by immunoblotting and real-time RT-PCR.
Results: EGF or HB-EGF increased the amount of active form of Rap1 (GTP-Rap1) in HTR8/SVneo. Serum-, EGF- or HB-EGF-stimulated proliferation and invasion were significantly abrogated in Rap1 siRNA transfected cells. Knockdown of Rap1 inhibited EGF- or HB-EGF-induced phosphorylation of AKT, ERK1/2 and p38MAPK. Furthermore, Rap1 knockdown significantly decreased EGFR protein level but not its mRNA level.
Conclusions: These results suggest that Rap1 may function as a mediator of EGF signaling pathway and a regulator of EGFR protein level in EVT.

P1.96

THE EFFECT OF STEROID HORMONES ON THE PROLIFERATION ABILITY AND ANGIOGENIC DIFFERENTIATION OF HUMAN PLACENTA-DERIVED MESENCHYMAL STEM CELLS

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Objectives: Gender difference seemed to exist in MSCs on the recovery from cardiac infarction, acute inflammation, and osteogenic differentiation. In general, female-derived MSCs have better cell proliferation and cell differentiation ability compared with male-derived MSCs. Limited information was available to explore the effect of sex hormones on MSCs' ability of angiogenic differentiation. Therefore, we explored if sex hormones and their antagonists interfere the cell proliferation and angiogenic differentiation abilities of human placenta-derived MSC (PDMSCs).
Materials and Methods:

(A) **Sex hormones' effects on MSC proliferation:** PDMSCs were isolated from human term placenta tissue, and were used to assess the characteristics of MSC at passage 5. PDMSCs were exposed to culture medium (DMEM-LG) supplemented with different concentrations of sex hormones. When cell growth reached subconfluence, cell number was analyzed by cell counting.
(B) **Induction of MSC into angiogenic differentiation:** P5-PDMSCs were cultured with induction medium (EGM-2) supplemented with different concentrations of sex hormone for 14 and 21 days. Total RNA was extracted, reverse-transcribed to cDNA and quantified as standard procedure. KDR, vWF, CD31 were used as markers of angiogenic differentiation by real-time PCR.

Results:
In this study, our findings are summarized as below:

(1) Although estrogen did not promote MSC proliferation, its antagonist (ICI-182 780) inhibited cell proliferation. Both estrogen and its antagonist did not have significant effect on angiogenic differentiation of PDMSCs. (Fig 1)
(2) Progesterone promoted cell growth, whereas its antagonist (mifepristone) inhibited cell proliferation (Fig 2). Progesterone, in another way, inhibited angiogenic differentiation, but mifepristone reversed the effect and further promoted MSCs' angiogenic differentiation. (Fig 3A, 3B)
(3) Testosterone increased proliferation of PDMSCs, and also enhanced angiogenic differentiation. (Fig 4A, 4B)

Conclusion: Sex hormones (estrogen, progesterone and androgen) promote cell proliferation of PDMSC in some situations and interfere their angiogenic differentiation. Progesterone inhibited angiogenic differentiation of PDMSC, but testosterone enhanced the ability. In conclusion, sex hormones affect the abilities of cell proliferations and angiogenic differentiation of PDMSC in different way.

P1.97

SYNEPITHELIOCHORIAL PLACENTAL INTERACTIONS IN EGFP CLONED CATTLE MODEL

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Objective: To investigate the mechanisms by foetal cells interact and transfer their contents to the maternal compartment in the bovine placenta using a model of cloned transgenic enhanced Green Fluorescent Protein (eGFP) expressing bovine embryos.
Methods: EGFP-expressing embryos were produced and transfer into surrogate cow. The uteri were surgically recovered at days 60 (N=3) and 90 (N=3) of pregnancy. Placentome/endometrium samples and maternal peripheral blood leukocyte (PBL) samples were collected to assess the presence of fDNA by nested-PCR for eGFP and testis-specific Y-encoded protein (TSPY), immunohistochemistry, transmission electron microscopy and western blotting analysis.
Results: eGFP DNA was present in PBL at day 60 and 90 of pregnancy confirming that there is transplacental transfer of fDNA to the mother. Interestingly, eGFP was found to be present at protein and DNA levels in the intercaruncular epithelium, suggesting that eGFP is delivered to the maternal side even in the regions where the cells are loosely attached. Moreover, at local level eGFP was highly expressed by the trophoblast; however it was also present in the uterine epithelium (UE) facing the trophoblast. In the arcade zone at the syncytial plaques, the expression of eGFP by both tissues was less intense than in the neighbouring cells. Our eGFP construct possessed an ubiquitin promoter. Ubiquitin is not expressed by invasive cells in human, which might explain the weak staining of eGFP in the syncytial plaques. Additionally, trophoblast cells closer to UE showed decreased cytoplasmic area and flattened nucleus, suggesting that maternal cells respond to foetal signals undergoing to apoptosis.
Conclusion: Our results showed that the eGFP is delivered to maternal side at local and systemic levels. Since eGFP does not have a signal peptide, it is suggested that eGFP could be delivered to the maternal side through transtrophoblastic water channels that are present in the ruminant placenta.

P1.98

IGF-II ANALOGUE ENHANCES PLACENTAL EFFICIENCY & REDUCES FETAL WEIGHT DISTRIBUTION IN NORMAL MICE

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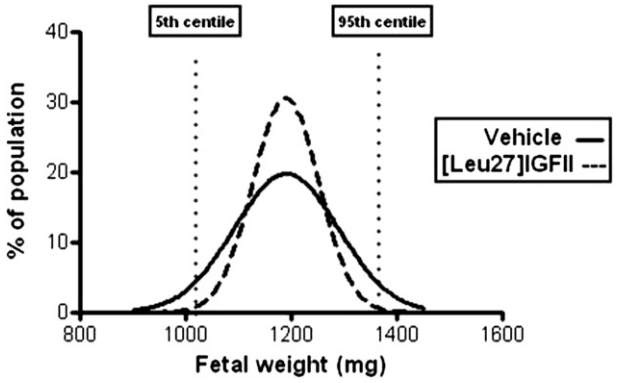
Objectives: Insulin-like growth factors (IGF-I & IGF–II) and their cell surface receptors (IGF-R1 & IGF–R2) are critical regulators of fetal and placental growth. Administering IGF-I or –II in pregnancy appears an attractive therapeutic strategy for improving outcomes in fetal growth restriction (FGR), but IGF receptors are ubiquitously expressed, with potential off-target effects. An alternative approach is to enhance the efficacy of endogenous IGF already present at the materno-fetal interface. This could be achieved by treatment with Leu^{27}IGF-II, an IGF-II analogue that binds the IGF-R2 clearance receptor, thus blocking IGF-II removal, allowing IGF-II to bind IGF-R1, thereby promoting its downstream effects. Previous studies in the guinea pig suggest that such treatment improves fetal weight (Sferruzzi-Perri *et al* 2008). Here we have investigated the effects of constant Leu^{27}IGF-II infusion into C57BL/6 mice from mid-late gestation.
Methods: 1mg/kg/day Leu^{27}IGFII (n=10 pregnancies, 80 fetuses) or vehicle (n=12, 83 fetuses) was delivered via a subcutaneous mini-osmotic pump from E12.5-E18.5, at which time fetal and placental weights were recorded. Frequency distribution curves of fetal weight were produced and values calculated for 5th and 95th centiles. Fetuses below/above these weights were deemed SGA or LGA, respectively, as per the human criterion.
Results: Leu^{27}IGFII treatment reduced fetal weight distribution, by reducing the number of fetuses below the 5th centile (6 vs. 2) and similarly reducing those above the 95th centile (5 vs. 0) (see figure). Placental weights were significantly decreased in the treated group (83.2 ±2.4mg treated vs. 90.1 ±2.6mg vehicle, $p<0.05$), and an exaggerated fetal:placental weight ratio ($p<0.05$) suggested enhanced placental efficiency.



Conclusion: These results indicate that although smaller, the placentas of Leu^{27}IGFII-treated C57 mice have greater capacity for supporting normal fetal growth. The reduction in SGA and LGA fetuses post-treatment supports the potential use of this analogue as a means of standardising fetal growth.

P1.99

PLACENTAL DEVELOPMENT IN *NECROMYS LASIURUS* (RODENTIA, CRICETIDAE) - FUNCTIONAL MORPHOLOGY USING STEREOLOGICAL APPROACH

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The mature chorioallantoic placenta in rodents is organized in labyrinth zone (Lz), junctional zone (Jz), and decidua (Dd). Within rodents, the placental development of murids has been well described but that of cricetids remains less investigated.
Objectives: We described the development of the main placental regions in a wild Brazilian rodent *Necromys lasiurus*.
Methods: The volume fraction (VF) of different cell types which constitute the labyrinth was quantified by the one stop stereology using 31 placentae at different stages of gestation. In addition, we investigated the volume changes of the Lz, Jz, and Dd in samples of early gestation (10-11 days, n=3), mid-gestation (15-16 days, n=4), and near term (21 days, n=3) using the Cavalieri principle. 5 μm sections were stained using H&E and fetal vessels were localized by immunohistochemistry using vimentin. Samples were analyzed using the Mercator® software and statistical analysis (one-way ANOVA and Mann and Whitney test) was performed using Graphpad Prism®.
Results: There was a significant increase in absolute placental volume from early (4.427±0.282mm^3) to mid-gestation (12.98±1.305mm^3, $p<0.01$), followed by a reduction near term (7.52±0.155mm^3, $p<0.05$). The absolute volume of the Lz, Jz, and Dd followed this same trend. The VF of the labyrinth components (fetal vessels, maternal compartment, giant cells and trophoblast) differed according to gestational age with a continuous increase in the proportion of fetal vessels from day 10 (7.38±0.49%) to 21 (34.05±0.876%, $p<0.01$) and in that of giant cells from mid- (11.66±0.286%) to late gestation (19.26±1.344%, $p<0.01$). In contrast, the VF of trophoblast decreased from mid- (37.29±1.013%) to late gestation (21.16±0.658%, $p<0.01$). There was no significant variation in the VF of maternal compartment throughout pregnancy.
Conclusion: Although the placental volume in *N. lasiurus* is smaller than murine placenta, the dynamics of placental development appears similar between these rodents.

P1.100

EARLY YOLK SAC DEVELOPMENT IN *GALEA SPIXII* (RODENTIA, CAVIIDAE)

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Placentation in caviomorphs is characterized by key characters such as a yolk sac that is inverted and temporarily associated to the uterus and placenta. However, ground laying processes are not well understood.
Objectives: Herein, we investigated the yolk sac inversion in a small cavy native to Brazil, the preá *Galea spixii* (Wagler, 1831).
Methods: Data were obtained from 30 females of gestational days 6, 10-15, 20, 25, and 30. Samples were investigated by means of histology (haematoxilin and eosin, Toluidine blue and periodic acid Schiff), immunohistochemistry for vimentin (1:200) and PCNA (1:800), and scanning and transmission electron microscopy.
Results: Implantation had been done on day 6, resulting in an inner embryoblast and the outer trophectoderm. From day 10 onward, an ectoplacental cone as precursor of the placenta and the first development of hypoblast cell groups as precursors of the yolk sac tissues were present. Following implantation, a rapid differentiation of the yolk sac tissues took place in the blastocysts. At day 13, both the visceral and parts of the parietal yolk sac layers occurred, surrounding a yolk sac cavity. On day 14, an exposition of the visceral yolk sac endoderm to the outside was present, which characterizes yolk sac inversion, associated with degeneration of the parietal endoderm. The visceral yolk sac near the chorioallantoic placenta became highly villous, proliferative, vascularized and attached to the uterus and placenta.
Conclusion: *Galea* showed the caviomorph conditions in blastocyst development as well as in the nature and position of the definitive yolk sac. However, data suggest that the early yolk sac development in caviomorphs is more diverse than thought before.

P1.101

DEVELOPING THE BRAIN: A POTENTIAL ROLE FOR THE PLACENTA IN HOMININ BRAIN EVOLUTION?

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Objectives: Of all primates, humans produce the largest-bodied and –brained neonates, relative to maternal size. This suggests that the intrauterine supply of nutrients available to the developing fetus by the placenta has increased or been made more efficiently available in hominins compared to other primates. The monkeys, apes, and humans all have hemochorial placentas: within that framework, do they differ from one another in ways that could explain differences in fetal brain growth?
Methods: 1) We compared descriptions and consequences of placental invasion and vascular remodeling across primate taxa. 2) To calculate placental efficiencies at term we performed literature searches to extract data regarding placental, neonatal body, and brain weights in as many primate taxa as we could find. 3) We compared the magnitude in increases in efficiency across late gestation between humans and vervet monkeys. 4) We compared increases in villous surface area relative to fetal growth across late gestation between humand and vervet monkeys.
Results: Several lines of evidence suggest that human and ape placentation is more invasive than monkey placentation. Apes and humans, the largest-brained primates, have the most efficient placentas, both in terms of supporting overall fetal growth and brain growth specifically. While placental efficiency increases across gestation for both the vervet and the human, the magnitude is significantly greater in humans (65% vs. 48%). It takes 8 times as much surface area to build one gram of human fetus as it does vervet fetus (4.0 mm^2 vs. 0.5 mm^2, $p<0.0001$).
Discussion: Metabolic investment in the human fetal brain is high. These preliminary findings suggest that the more expensive the fetal brain, the more the hemochorial placenta exploits existing mechanisms (i.e. invasiveness, efficiency, surface area) to enhance energetic investment.

P1.102

REDUCED TAUT IN PRETERM SYNCYTIOTROPHOBLAST MAY ACCOUNT FOR REDUCED PLACENTAL TAURINE AND POTENTIAL INCREASED NEURODEVELOPMENTAL RISK IN PRETERM INFANTS

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Background: The amino acid taurine promotes neuronal growth and development before and after birth. The placenta actively transports taurine from maternal blood to the fetus via a taurine specific transporter protein (TauT). Oxidative stress and acute infections, the two most common causes of preterm birth, affect placental structure and/or function, and thus may result in reduced trans-placental taurine transfer and lower fetal taurine levels, increasing risk for poor neurodevelopmental outcome. Here we test the hypothesis that placentas delivered preterm, previously shown to have reduced taurine concentrations, have lower syncytiotrophoblast TauT protein compared to term controls.
Methods: Details of placental samples analysed included 12 term controls and 17 preterm (PT, 10 preeclamptic, 11 infected and 4 with both pathologies). TauT protein was detected by immunohistochemistry; slides were digitized. Three representative regions of interest (ROI) were extracted from the slides by a blinded reviewer. Segmentation was performed using linear discriminant analysis and normalized to villous area.
Results: The correlation between TauT and taurine level overall was 0.303. However, two distinct clusters are present in the scatter plot. Within each cluster, the correlations are 0.732 and 0.818 (p-values < 0.002) with linear regressions:
TauT $= 0.089758 \cdot$(taurine level) $+ 0.217548$,
TauT $= 0.109059 \cdot$(taurine level) - 0.165649.
In this pilot sample, differences in TauT normalized to villous area are seen as follows:
PT 0.35$\pm$0.19 Infected PT 0.31$\pm$0.17 Preeclamptic PT 0.34$\pm$0.20
Term 0.38$\pm$0.17 Non-infected PT 0.39$\pm$0.18 Non preeclamptic PT 0.37$\pm$0.16
Conclusion: Lower taurine concentrations in placentas delivered preterm is associated with reduced syncytial TauT expression. Compromised trans-placental taurine transfer capacity could limit taurine availability for neuronal development, increasing risk for poor childhood neurological function. In uncomplicated pregnancies, placental TauT expression and taurine concentrations are comparable between early and late gestation. Factors responsible for altered placental taurine transfer in preterm birth require further investigation.

P1.103

ROLES OF HTRA1 AND HTRA3 IN THE DEVELOPMENT OF PLACENTA

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Objectives: The HtrA (High Temperature Requirement Factor) family of secretory serine proteases has been found in different species ranging from bacteria to human. Mammals have four HtrA members, HtrA1-4. HtrA1 and HtrA3 are expressed most abundantly in placenta and have been implicated in placenta development. Both HtrA1 and HtrA3 were upregulated during implantation and first-trimester pregnancy at placentation in the decidua. Some previous researches showed that HtrA1 expressed in the ectoplacental cone around embryonic day 6.5-8.5, whereas HtrA3 expressed from day 8.5 to 12.5 in the deciduas in mouse. Deregulation of both HtrA1 and HtrA3 expression has been reported in human preeclampsia with Intra uterine growth restriction. But the molecular mechanism is yet to be discovered.
Methods: We developed HtrA1 -/-, HtrA3 -/- and HtrA1/3 double -/- mice. We isolated placentas from these KO mice on different gestational period and examined histology by various staining methods.
Results: Pups of HtrA1 -/-, HtrA3 -/- and HtrA1/3 -/- mice had lower body weight than pups from wild type mothers. Pups of HtrA1/3 -/- were smallest. The differences in body weight persisted for 1-2 months after birth. The HtrA1 -/-, HtrA3 -/- and HtrA1/3 -/- mice had shorter labyrinth and spongiotrophoblast layer compare with wild type mice. Vasculogenesis was decreased in these KO placentas and HtrA1/3 -/- placenta showed the most severe abnormality. We observed presence of large trophoblast islands in the labyrinth at embryonic day 14.5 placentas in all knockout mice, but not in wild type mice. Interestingly, we found some empty vacuole-like structure in the spongiotrophoblast layer of HtrA1 -/-, HtrA3 -/- and HtrA1/3 -/- mouse placentas at E 14.5, whereas those spaces were filled with spongiotrophoblast cells in wild type mice.
Conclusion: HtrA1 and HtrA3 are necessary for the normal development of placenta and embryo in mice, especially in vasculogenesis in the labyrinth and formation of mature junctional zone.

P1.104

TCFAP2C DEPENDENT TROPHOBLAST LINEAGE DIFFERENTIATION DURING PLACENTAL DEVELOPMENT

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Objectives: The placenta is the first organ to be formed during pregnancy and impairment of placental formation leads to various pregnancy complications. It has been shown that the highly conserved family of Activator Protein-2 (AP-2/Tfap2) is important for placenta development. We could demonstrate a marked downregulation of Tfap2c expression level in lineage development of trophoblast stem cells lacking connexin 31 (Cx31). This could cause the placental phenotype observed in Cx31 deficient mice. Since complete Tfap2c deficiency causes early embryonic death on day 7.5 of gestation due to a loss of extraembryonic cell lineage development we investigated mice heterozygous for Tfap2c.
Methods: To investigate the phenotype and genotype of the Tfap2c heterozygous placentas we performed immunohistochemistry of formalin-fixed and paraffin-embedded samples and investigated various expression levels with quantitative real time PCR.
Results: We found that the failure of an appropriate lineage development in *Cx31* deficient trophoblast stem cells is probably due to a reduced expression level of *Tfap2c*. To investigate the underlying mechanisms we have started to characterize the placentas of *Tfap2c* heterozygous mice which express reduced levels of *Tfap2c* and revealed enhanced resorption sites at ED 14.5 (52% compared to 5.7% in wild type matings). Phenotypic analysis of ED 14.5 placentas showed a reduced spongiotrophoblast layer which was identified by using the marker gene *tpbpa*. Moreover development of the labyrinth was impaired indicated by higher proliferation rates and large clusters of undifferentiated cells, both signs of delayed differentiation. The clusters showed staining for *tpbpa*, PAS and Ki67 which indicates an accumulation of glycogen rich and proliferative cell populations. In addition, staining for CD31 revealed less and poorly branched embryonic vessels in the labyrinth.
Conclusion: These findings point to an impaired trophoblast lineage differentiation in heterozygous *Tfap2c* mice leading to reduced spongiotrophoblast formation combined with a delay in labyrinthine development which could be the reason for embryonic lethality.

P1.105

MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) PRODUCED BY TROPHOBLAST CELLS AT MATERNAL-FETAL INTERFACE AS A POSSIBLE SURVIVAL FACTOR FOR DECIDUAL CELLS

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Previous studies in our laboratory showed the expression pattern of macrophage migration inhibitory factor (MIF) by trophoblast cells and, their receptors (CD44/CD74) by decidual cells during post implantation stages in mice. These results support the idea of decidual cells as potential targets of this cytokine. MIF plays relevant roles as pro-inflammatory factor and mediator of the immune system. Recently also, functions in cell survival and proliferation has also been demonstrated. In this context, we analyzed the possible role of MIF in mouse decidual cells in vitro. Decidual primary cell cultures were treated with mouse recombinant (mr) MIF with or without PI3K/AKT inhibitors (Wortmannin and LY294002). Cultures were examined by immunohistochemical reactions and Western blot after hydrogen peroxide treatment used as cell death inducer. MIF receptor complex CD74/CD44 was immunolocalized in the cultured decidual cells MIF. The addition of mrMIF reduced significantly the apoptosis levels and was positively correlated to the increase of pAkt expression. After hydrogen peroxide treatment, the apoptotic and necrotic levels increased significantly; apoptosis, however, was completely abolished when MIF was added to the assay. The levels of necrosis did not change after MIF treatment. Our results therefore indicate that MIF can reduce the spontaneous and induced apoptotic indexes in decidual cells, activating an AKT route, a presumptive pathway associated to cell survival in different cell types. Notably, this finding suggests a role for MIF in decidual homeostasis; insofar it ensures the integrity of the maternal-fetal interface.
Financial Support: FAPESP and CNPq

P1.106

THE POSSIBLE ROLE OF PIM KINASES (PIM1, PIM2 AND PIM3) UPON LIF STIMULATION IN TRANSFORMED TROPHOBLAST CELLS AND CHORIOCARCINOMA CELLS

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Objective: Proviral Integrations of Moloney (PIM) kinases belong to the family of serine/threonine kinases and in human are encoded by Pim1, Pim2, Pim3 genes which are associated with tumorigenesis especially in solid tumours and hematologic malignancies. The expression of PIM is mediated by the Signal Transducer and Activators of Transcription/Janus kinase STAT/JAK signalling pathway. PIM regulate cell progression and survival. PIM kinases are constitutively active and their activity supports *in vitro* tumour cell growth and survival. So far, PIM kinases have not been reported in trophoblast cells. Therefore, we aimed to identify PIM expression in trophoblast.
Methods: The techniques performed in this study were cell culture (HTR8/sv-neo and Jeg-3), LIF stimulation, Western blotting, and real time polymerase chain reaction (PCR). HTR8/sv-neo and Jeg-3 were seeded into 6-well plates and stimulated with LIF for different time intervals. The treated cells were harvested, lysed and PIM1/2/3 and STAT3 expression was assessed by Western blot. Finally, total RNA was extracted and quantified followed by real time PCR for quantification of *Pim1/2/3* gene expression.
Results: HTR8/Sv-neo and Jeg-3 cell lines express PIM kinases. Upon LIF stimulation and Phosphorylation of STAT3, Western blots and real time PCR displayed a relatively low expression of Pim1 compared with Pim2 and Pim3.
Conclusion: PIM kinases are present in trophoblastic cells and may be involved in the regulation of their functions. It may be concluded that PIM dysregulation is involved in placenta disorders and subsequent pathologies.

P1.107

ERK1/2 CROSSTALKS WITH STATS IN TROPHOBLASTIC CELLS

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Objectives: Pregnancy tolerance is regulated by mediators expressed by maternal and fetal cells, like Leukemia Inhibitory Factor (LIF), Epidermal Growth Factor (EGF) and Oncostatine M (OSM). Signal Transducer and Activators of Transcription (STAT)-1, -3 and -5 play important roles in trophoblast proliferation and regulation of functions. They are activated by a plethora of cytokines and can form homo- and heterodimers. ERK1/2 is a molecule of the MAPK cascade recognized as a mediator of trophoblast proliferation. The aim of this study was to analyze the effects of LIF, EGF and OSM on ERK1/2, STAT-1, -3 and -5 activation and the possible crosstalk between ERK1/2 and the STATs in trophoblastic cell lines.
Methods: The trophoblastic cell lines JEG-3 and HTR-8/SVneo were stimulated with EGF, LIF and OSM. Subsequently, ERK1/2, STAT-1 and -5 activation was inhibited by U0126, Fludarabine and siRNA, respectively. Phosphorylation of STAT-1, -3, -5 and ERK1/2 was assessed by Western blotting. Finally, STATs DNA-binding capacity and cell proliferation were analyzed in presence/absence of cytokines and inhibitors.
Results: LIF and OSM induce STAT1- and -3 phosphorylation and DNA-binding in HTR-8 and JEG-3 cells. EGF is a major inducer of STAT5 phosphorylation in both cell lines. ERK1/2 phosphorylation was induced with LIF, OSM and EGF. Suppression of STAT-1 and -5 elevated ERK1/2 activity, whilst abrogation of p-ERK1/2 results in an increase of STAT-3 activation and DNA-binding capability. Proliferation increases in all cell lines after treatment with LIF, EGF and OSM, while STAT-5 silencing resulted in a decrease. Invasion was decreased by STAT-1, -3 inhibition while STAT-5 silencing did not result in significant changes.
Conclusion: LIF and OSM signal through STAT1 and -3, while EGF uses STAT5. All applied cytokines activate ERK1/2, and induce STAT-mediated proliferation and invasion in trophoblastic cells. Crosstalks between JAK/STAT and ERK1/2 MAPK pathways are evident.

P1.108

THE CCN3 PROTEIN REGULATES PROLIFERATION AND MIGRATION PROPERTIES IN JEG3 TROPHOBLAST CELLS VIA ERK1/2, AKT AND NOTCH SIGNALLING

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Objectives: Previous studies showed that the matricellular CCN3 is upregulated in Jeg3 trophoblast cells as well as in the invasive trophoblast of placental explants by low oxygen and is found to be deregulated in early-onset preeclampsia. We identified the glycosylated CCN3 (g-CCN3) as well as the non-glycosylated CCN3 (ng-CCN3) protein as a key factor in regulating trophoblast proliferation and invasion. Jeg3 cells revealed decreased proliferation upon both forms of CCN3 but enhanced migration and invasion properties combined with an elevation of MMP-2 and MMP-9 activity was only found for ng-CCN3.
Methods and Results: Here we focused on signalling cascades MAPK, PI3 kinase/Akt and Notch/p21 for mediating the dual function of CCN3 for trophoblast proliferation versus migration/invasion in Jeg3 cells upon stimulation with glycosylated and non-glycosylated recombinant CCN3 (g-/ng-rCCN3). Analysis of the CCN3-mediated signalling pathways showed that ng-rCCN3 stimulated migration properties by activating two different signalling pathways, the PI3 kinase/Akt and the MAPK cascade and these two signalling cascades worked independent from each other. Moreover, ng-rCCN3-stimulated trophoblast cell migration - but not the antiproliferative capacity of both forms of CCN3 - was mediated via Integrin $\alpha5\beta1$ signalling using siRNA transfection. The Notch signalling pathway contributes to the antiproliferative properties of both forms of CCN3 evidenced by an increased expression of Notch1 and its target gene, the cell cycle inhibitor p21.
Conclusion: Our data showed that the presence of both forms of CCN3 in trophoblast cells is associated with a balance in proliferation and migration/invasion properties which are triggered by different signalling pathways. A deregulation of the CCN3 forms in preeclampsia could lead to an imbalance in proliferation versus invasion which might contribute to the shallow invasion observed in this disease.

P1.109

ELASTIN-DERIVED PEPTIDE MEDIATED SIGNALLING IN EXTRAVILLOUS TROPHOBLAST

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Objectives: Elastin breakdown in the walls of the spiral arteries during early pregnancy facilitates their transformation into dilated, high-flow, low-resistance channels. Elastolysis is known to generate biologically active elastin-derived peptides (EDP). We have previously demonstrated that EDP promote extravillous trophoblast (EVT) invasion, migration and upregulation of elastolytic enzymes. We hypothesise that EDP release during elastolysis orchestrates a positive feedback loop that promotes EVT invasion and further elastin breakdown, completing the process of vascular remodelling. Here we investigate expression of potential receptors and intracellular signalling events mediating EDP effects in EVT.
Methods: First trimester placenta and decidua basalis were processed for immunohistochemistry, to examine expression of galactin 3 and the elastin receptor complex, receptors known to bind EDP in other cell types. Primary EVT isolated from first trimester placenta (7-12 weeks gestation) were exposed to 1μg/ml EDP or vehicle control (0.1% DMSO) for 30 minutes before lysing for parallel determination of the relative levels of protein phosphorylation using a Human Phospho-Kinase Array Kit (R&D Systems). Lysates from three separate cell preparations were pooled for analysis.
Results: Expression of the elastin receptor complex was observed in villous cytotrophoblasts (CTB), syncytiotrophoblast, and EVT in placental cell columns. Galectin 3 was expressed in villous CTB, and to a much lesser extent in cell columns. Interstitial and endovascular EVT did not express galectin 3; however, a proportion of endovascular EVT expressed the elastin receptor complex. Following exposure of primary EVT to EDP, phosphorylation of p38 alpha and endothelial nitric oxide synthase (eNOS) was increased by 85% and 130%, respectively.
Conclusion: The EDP receptors galactin 3 and the elastin receptor complex are expressed in human first trimester placenta. The biological effects of EDP on EVT are mediated via phosphorylation of p38 and eNOS, intracellular signalling molecules that are also associated with tumour cell migration and invasion.

P1.110

AN IGF/MIR483-3P REGULATORY FEEDBACK LOOP INFLUENCES HUMAN PLACENTAL DEVELOPMENT

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Objectives: Fetal growth complications such as FGR and macrosomia, are associated with altered placental development and function. IGF-I and -II are required for optimal cytotrophoblast (CT) proliferation thus they are important mediators of both placental and fetal growth. We have recently demonstrated that microRNAs (miRs) are also important mediators of CT proliferation. In this study, we explored the possibility that IGFs and miRs interact to regulate placental growth.
Methods: First trimester placental explants were cultured in serum-free conditions for 24 h and then exposed to IGF-I (10nM) or IGF-II (10nM) for a further 24 h (n=6). miR expression was profiled using arrays and data were validated by QPCR. In-situ hybridisation was used to localise miRs within placenta. miR-specific inhibitors (30-100nM) were introduced into placental explants by nucleofection. Following confirmation of miR inhibition (QPCR), levels of proliferation (Ki67 and BrdU) were assessed by immunohistochemistry and miR target expression was analysed by arrays, western blotting and QPCR. Direct interaction of miRs with target genes was assessed by RNA-inducing silencing complex immunoprecipitation (RIP) followed by gene specific PCR (RIP-chip).
Results: IGF (-I and –II) significantly decreased expression of two miRs ($P<0.001$); one of these, miR-483-3p was expressed throughout the villous stroma and within cytotrophoblast in first trimester placenta. Analysis of CT proliferation revealed that inhibition of miR-483-3p expression (using a miR-specific inhibitor) significantly enhanced levels of CT proliferation ($P< 0.01$; n=6). One predicted target of miR-483-3p is IGF-I; protein arrays, western blotting and QPCR confirmed that miR-483-3p negatively regulates IGF-I expression. RIP-Chip demonstrated that the miR483-3p/IGF-I interaction is direct.
Conclusion: Here we demonstrate that maternal IGF-I acts via miR-483-3p to potentiate placental IGF-I expression. This positive regulatory feedback loop appears to influence CT proliferation. Interestingly, we have preliminary data demonstrating that this feedback loop is dysregulated in pregnancies complicated by macrosomia.

P1.111

THE MIRNA SIGNATURE OF TROPHOBLAST CELLS

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Objectives: MicroRNAs (miRNAs) are small single-stranded RNA molecules which are important post-transcriptional modulators of gene expression. Trophoblast cells are a group of fetal cells in the placenta displaying important functions in the establishment and maintenance of pregnancy in humans. We aimed to compare the miRNA profile (MiRNome) of primary first and third trimester trophoblast cells with that of different trophoblastic cell lines. We also analyzed miRNA expression in four trophoblastic cell lines (JEG-3, ACH-3P, AC1-M59, HTR8/SV neo) before and after Leukemia Inhibitory Facor (LIF) challenge.
Methods: Total RNA was isolated from cytotrophoblast cells from healthy first and term placentae and the cell lines HTR-8/SVneo (immortalized trophoblast cells), JEG-3 (choriocarcinoma), ACH-3P and AC1-M59, which are choriocarcinoma cells fused with first and third trimester trophoblast cells, respectively. Furthermore, total RNA was isolated from cells stimulated with LIF for 4h. The expression level of 762 different miRNAs was quantitatively analyzed by using a TaqMan Human MicroRNA Array. Results for 10 important miRNAs were confirmed by individual qPCR and expression kinetics were analyzed after LIF stimulation. As being one of the most affected, miR-141 has been silenced by RNA interference or overexpressed to test its role in proliferation of JEG-3 cells after 24h and 48h. Finally, HTR-8 and JEG-3 cells were karyotyped through Multiplex-Fluorescence In Situ Hybridisation (M-FISH).
Results: MicroRNA expression profiles of first and third isolated trophoblasts were more similar to those in JEG-3, ACH-3P and AC1-M59 exhibiting high expression of the chromosome 19 miRNA cluster (C19MC), known to be almost exclusively expressed in the placenta, as well as the three miRNAs of the cluster miR-371, recognized as specific markers for human embryonic stem cells. Conversely, HTR-8 exhibits almost no expression of the C19MC miRNAs but instead high expression of C14MC. These results correlate with those observed in the M-FISH karyotype of HTR-8 cells, where both chromosomes C19 and C14 were found to be physically altered. MicroRNAs change differentially between cell lines under LIF stimulation. Only a small group of miRNAs was simultaneously altered in all tested cells. Silencing of miR-141 completely inhibited proliferation of JEG-3 cells, while overexpression had no effect.
Conclusion: LIF modulates microRNA expression in trophoblastic cells in a cell type-dependent manner. The miRNA expression profile provides a tool for the identification of trophoblast cells and their malignant derivates. Several miRNA highly expressed in trophoblastic cells have been previously detected in pregnancy serum. MiR-141 may have an essential role in regulation of functions in trophoblastic cells.

P1.112

TARGET GENE-SPECIFIC RNAI FOR PLACENTA RESEARCH

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Objectives: RNA interference (RNAi) is the process through which small interfering RNA (siRNA) induces sequence-specific posttranscriptional gene silencing. RNAi is widely recognized as a powerful tool not only for functional genomics but also for therapeutic applications, since the silencing effect of siRNA has been assumed to be extremely specific. However, accumulated evidences revealed that siRNA could also down-regulate many unintended genes. This phenomenon is referred to as off-target effect. For understanding accurate target gene function and successful therapeutic application, it may be critical to select functional siRNAs with minimized off-target effects. Here I show our elaborative siRNA selection algorithm based on our mechanistic analysis of RNAi machinery.
Methods: We determined the relationship between siRNA seed sequences and off-target effects by introducing each of seed-matched target sequences into a luciferase reporter plasmid, and examined the change in luciferase activity in transfected human HeLa cells as a function of siRNA concentration. The result was also confirmed by genome-wide experiments using microarray.
Results: The seed-dependent off-target effects were apparently different according to siRNA seed sequences. The capability to induce off-target effect of each siRNA was revealed to be strongly correlated to the thermodynamic stability in the duplex formed between siRNA seed and its target mRNA. The seed-dependent off-target effect is almost completely eliminated with G:U pairing in the seed-target duplex. In this case, off-target effect is avoided probably because of the structural perturbation but not stability in seed-target duplex.
Conclusion: We revealed that the seed-dependent off-target effect is induced by strong stability in siRNA seed-target duplex. This means siRNA with low stability in seed-target duplex has little or no seed-dependent off-target gene silencing activity. Thus, our strategy to select target-specific siRNA may be applicable for functional genomics and safety therapeutic applications in placenta research.

P1.113

EXPRESSION AND FUNCTION OF THE IRON-REGULATORY PROTEIN HEMOJUVELIN IN THE UTERO-PLACENTAL UNIT

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Objectives: Several proteins maintain iron homeostasis in body; mutations in HFE, hepcidin, ferroportin, transferrin receptor-2, and hemojuvelin (HJV or HFE2), disrupt iron homeostasis causing the iron-overload disease hemochromatosis. There have been some studies on the expression of iron-regulatory proteins in placenta, but none in uterus. HFE, ferroportin, and hepcidin are expressed in placenta. Here we examined the expression of HJV in placenta and uterus, and determined the effects of HJV deletion on the utero-placental unit in mice.
Methods: RNA from human placenta, primary human placental trophoblast cells, and the human placental cell lines BeWo and Jar was used for RT-PCR to examine HJV expression. Cell lines and primary cells were used to determine HJV expression in uterine myometrium and blood vessels. RNA samples from wild type and HJV-knockout mouse placenta and uterus were used for RT-PCR and tissue sections for immunofluorescence to examine HJV expression in utero-placental unit. The functional consequences of deletion of HJV were assessed in HJV-knockout mice.
Results: HJV mRNA and protein are expressed in human and mouse placenta and uterus. The expression is evident in BeWo cells but not in Jar or primary trophoblast cells, suggesting that HJV expression is likely restricted to syncytiotrophoblast. In uterus, HJV is expressed only in endometrium but not in muscle cells. HJV-knockout mice are fertile; however, at older age (16-22 months), knockout mice have drastic morphological changes in uterus, characterized by angiomatous polyps. Interestingly, cultured endothelial cells and vascular smooth muscle cells do not express HJV.
Conclusions: The iron-regulatory protein HJV is expressed in placenta and uterus; its expression is restricted to syncytiotrophoblast. Deletion of the protein in mice causes abnormal endothelial cell proliferation, leading to angiomas. Since blood vessels do not seem to express HJV, angiomas seen in HJV-knockout mice occur most likely through modulation of pro-angiogenic factors.

P1.114

BEWO-CELL-DERIVED *MIR-517A* MODULATES THE EXPRESSION OF *PRKG1* IN JURKAT CELLS VIA EXOSOMES

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Objectives: MicroRNAs (miRNAs) are small non-coding RNAs that repress gene expression by binding to the complementary sequences of 3′-UTRs of their target mRNAs. We have recently developed an *in vitro* model system for analysis of placenta trophoblast cell and T cell communication via exosomes (manuscript in preparation). In this study, we identified a target mRNA of placenta-specific miRNA *miR-517a* and investigated whether *miR-517a* was capable of modulating its target gene expression in recipient cells via exosomes using the *in vitro* model system.
Methods: Microarray analysis on Jurkat cells (human T cell lymphoblast-like cell line) overexpressing *miR-517a* was performed with Agilent DNA microarrays. *MiR-517a* target genes were validated by 3′-UTR-luciferase reporter assay. pMIR-REPORT-luc reporter plasmid was used for luciferase assay (Ambion). Exosomes isolated from the culture supernatant of BeWo cells (human trophoblast cell line) were incubated with Jurkat cells. After incubation with the BeWo-exosomes, the expression levels of the targets were examined by real-time PCR.
Results: Microarray analysis showed that 7 genes were downregulated after *miR-517a* overexpression in Jurkat cells. One of these genes was *PRKG1* encoding cyclic GMP dependent protein kinase 1 that is essential for numerous physiological processes. We found that *PRKG1* was a target candidate of *miR-517a in silico*. *miR-517a* overexpression significantly decreased luciferase activity in Jurkat cells cotransfected with pMIR-REPORT-*PRKG1*. Exosome analysis using the *in vitro* model system revealed that the expression level of *PRKG1* mRNA was significantly downregulated in Jurkat cells after treatment with the BeWo-exosomes.
Conclusion: We demonstrate that *PRKG1* is indeed a target of *miR-517a* in Jurkat cells. These *in vitro* findings suggest that placenta-specific miRNAs can modulate their target mRNA expression in maternal recipient cells via exosomes. We provide a new insight into cell-cell communication via exosomes between placenta and lymphocytes.

P1.115

PLACENTAL EXTRACELLULAR VESICLES' MICRO-RNA PROFILE, IS ALTERED BY FREE HEMOGLOBIN AND RESTORED BY ALPHA-1-MICROGLOBULIN

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Objectives: We previously showed that free fetal hemoglobin (HbF) plays a role in the pathophysiology of preeclampsia (PE). Free Hb causes functional and structural damage to the placenta, effects that can be counteracted by the endogenous heme scavenger alpha-1-microglobulin (A1M). Extracellular vesicle (EV) release and placental micro-RNA (miRNA) expression is altered in PE. We hypothesize that these changes are caused by the toxic effects of free Hb. In this study we examined the miRNA profile in EVs released from placental tissue perfused ex-vivo with free Hb. The therapeutic effect of A1M was also evaluated.
Methods: Human term placentas were perfused using the dual placental perfusion system previously described. Perfusion media were supplemented with free Hb and/or A1M. Control experiments were performed using medium only.
To isolate EVs, perfusion medium was centrifuged at 3,500g for 30min and ultra-centrifuged at 110,000g for 3hrs. Total RNA was extracted from the EVs using mirVana miRNA isolation kit. RNA quality was determined using Bioanalyzer, followed by hybridization to Affymetrix GeneChip® miRNA 2.0 Array (Affymetrix Inc).
For the basic data analyses, background correction, normalization, and probe summarization, miRNA QC tool (Affymetrix Inc) was used. SAM (Significance Analysis of Microarrays) method was used to identify significantly differentially expressed miRNAs.
Results: 149 miRNAs were expressed in over 70% of the samples. Four of these (mir-373, mir-424*, mir-372 and mir-527) were identified as significantly up-regulated after Hb perfusions compared to controls (q-value 0%), whereas no significantly different miRNA expression was seen in HbA1M and control samples.
Conclusion: Free Hb altered the miRNA expression of placental EVs. The up-regulated miRNAs; mir-373, mir-372 and mir-527 are all expressed on chromosome 19, close to the placental imprinted gene cluster C19MC. Mir-424 is down-regulated in hypoxic trophoblasts. A1M reduced the Hb induced changes, suggesting heme scavenging as a potential therapeutic approach in PE.

P1.116

CIRCULATING PLACENTA-SPECIFIC MIRNAS ARE DELIVERED TO MATERNAL IMMUNE CELLS DURING PREGNANCY

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Objectives: MicroRNAs (miRNAs), small non-coding RNAs of approximately 22 nucleotides in length, play a critical role in post-transcriptional gene regulation. Human placenta expresses the unique miRNAs that are derived from the miRNA cluster in human chromosome 19. These miRNAs are secreted extracellularly via exosomes, which enter into maternal circulation (Luo et al. Biol Reprod, 2009). However, little is known about the role[s] of placenta-specific miRNAs during pregnancy. Some of miRNAs are known to transfer into their target cells by exosomes, therefore we hypothesized that the placenta-specific miRNAs in maternal circulation may be delivered to immune cells in peripheral blood and work in immune system during pregnancy. In this study, we tested the presence of placenta-specific miRNAs in immune cells of pre- and post-delivery maternal blood.
Methods: Pre- and post-delivery maternal blood samples were obtained from the full-term women who gave informed consent according to protocols approved by the Nippon Medical School Ethics Committee. Mononuclear cells (MNCs), natural killer (NK) cells and regulatory T (Treg) cells were isolated from the samples by using magnetic beads. Quantification of miRNA expression was performed by real-Time PCR.
Results: Placenta-specific miRNAs were detectable in maternal MNCs before delivery. In NK cells, abundant placenta-specific miRNAs were detected before delivery; the levels of these miRNAs were significantly reduced 4 days after delivery. In contrast, Treg cells showed a little uptake of placenta-specific miRNAs even before delivery; no differences were found in the miRNA uptake between pre- and post-derivers.
Conclusion: We showed the presence of placenta-specific miRNAs in maternal immune cells of peripheral blood during pregnancy and their rapid clearance from the cells after delivery. These data lend support to the assumption that circulating placenta-specific miRNAs are delivered to maternal immune cells via exosomes and modulate their target mRNAs that participate in immune tolerance during pregnancy.

P1.117

ABERRANT EXPRESSION OF TRAIL IN PLACENTA AND MATERNAL SERUM IN EARLY PREGNANCY COMPLICATIONS

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Recurrent miscarriage (RM; $\geq$3 pregnancy losses) occurring in 1-3% of fertile couples has a heterogeneous background with contribution from both genetic and environmental factors. No biomarkers with high predictive value of threatening miscarriage have been identified.
Objectives: We aimed to (i) perform whole-genome gene expression profiling in the placental tissues from RM patients and elective termination of first trimester pregnancy, (ii) determine the protein levels in maternal sera during early pregnancy for the loci with differential expression in placenta.
Methods: GeneChips (Affymetrix®) were used for discovery and Taqman RT-qPCR assays for replication of mRNA expression in placentas from RM cases (n=13) compared to uncomplicated pregnancies matched for gestational age (n=23). Concentrations of soluble TRAIL (sTRAIL) and calprotectin (S100A8/A9) in maternal serum in normal first trimester (n=35) and failed pregnancies: early miscarriage (n=18), late miscarriage (n=4), tubal pregnancy (n=11) were determined by ELISA.
Results: In RM placentas 30 differentially expressed transcripts were identified. Significantly increased placental mRNA expression of TNF-related apoptosis-inducing ligand *TRAIL* (p=1.4x10-3; fold change 1.68) and *S100A8* (p=7.9x10-4; fold change 2.56) encoding inflammatory marker calprotectin (S100A8/A9) was confirmed by RT-qPCR. Compared to normal pregnancy (sTRAIL 16.1$\pm$1.6pg/ml), significantly higher maternal serum sTRAIL was detected at the RM event (33.6$\pm$4.3pg/ml, p=0.00027), tubal pregnancy (30.5$\pm$3.9pg/ml, p=0.035) and also in pregnant women, who developed an unpredicted miscarriage after serum sampling (28.5$\pm$4.4pg/ml, p=0.039). Maternal serum levels of calprotectin were neither diagnostic nor prognostic of early pregnancy failures (P>0.05).
Conclusion: The current study identified sTRAIL as a potential biomarker in maternal serum for prediction of early pregnancy complications.

P1.118

MIR-514: A NOVEL REGULATOR OF GROWTH FACTOR SIGNALLING IN THE HUMAN PLACENTA

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Objectives: Growth factors such as IGF-I and -II promote placental growth by inducing activation of receptor tyrosine kinase (RTK)/ERK signalling cascades. We have recently demonstrated that SHP-2 is a critical regulator of RTK activation status in the placenta, so regulation of placental SHP-2 expression is important for normal pregnancy outcome. Endogenously, gene expression is regulated by microRNAs (miRs); the human placenta contains high levels of these molecules thus we investigated whether miRs can modulate SHP-2 expression and placental growth.
Methods: To identify candidate miRs that regulate SHP-2 expression bioinformatics was performed to identify miR transcriptome PCR arrays (a library of miR mimetics; SABiosciences) enriched for miRs predicted to target SHP-2 (identified from miRecords). SHP-2 specific primers were applied to the arrays and miRs found to alter SHP-2 mRNA expression were defined as candidate SHP-2 regulators; the expression of these within the first trimester placenta was confirmed by QPCR. miR specific mimetics were transfected into first trimester placental explants (n=6) by nucleofection and explants cultured for up to 4 days (+/-IGF-I; 10nM). QPCR and western blotting were undertaken to assess the effect on mRNA and protein expression and immunohistochemistry (Ki67) was performed to assess proliferation.
Results: Transcriptome arrays revealed two novel candidate SHP-2 regulatory miRs: miR-754 overexpression increased SHP-2 mRNA expression (2.16 fold) whilst miR-514 mimetics reduced SHP-2 mRNA expression (3.4 fold), suggesting that these were positive and negative regulators respectively. Both miR-754 and miR-518 were expressed in the placenta. Overexpression of miR-514 (6 fold; $P<0.05$; n=5) in first trimester placental explants significantly reduced SHP-2 mRNA and protein expression and significantly attenuated IGF-I-induced cytotrophoblast proliferation ($P<0.05$; n=6).
Conclusion: By modulating SHP-2 expression, miR-514 has been identified as a novel growth factor regulatory molecule in the human placenta. Ongoing studies will establish whether alterations in placental SHP-2 and/or miR-514 expression are associated with fetal growth disorders.

P1.119

THE ROLES OF CAMP-DEPENDENT PROTEIN KINASE A (PKA) AND EXCHANGE PROTEIN DIRECTLY ACTIVATED BY CAMP (EPAC) FOR THE SYNCYTIALIZATION IN HUMAN PLACENTAL BEWO CELLS

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Background: The mononuclear villous cytotrophoblast fuses and differentiates to the multinucleated syncytiotrophoblast (syncytialization), which produces hCG and other syncytialization marker proteins. However the syncytialization process is poorly understood. Syncytin-1, one of the human endogenous retrovirus envelop protein, is known as a contributor in the cell fusion process. Forskolin (FSK) elevates intracellular cAMP concentration and PKA activity, then up-regulated syncytin-1 causes syncytialization and the secretion of hCG. Classically, cAMP signaling cascade was considered to be mediated only by PKA, but recently PKA-independent mechanisms, including the exchange protein directly activated by cAMP (Epac), were identified. The aim of our study was to investigate the participation of PKA and Epac signal cascade in syncytialization of human placental BeWo cells.
Methods: BeWo cells were treated with PKA selective analog (N6-phenyl-cAMP, Phe), Epac selective cAMP analog (8-pCPT-2'-O-Me-cAMP, CPT). PKA inhibitor (H-89) was incubated with FSK. The syncytialization activity was evaluated by the expression of syncytin-1, hCGβ mRNA and protein, production of hCGβ. Cell fusion was determined by a quantitative flow cytometry assay.
Results: Phe increased the expression of syncytin-1/hCGβ mRNA, protein and hCGβ secretion in the same level of FSK stimulation. Cell fusion was also increased by Phe and FSK, whereas CPT did not stimulate mRNA expression or cell fusion. H89 suppressed the expression of syncytin-1/hCGβ mRNA and cell fusion, to the same level of non-stimulated FSK (Control).
Conclusions: cAMP-mediated syncytialization is caused by PKA signaling passway dominantly.

P1.120

SYNEPITHELIOCHORIAL PLACENTAL INTERACTIONS IN EGFP CLONED CATTLE MODEL

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Objective: To investigate the mechanisms by foetal cells interact and transfer their contents to the maternal compartment in the bovine placenta using a model of cloned transgenic enhanced Green Fluorescent Protein (eGFP) expressing bovine embryos.
Methods: EGFP-expressing embryos were produced and transfer into surrogate cow. The uteri were surgically recovered at days 60 (N=3) and 90 (N=3) of pregnancy. Placentome/endometrium samples and maternal peripheral blood leukocyte (PBL) samples were collected to assess the presence of fDNA by nested-PCR for eGFP and testis-specific Y-encoded protein (TSPY), immunohistochemistry, transmission electron microscopy and western blotting analysis.
Results: eGFP DNA was present in PBL at day 60 and 90 of pregnancy confirming that there is transplacental transfer of fDNA to the mother. Interestingly, eGFP was found to be present at protein and DNA levels in the intercaruncular epithelium, suggesting that eGFP is delivered to the maternal side even in the regions where the cells are loosely attached. Moreover, at local level eGFP was highly expressed by the trophoblast; however it was also present in the uterine epithelium (UE) facing the trophoblast. In the arcade zone at the syncytial plaques, the expression of eGFP by both tissues was less intense than in the neighbouring cells. Our eGFP construct possessed an ubiquitin promoter. Ubiquitin is not expressed by invasive cells in human, which might explain the weak staining of eGFP in the syncytial plaques. Additionally, trophoblast cells closer to UE showed decreased cytoplasmic area and flattened nucleus, suggesting that maternal cells respond to foetal signals undergoing to apoptosis.
Conclusion: Our results showed that the eGFP is delivered to maternal side at local and systemic levels. Since eGFP does not have a signal peptide, it is suggested that eGFP could be delivered to the maternal side through transtrophoblastic water channels that are present in the ruminant placenta.

P1.121

INDUCTION OF IGFBP-1 EXPRESSION BY CAMP IS ASSOCIATED WITH HISTONE ACETYLATION STATUS OF THE PROMOTER REGION IN HUMAN ENDOMETRIAL STROMAL CELLS

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Objectives: Many genes are up or down-regulated in human endometrial stromal cells (ESC) undergoing decidualization. Expressions of insulin-like growth factor binding protein-1 (IGFBP-1) and prolactin (PRL) are preferentially induced with decidualization, and are recognized as specific markers of decidualization. This study investigated the involvement of epigenetic mechanisms in the regulation of IGFBP-1 and PRL induction by decidualization in ESC.
Methods: (1) ESC isolated from the proliferative phase endometrium were incubated with cAMP to induce decidualization. To examine whether IGFBP-1 and PRL expressions can be induced by cAMP in the cell that is not derived from the endometrium, human dermal fibroblasts (HDF) were cultured with cAMP. (2) DNA methylation and histone acetylation status of the promoter were analyzed by sodium bisulfite genomic sequencing and ChIP assay, respectively. (3) FBC were cultured with cAMP in the presence or absence of a histone deacetylase (HDAC) inhibitor and thereafter IGFBP-1 mRNA expression and C/EBP-b promoter binding were examined.
Results: (1) IGFBP-1 and PRL expressions were induced by cAMP in ESC. PRL mRNA expression, but not IGFBP-1, was induced by cAMP in HDF. (2) Histone acetylation levels of the IGFBP-1 promoter region were higher in ESC compared with HDF. Both cells showed similar DNA hypomethylation status of the IGFBP-1 promoter region. There were no differences in histone acetylation levels and DNA methylation status of the promoter and enhancer region of PRL between both cells. (3) Co-treatment with cAMP and HDAC inhibitor in HDF induced IGFBP-1 expression with increased histone acetylation levels and recruitment of C/EBP-b to the promoter region of IGFBP-1.
Conclusions: Histone acetylation status of the IGFBP-1 promoter region is important for IGFBP-1 gene expression. High histone acetylation status of the IGFBP-1 promoter region induces loose chromatin structure of the promoter region, which allows the recruitment of transcription factors to the promoter region.

P1.122

ROLE OF *RTL1* IN HUMAN PLACENTA: PLACENTAL STUDY IN AFFECTED CASES WITH STRUCTURAL ABNORMALITY OF THE IMPRINTED REGION ON HUMAN CHROMOSOME 14

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Human chromosome 14q32.2 region carries a cluster of imprinted genes including paternal expressed genes (*PEGs*) such as *DLK1, RTL1*, *DIO3,* and maternal expressed genes (*MEGs*) such as *MEG3, RTL1as, MEG8*, *microRNAs* and *snoRNAs*, together with *DLK1-MEG3* intergenic differentially methylated region (IG-DMR) and *MEG3*-DMR. Paternal uniparental disomy for human chromosome 14 (UPD(14)pat) in which the expression of *PEGs* and *MEGs* are increased and absent, respectively, results in placentomegaly. However, the functions and the regulatory mechanisms of the imprinted genes at 14q32.2 are still unknown. Here, we performed placental study of two cases with UPD(14)pat, a case with maternal microdeletion involving *DLK1*, DMRs and *MEG3* and a case with paternal duplication of 14q32.2 imprinted region. *RTL1* and *DLK1* expressed only in endothelial cells and pericytes of villous vessels. In the placentas of UPD(14)pat revealing placentomegaly, the expression of *DLK1* and *RTL1* increased, even though in the placenta of a case with maternal microdeletion revealing placentomegaly, only the expression of *RTL1* increased. Electron microscopic study showed hyperplasia of the endothelial cells and the pericytes in the placentas of UPD(14)pat. Expression analysis using fresh placental samples of UPD(14)pat showed excessive *RTL1* expression from 6 to 9-folds above the normal control levels because of a synergic effect between the biallelic activation of *RTL1* and loss of functional microRNA-containing *RTL1as* as a repressor for *RTL1*. These results indicate that excessive *RTL1* expression causes placentomegaly and *RTL1* expression is regulated through an RNAi mechanism. *RTL1* may play an essential role in the development of human placenta.

P1.123

METHYLATION PROFILE OF C19MC-MIRNA CPG ISLAND IN TROPHOBLAST CELL LINES

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Objective: MicroRNA (miRNA) is noncoding RNAs that bind to the 3' untranslated region of target mRNA and regulate its translation. MiRNAs derived from the chromosome 19 miRNA cluster (C19MC-miRNAs) are exclusively expressed in the human placenta. The expression of C19MC-miRNAs has been considered to be highly correlated with the methylation state of a CpG island (cytosine/guanine-rich regions of DNA) located about 18 kb upstream of the C19MC-miRNA genes (Noguer-Dance *et al. Hum Mol Genet* 19: 3566-3582, 2010). In this study, we examined the methylation status of the CpG-island with C19MC-miRNA expression levels in trophoblast cell lines.
Methods: HTR8/SVneo, BeWo, and JEG3 were used to assess methylation status. Genomic DNA was extracted from samples. After sodium bisulfite treatment, the DNA regions of C19MC were amplified and sequenced to identify the CpG methylation profile. As inhibitor of DNA methylation, 5-aza-2'-deoxycytidine was used. The expression levels of C19MC- miRNAs were quantified by real-time PCR. Human full-term placentas were also obtained according to protocols approved by the Nippon Medical School Hospital Ethics Committee and the Jichi Medical University Ethics Committee.
Results: In BeWo and JEG3 cells as models of villous trophoblast, the CpG island located about 18 kb upstream of the C19MC-miRNA genes was hypomethylated, and C19MC- miRNAs (e.g., *miR-517a*) were highly expressed. This is consistent with the in vivo findings that the CpG island was hypomethylated in human full-term placentas that were highly expressed C19MC- miRNAs. On the other hand, in HTR8/SVneo cell as a model of extravillous trophoblast, the CpG island was hypermethylated, and C19MC- miRNAs were hardly detected. After the treatment with 5-aza-2'-deoxycitidine, the expression of C19MC-miRNAs was rejuvenated in HTR8/SVneo cells.
Conclusion: Our in vitro findings suggest that the methylation status of the CpG-island with C19MC-miRNA expression levels is altered with trophoblast differentiation along the invasive pathway, i.e., extravillous.

P1.124

DIFFERENTIAL EXPRESSION OF MIRNAS IN PLACENTA FROM EARLY AND LATE ONSET PREECLAMPSIA VERSES CONTROLS

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Objectives: miRNAs are a group of small non-coding RNAs that play important roles in the regulation of physiological functions both in health and disease by post-transcriptional silencing of target mRNAs. miRNAs are abundantly expressed in placenta with a large cluster of miRNAs on chromosome 19 being highly specific for placenta. A few studies have investigated the differential expression of miRNAs in preeclamptic (PE) placenta, but with somewhat conflicting results. The aim of this study was to investigate the differential expression of miRNAs in early and late-onset PE compared to controls using high throughput sequencing.
Methods: Preeclamptic and control placenta samples were obtained during caesarean section from an ongoing biobank collection of patient samples at Oslo University Hospital, Ullevaal. Following sample preparation, 23 normotensive controls, 23 early-onset preeclamptic (gestational week <34) and 26 late-onset preeclamptic (week ≥ 34) samples were sequenced using Illumina sequencing technology. The reads were assigned to known human miRNAs from miRBase (release 18) using miRanalyzer and differential expressed miRNAs were identified using the DESeq package in R.
Results: Of the $\sim$1500 miRNAs identified in humans, $\sim$800 miRNAs were detected in our placenta samples. There were 10 miRNAs that were differentially expressed between controls and PE (p-value <0.1). Nine of the differentially expressed genes were found between controls and early-onset preeclampsia patients, while only miR-210 was differentially regulated between late-onset preeclampsia patients and controls. Approximately 20% of the reads mapping to miRNAs stemmed from the placenta specific chromosome 19 cluster, confirming the reliability of the results.
Conclusion: The preliminary results confirmed some of previous results and provide new insights about miRNA regulation in preeclampsia. All these results suggest that miRNAs are involved in the regulation of mRNAs in preeclampsia, especially in the early-onset type.

P1.125

THE MICRORNAS MIR27A AND MIR143 REGULATE PLACENTAL AMINO ACID TRANSPORTER MRNA LEVELS

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Objective: The amino acid transporter TAT1 mediates efflux of specific amino acids across the basal membrane (BM) of the placental syncytiotrophoblast into the fetal circulation. In placentas from Southampton Women's Survey (SWS) pregnancies the mRNA levels of TAT1 are positively related to several measures of fetal growth. The small non-coding microRNAs (miRNAs) miR27a and miR143 are predicted to regulate TAT1 expression. This study investigated whether miR27a and miR143 are related to placental mRNA expression levels of TAT1 which may have sex specific expression, as well as the amino acid transport systems A, L and ASC.
Methods: Ethical approval for this study was granted by the Southampton and Southwest Hampshire Local Research Ethics Committee. Tissue samples were collected from human placentas (53 male, 47 female infants) from the SWS. Quantitative real-time PCR (qPCR) was used to measure the expression of the miRNAs miR27a and miR143 normalized to the geometric mean of the housekeeping genes SNORD6-1 and RNU6-2. Pearson's correlation (r_p) was used to explore the relationship between placental miRNA expression levels and amino acid transporter mRNA levels.
Results: MiR27a expression negatively correlated with TAT1 (r_p=-0.28, p=0.04) and ASC1 (r_p=-0.33, p=0.02) mRNA expression in male but not female placentas. MiR27a positively correlated with LAT2 mRNA in both male and female placentas (r_p=0.34, p=0.01; r_p=0.35, p=0.02 respectively). MiR143 correlated with placental/fetal birth weight ratio (r_p=-0.28, p=0.04) in male placentas.
Conclusions: This data suggest that these miRNAs are involved in the regulation of amino acid transporter mRNA expression and may influence the transfer of amino acids to the fetus. By influencing the supply of amino acids to the fetus placental miRNAs may regulate fetal growth and underpin sex differences in mRNA levels and placental function.

P2.1

INCREASING EVIDENCE FOR THE ROLE OF PLACENTAL RENIN ANGIOTENSIN SYSTEM (RAS) IN PRE-ECLAMPSIA

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Objectives: Oxidative stress and increased prorenin receptor (PRR) both enhance the cleavage of angiotensin I from angiotensinogen (AGT). We have previously reported specific clustering of PRR in conjunction with reduced antioxidant glutathione peroxidase (GPx) activity[1]. Within the pre-eclamptic placenta, an increase in angiotensin II acting at the type 1 receptor (AT1R) may lead to increased vasoconstriction with a reduction in the growth of the fetus.
Hypothesis: Pre-eclampsia (PE), an increased state of oxidative stress, will be associated with increased placental AT1R protein expression leading to reduced infant birthweight.
Methods: Biopsies were taken 1cm from the placental edge from 27 normotensive (NT) and 23 PE White European women following informed written consent. Immunohistochemistry was performed on paraffin-embedded serial sections using antibodies to AGT, PRR, AT1R and AT2R. Protein expression was semi-quantitatively assessed (H-score) and compared between groups, to birthweights and to previously measured placental GPx 3 protein expression[2].
Results: AT1R expression was increased in PE placentae with a negative association with birthweight (r= -0.529, P=0.009). In NT, AT1R expression was negatively correlated with GPx3 expression (r=-0.865, P=0.003), an association not seen in PE. All other RAS components were similarly expressed in both groups.

Protein expression	H-score (median [interquartile range])	
	NT	PE
AGT	34 [10,95]	30 [9,60]
PRR	117 [16,144]	139 [72,187]
AT1R	40 [10,70]	68 [30,112]*
AT2R	152 [120,180]	184 [74,220]

*$P = 0.032$, NT compared to PE.

Conclusion: PE is associated with raised AT1R expression. In NT placentae, GPx3 may attenuate the vasocontrictive action of the AT1R whereas in PE, GPx3 levels are significantly lower and therefore may be insufficient to provide this inhibition thus compromising fetal growth.

1. Kurlak LO et al, (2011) Preg Hyper 1;278
2. Mistry HD et al, (2010) Placenta 31:401-408

P2.2

DISRUPTION OF NRF2 SIGNALING DUE TO DECREASED EXPRESSION OF LECTIN-LIKE OXIDIZED LDL RECEPTOR 1 (LOX-1) IS INVOLVED IN PREECLAMPSIA

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Objectives: The serum concentration of oxidized LDL (oxLDL) is higher in women with preeclampsia than in normal pregnant woman. Lectin-like oxLDL receptor-1 (LOX-1) is one of the scavenger receptors for oxLDL and is abundantly expressed in placenta. Oxidized LDL activates nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of antioxidant and cytoprotective genes such as heme oxygenase-1 (HO-1). However it has yet to be elucidated whether LOX-1, along with Nrf2, participates in the pathology of preeclampsia. Our objective is to assess LOX-1 expression and Nrf2 signaling in preeclamptic placentas and to manifest their physiological roles in preeclampsia.
Methods: Expressions of LOX-1, HO-1, and Nrf2 activation were evaluated by quantitative RT-PCR or Western blotting in placental tissues and JAR, a choriocarcinoma cell line. The functions of LOX-1 and Nrf2 were examined using an anti-LOX-1 antibody and Nrf2 activator in JAR cells or placental explants.
Results: LOX-1, HO-1 mRNA and Nrf2 activation were significantly decreased in preeclamptic placentas compared with normal controls. Significant decrease in LOX-1 mRNA was found in placental explants under hypoxic conditions. Activation of Nrf2 up-regulated HO-1 expression in both explants and JAR cells. Furthermore, oxLDL enhanced nuclear accumulation of Nrf2, and increased HO-1 expression, whereas blockade of LOX-1 inhibited the increase of HO-1 mRNA in JAR cells.
Conclusion: Nrf2 activation is an appropriate reaction to oxidative stress. However, decreasing LOX-1 expression in preeclamptic placenta can inhibit Nrf2 activation, and can contribute to reduced HO-1 expression. Moreover, reduced LOX-1 expression in preeclamptic placenta may cause maternal high oxLDL concentration. These findings provide novel insights into the crucial role of LOX-1 and Nrf2 in the pathogenesis of preeclampsia.

P2.3

SOLUBLE ENDOGLIN INHIBITS EVT FUNCTIONS VIA AUTOPHAGY UNDER HYPOXIA, RESULTING IN PREECLAMPSIA

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Objectives: Impaired extravillous trophoblast (EVT) invasion of myometrial spiral arteries is believed to be the main cause of preeclampsia (PE), although the cause of poor placentation remains largely unknown. In early pregnancy, trophoblast and fetus experience hypoxic and low-nutrient condition. We have studied whether autophagy, an intracellular bulk degradation system, play some roles in the early placentation. METOHDS: We have established autophagy-defect EVT cell lines by Atg4B-mutant transfer.
Results: Autophagy was detected in primary cultured EVT and EVT cell lines under hypoxia (2% O_2) and EVT in early pregnancy decidual tissue at a distal site by Western blot analysis, immunofluorescent staining and/or election microscope. The invasion of autophagy-defect EVT cells was impaired in hypoxic condition (2% O_2) (142±50 vs 265±73, $p=0.0001$), but not in 20% O_2. To investigate the vascular remodeling by EVT, tube formation assay by EVT and HUVEC was performed under 8% oxygen tension. The vascular remodeling rates by autophagy-defect EVT were significantly lower than those by wild type cell (80±4% vs 42±6%, $p<0.001$). Interestingly, 100ng/ml soluble endoglin, which is close to the serum level of severe preeclampsia patients, remarkably inhibited hypoxia-induced autophagy in EVT (52±3.2% suppression, $p<0.01$). In the functional assays, sEng significantly decreased the number of invaded cell in wild type EVT cell, but not in autophagy-defect EVT under hypoxia. Furthermore, the vascular formation rates by wild type EVT was significantly suppressed by sEng under hypoxia, similar to the level by autophagy-defect EVT (80±4% vs 35±5%, $p<0.001$). Finally, the expression of p62, an substance selectively degraded by autophagy, was significantly increased in EVT cells of placental bed biopsy samples obtained from preeclampsia patients, compared to that in normal pregnancy (32±16.7% vs 8.5±5.8%, $p=0.0005$), suggesting the impaired autophagy in EVT in preeclampsia.
Conclusion: This is the first report showing that autophagy impairment by sEng contributes to shallow placentation in preeclampsia.

P2.4

EXPRESSION OF ANGIOGENESIS-RELATED FACTORS AND INFLAMMATORY CYTOKINES IN PLACENTA AND UMBILICAL VESSELS IN PREGNANCIES WITH PREECLAMPSIA AND CHORIOAMNIONITIS/FUNISITIS

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Objective: We hypothecate that gene expression in placenta and umbilical vessels are affected by intrauterine environment and some of expression in umbilical vessels originating from the fetus could reflect fetal condition of these complicated pregnancies. Expression of angiogenesis-related factors and inflammatory cytokines were examined in placenta and umbilical vessels in order to clarify the effects of intrauterine environment of pregnancies complicated by preeclampsia and chorioamnionitis/funisitis.
Study Design: Forty-six preterm Caesarean section deliveries were classified into three groups based on maternal condition during prenatal monitoring: preeclampsia (PE) (n=11), chorioamnionitis/funisitis (CAM) (n=8), and preterm control (PC) (n=27). Angiogenesis-related factors and inflammatory cytokines in placenta, umbilical arteries and umbilical veins were analyzed by RT-PCR and immunohistochemistry.
Results: We demonstrated that Ang-2, Tie-2, and Dll4 increase in the placentas of PE compared to PC for the first time, and we confirmed the findings of previous reports showing the high expression of HIF-1α, sFlt-1, endoglin, leptin, and AT1R. Expression of Angiogenesis-related factors, including HIF-1α, VEGF, angiopoietin, and TGF-β systems, and inflammatory cytokines, such as TNF-α and IL-6, increased in umbilical vessels of PE. Umbilical veins of CAM showed a higher Dll4 level than did PC.
Conclusions: In preeclampsia, abnormal expressions of angiogenesis-related factors related to lifestyle diseases in adulthood were seen in the placenta and umbilical vessels as compared to PC. Chorioamnionitis/funisitis showed only upregulation of Dll4 in umbilical veins.

P2.5

CIRCULATING ENDOTHELIAL PROGENITOR CELLS AND PLACENTAL ABRUPTION IN WOMEN WITH PREECLAMPSIA

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Objectives: Placental abruption is one of the most significant causes of both maternal and perinatal mortality and morbidity. However, prediction marker has not been established. Abnormalities in circulating angiogenic factors and endothelial progenitor cells (EPCs) have been reported in preeclampsia and placental abruption. The objective of this study is to compare the level of EPCs in women with preeclampsia and in women with preeclampsia complicated by placental abruption.
Methods: Subject are Japanese singleton pregnant women with preeclampsia (n = 27) and without any complications (n = 15). The EPC ($CD45^{low}CD34^{+}CD133^{+}$ cells) counts were examined by flow cytometry in peripheral blood collected from 27 women with preeclampsia and 15 normal pregnants. Out of 27 women with preeclampsia, five women subsequently developed placental abruption. All subjects were then divided into three groups: normal pregnancy (NP, n=15), preeclampsia without placenta abruption (PE, n=22), and preeclampsia with placental abruption (PA, n=5).
Results: The EPC counts in the PE group significantly decreased compared to the NP group (620 counts/ml versus 1918 counts/ml, $P<0.01$). In the PA group, the EPC counts markedly decreased compared to the PE group (221 counts/ml, $P<0.05$).
Conclusions: The numbers of EPCs were significantly decreased in the preeclamptic women who subsequently developed placental abruption. The circulating EPCs might serve as a prediction marker of placental abruption.

P2.6

HYDROXYSTEROID (17-BETA) DEHYDROGENASE 1 (*HSD17B1*) IS DYSREGULATED BY *MIR-210* AND *MIR-518C* IN THE HUMAN PLACENTA COMPLICATED WITH PREECLAMPSIA: PLASMA LEVELS OF HSD17B1 AS A NOVEL MARKER FOR PREDICTING LATE-ONSET PREECLAMPSIA

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Objectives: The purpose of this study was to elucidate novel mechanisms of microRNA (miRNA) underlying the molecular pathology of preeclampsia (PE), and identify novel prognostic factors for PE.
Methods: We performed a comparative analysis of miRNA expression between normal and preeclamptic placentas by the combination of large-scale, high-throughput sequencing and quantitative PCR-based array analysis to identify miRNAs that were aberrantly expressed in preeclamptic placentas, i.e., PE-related miRNAs. We validated a target for these miRNAs. Furthermore, we investigated whether the target can serve as a novel marker for the prediction of PE, especially late-onset PE.
Results: By comprehensive analyses of miRNA expression, we identified 22 miRNAs significantly upregulated in preeclamptic placentas, five of which were predicted *in silic*o to commonly target the mRNA encoding hydroxysteroid (17-beta) dehydrogenese 1 (HSD17B1), a steroidogenetic enzyme expressed predominantly in the placenta. *In vivo* HSD17B1 expression, at both the mRNA and protein levels, was significantly decreased in preeclamptic placentas. Of these miRNAs, *miR-210* and *miR-518c* were experimentally validated to target *HSD17B1* by luciferase assay, real-time PCR and enzyme-linked immunosorbent assay. Furthermore, we found that plasma HSD17B1 protein levels in preeclamptic pregnant women reflected the decrease of its placental expression. Moreover, a prospective cohort study of plasma HSD17B1 revealed that there was a significant difference in plasma HSD17B1 protein levels between the two groups even at 20–23 weeks of gestation prior to the onset of PE, especially late-onset PE.
Conclusion: We conclude that *HSD17B1* is dysregulated by *miR-210* and *miR-518c* that are aberrantly expressed in preeclamptic placenta, and that reducing plasma level of HSD17B1 precedes the onset of PE and is a potential prognostic factor for PE.

P2.7

INVESTIGATING GENETIC PREDISPOSITION AND CLINICAL OCCURRENCE OF PREECLAMPSIA AND CARDIOVASCULAR DISEASE THROUGH A FAMILY-BASED DESIGN

The Norwegian Preeclampsia Family Biobank
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Objectives: Preeclampsia is the most frequent pregnancy complication in the Western world (2-8%) and a major cause of maternal and fetal morbidity and mortality. A woman with a preeclamptic pregnancy has an increased risk (2-8 fold) of developing atherosclerosis/cardiovascular disease (CVD) later in life. Preeclampsia is a complex genetic syndrome and clustering of the condition in families indicates disease predisposition. Family studies are recognized as valuable resources to investigate underlying genetics. To examine genetic mechanisms involved in the syndrome we used a population-wide registry to establish a biobank founded on a Norwegian family-cohort with increased occurrence of preeclampsia.
Methods: Familial predisposition to preeclampsia was defined as when a woman and her mother, daughter or sister were registered in the Medical Birth Registry of Norway as having the disease. The diagnoses were validated by reviewing hospital records. 426 women with validated diagnoses were invited to participate and include relatives. Information on obstetric and medical history of participants and their families was gathered and peripheral blood samples collected from participants. Pedigrees were recorded and the participants were strictly characterized both as individuals and in a family setting according to phenotype, sub-grouped as: preeclampsia according to severity, preeclampsia with or without growth restriction in the offspring (FGR), and preeclampsia with or without CVD or diabetes development.
Results: The Norwegian Preeclampsia Family Biobank contains information on and samples from 110 families (504 individuals). Majority of the families contains cases of serious preeclampsia, has several affected family members, and an identified accumulation of CVD. The families are further subdivided according to clinical phenotype as families of aggregated early/late preeclampsia with or without FGR and CVD.
Conclusion: The Norwegian Preeclampsia Family Study, a population- and family-based cohort study, has led to the founding of a substantial high-quality biobank to benefit and promote future research on preeclampsia.

P2.8

EXPRESSION OF NON-MUSCLE MYOSIN IIA AND IIB IN NORMAL AND PREECLAMPTIC HUMAN PLACENTA AND THEIR ROLE IN TROPHOBLAST CELL MIGRATION

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Objective: Non-muscle myosin proteins, NMHC-IIA and -IIB, are known to be important in the regulation of cell motility and chemotaxis. Our aim was to study expression of NMHC-IIA and -IIB in normal and preeclamptic human placentas and to elucidate their possible role in trophoblast cell migration.
Methods: Placental samples obtained from healthy (n=16) and severely preeclamptic (n=15) women after delivery were fixed, embedded in paraffin and sectioned. Immunostaining was performed using automated Dako Cytomation EnVision and Duel Link System. Expression of NMHC-IIA and –IIB in the various placental cell populations was analyzed by a pathologist who was blinded to the diagnosis and outcome of pregnancies.
First trimester primary trophoblast cell lines immortalized by SV40 virus (HTR-8/SVneo) used as a model to study extravillous trophoblast function, were transfected by siRNA for MYH9 (NMHC-IIA), MYH10 (NMHC-IIB) and scrambled control siRNA. Scratch wound healing assays were performed to assess the role of these proteins in trophoblast cell migration.
Results: NMHC-IIA was localized mainly in endothelial cells both in normal and preeclamptic placentas. It was strongly expressed in polymorphonuclear leucocytes (PMNC), which were present in higher numbers in preeclamptic placentas compared to normal. NMHC-IIB showed a heterogeneous but higher level of expression in preeclamptic placentas compared to NMHC-IIA. NMHC-IIB was expressed by almost all extravillous trophoblasts but not by PMNC. Subsyncytial trophoblasts showed reduced expression of NMHC-IIA compared to NMHC-IIB. Syncytial trophoblasts and syncytial knots did not express these markers except in some focal areas in case of NMHC IIB. Suppressing the MYH9 and MYH10 expression reduced protein expression as well as the migration capabilities of first trimester trophoblast cell lines.
Conclusion: Non-muscle myosin-IIA and -IIB are differentially expressed between normal and preeclamptic placentas and appear to affect the first trimester trophoblast cell migration. Their role in the pathogenesis of preeclampsia needs to be further elucidated.

P2.9

ATORVASTATIN DECREASE SOLUBLE FMS-LIKE TYROSINE KINASE-1 (SFLT-1) IN HUMAN JEG-3 CHORIOCARCINOMA CELLS: ROLE OF ENOS/NO PATHWAY

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Objectives: Recently studies showed that elevated soluble fms-like tyrosine kinase-1 (sFlt-1) reduces bio-availability of trophoblast placenta growth factor (PGF) and vascular endothelial growth factor (VEGF) in Preeclampsia. Although Statins therapy abolishes sFlt-1expression in some animal model of preeclampsia. But the mechanism of their action is not known. This study aims to investigate the mechanism by which atorvastatin decrease sFlt-1expression in human JEG-3 choriocarcinoma cells.
Methods: JEG-3 choriocarcinoma cells were cultured under 21%O_2 or 1%O_2 conditions in the presence or absence of atorvastatin. Effects on PGF, VEGF, endothelial nitric oxide synthase (eNOS) and sFlt-1 mRNA expression were determined by quantitative real-time PCR. Changes in expression of PGF, VEGF, sFlt-1 protein were monitored by ELISA. NOx was determined in the culture media with Griess colorimetric assay.
Results: Hypoxia decreased PGF mRNA but increased VEGF, sFlt-1 mRNA expression in JEG-3 cells. Atorvastatin treatment under 1%O_2 culture conditions significantly reversed sFlt-1 mRNA and protein expression, Furthermore atorvastatin increased PGF, VEGF protein expression and nitric oxide production in culture supernatant also levels of eNOS mRNA expression increased. All of the effects of atorvastatin were attenuated by preincubation with NOS inhibitor L-NAME.
Conclusions: Atorvastatin decrease sFLT-1 production through up-regulation of eNOS in human JEG-3 choriocarcinoma cell culture. Our data support a role for statins in preeclampsia prevention.

P2.10

PAPPA2 REGULATES IGF PATHWAY IN PRE-ECLAMPTIC PLACENTA

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Objectives: In our current study, we performed genome-wide expression profiling of placental tissue samples, and pregnancy-associated plasma protein-A2 (PAPPA2) included putative candidate genes associated with the pathogenesis of pre-eclampsia. PAPP A2 is proteases that cleave insulin-like growth factor-binding protein-5 (IGFBP-5), resulting in local activation of IGF signaling pathways. Thus, we compared the placental expression level of PAPPA2 and IGFBP-5 at both the RNA and protein levels in women with uncomplicated pregnancies and in those with severe pre-eclampsia.
Methods: Placental biopsy samples were obtained during Caesarean sections from both normotensive patients ($n = 25$) and those with pre-eclampsia ($n = 21$) who gave consent for participating in this study. PAPP-A and IGFBP-5 mRNA and protein levels were determined in placental tissue and maternal sera using quantitative PCR, Western blot, ligand blot, immunohistochemistry, and enzyme-linked immunosorbent assay.
Results: We identified 137 genes as a result of microarrays analysis and focused on PAPPA2 as it was one of the most significantly up-regulated genes in the pre-eclamptic placentas. In the additional examination, PAPPA2 mRNA and protein levels is overexpressed in pre-eclamptic placental tissues ($p<0.01$). Maternal serum concentrations of PAPPA2 was also significantly elevated in pre-eclampsia as compared to uncomplicated pregnancy. Likewise, mRNA levels of IGFBP5 were also significantly increased, suggesting a potential role for IGFBP5 in fetal and placental growth suppression during pre-eclampsia. However, IGFBP5 protein was not elevated, possibly due to protein cleavage by PAPPA2.
Conclusion: These data suggest that PAPPA2 is a potential disease marker for pre-eclamptic pregnancy. Our data also suggest that PAPPA2 might be upregulated in order to compensate for IGFBP5-mediated inactivation of the local IGF pathway, although compensation may be inadequate to prevent low birth weight in pre-eclamptic pregnancy.

P2.11

MOLECULAR EVIDENCE THAT PLACENTAL PATHOLOGY IS MORE SEVERE IN EARLY-ONSET COMPARED TO LATE-ONSET PRE-ECLAMPSIA

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Objectives: Pre-eclampsia can be classified into two major subtypes dependent on the time of onset of the syndrome. Early-onset pre-eclampsia starts before 34 week of gestation, and is often associated with intrauterine growth restriction. In contrast, in late-onset pre-eclampsia, which starts after 34 week, birth weight is normal. Currently, there is debate as to whether these sub-types reflect the same pathophysiology, but with different severities, or different pathologies based on defective placentation and increased maternal susceptibility respectively. Here, we test between these two possibilities by examining molecular markers of placental endoplasmic reticulum (ER) and oxidative stress.
Methods: Placentas from early-onset (<34 week), late-onset pre-eclampsia (>34 week) and normotensive controls were collected from non-laboured, elective caesarean deliveries free of other complications with informed patient consent and local ethical approval. Western blotting analysis was used to examine changes of expression and phosphorylation of protein and kinases involved in ER and oxidative stress, and survival/growth signalling pathways. Differences were tested for using a one-way ANOVA.
Results: There were significant increases ($p < 0.05$) in ER stress markers P-eIF2a, GRP78 and GRP94, oxidative stress markers P-HSP27, HSP70 and HSP90, and stress kinase P-p38, in early onset pre-eclamptic placentas compared to normotensive controls. There was also a significant decrease of the growth and survival kinases P-AKT and P-ERK. No significant differences were observed between late-onset preeclampsia and normotensive controls.
Conclusion: These results demonstrate a clear difference in the molecular pathology of placentas from cases of early- and late-onset pre-eclampsia. Previous work has shown a strong correlation between activation of markers of ER stress and the severity of the initiating insult in trophoblast-like cells. We conclude that early-onset pre-eclampsia is likely to be associated with deficient placentation, whereas the late-onset subtype is due to increased maternal endothelial susceptibility.
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P2.12

FETAL BLOOD FLOW, OXYGEN DELIVERY AND CONSUMPTION IN SUB-CATEGORIES OF PREECLAMPSIA

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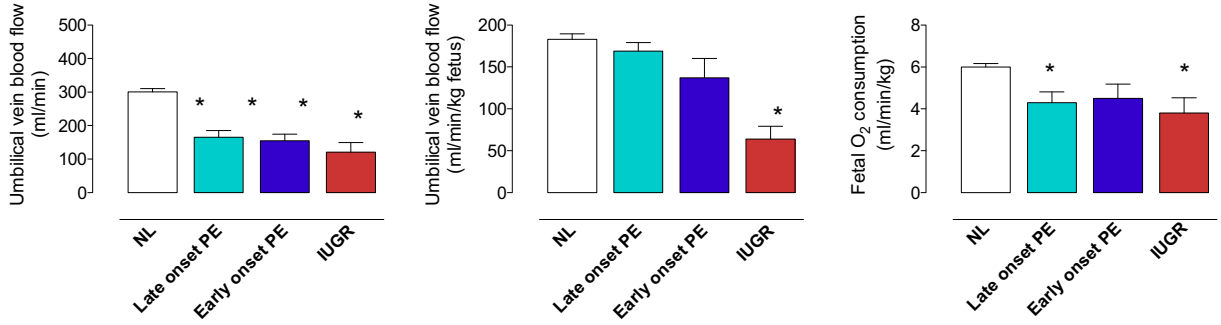
Objective: Lowered blood flow is thought to contribute to feto-placental hypoxia, reduced fetal growth and long-term health consequences in placental complications of pregnancy (preeclampsia–PE/Intrauterine growth restriction–IUGR). Early-onset PE is considered a more severe clinical entity. Disease severity is believed to correlate with the degree of compromise in blood flow, but this has never been formally tested.
Methods: We measured volumetric umbilical venous blood flow (Doppler and ultrasound, ml/min) in 122 normal (NL) and 48 complicated pregnancies (n=28 late-onset PE [≥34 wk], n=13 early onset PE, n=13 IUGR without PE) within 72 hours prior to cesarean delivery. Cord blood samples from the artery (umb a.) and vein (umb V.) were analyzed for blood gases, hemoglobin. Oxygen content was calculated as Hgb (gm/dL) * 1.36 * oxygen saturation (%). We then calculated fetal O_2 delivery and consumption. One-way ANOVA/Dunett's post-test was used to compare pathologies to the normal group and are presented as mean ± SEM.



Results: Abnormal umb. a. Dopplers were present in 0%, 46%, 72% and 57% of NL, late-, early-onset PE and IUGR, respectively. Birth weights were at the $51^{st}\pm8^{th}$ $26^{th}\pm5^{th}$, $25^{th}\pm7^{th}$, and $3^{rd}\pm0.8^{th}$ centile in NL, late-, early-onset PE and IUGR, respectively. Absolute Umb V. blood flow was reduced by 45-60%, but was similar across all pathologies (left panel). However, when flow was normalized to fetal weight, it was reduced only in IUGR (center panel). Fetal O_2 consumption was reduced by 28% late-onset PE and 37% in IUGR whilst the 25% difference in early onset-PE was not significant (right panel), due to greater variability in O_2 consumption in early-onset PE. IUGR in this study appears to have the greatest pathological reduction in flow and O2. The data also suggest that factor other than hypoxia/flow/O_2 delivery account for disease severity in PE. Support: NIH HD042737, HD046982, NSF BCS0309142.

P2.13

CELL-TYPE SPECIFIC ACTIVATION OF UNFOLDED PROTEIN RESPONSE PATHWAYS IN PRE-ECLAMPTIC PLACENTAS

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Objectives: The endoplasmic reticulum (ER) plays a crucial role in integrating cell responses to a variety of stressors through activation of the unfolded protein response (UPR). Three proximity sensors located at the ER membrane, including PERK-eIF2α, ATF6 and IRE1α-XPB1, are activated sequentially in response to increasing severity of stress. Although there is considerable overlap and cross-talk, these pathways mediate different aspects of the UPR, from suppression of translation to induction of apoptosis. We recently reported evidence of activation of the UPR in placentas from cases of intrauterine growth restriction, with and without pre-eclampsia. Here, we immunolocalised the UPR response pathways to specific cell types in pathological placentas.
Methods: Non-laboured placental samples were collected from normotensive controls and early-onset pre-eclamptic (PE) pregnancies delivered by caesarean section, with informed patient consent and local ethical committee approval. Samples were immediately fixed in paraformaldehyde for immunohistochemical analysis, or snap-frozen in liquid nitrogen for Western blotting. Immunoreactivity was assessed by H-score, by an observer blinded to the sample type.
Results: Total IRE1a was expressed ubiquitously in all cell types in both control and PE placentas, whereas immunoreactivity for P-IRE1α was observed only in smooth muscle cells (SMC) in the wall of stem villous arteries in PE placentas. In contrast, ATF6 was expressed in both endothelial cells and syncytiotrophoblast, but not in SMCs. Cleaved ATF6 is a potent transcription factor, and nuclear localisation of ATF6 was observed in endothelial cells in PE placentas, but not in syncytiotrophoblast. Western blotting detected a significant increase of full length ATF6 (p90), cleaved ATF6 (p50) and XBP-1 in pre-eclamptic placentas.
Conclusions: These results indicate differential activation of UPR signalling pathways in different placental cell types in pre-eclampsia. This finding indicates that responses to ER stress may be heterogeneous across placental cell types.
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P2.14

APOPTOTIC AND NECROTIC CELL DEATH OF TROPHOBLAST AND ADIPOSE TISSUE ON HYPOXIA AND PREECLAMPSIA – A PRELIMINARY OBSERVATION

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Objectives: Hypoxia is a key feature of preeclampsia (PIH) in the third trimester of pregnancy. However, there have been few studies about the effect of low oxygen environment on primary trophoblast from placenta. Additionally, there are new findings on adipose tissue-derived cytokines (adipocytokine) in preeclampsia have been reported. In this study, we performed primary trophoblast culture under hypoxic condition and adipose tissue culture with preeclamptic serum, and examined the cell death markers to investigate apoptosis / necrosis of the tissues in preeclampsia.
Methods: Placenta (38 weeks gestation, n=5) and subcutaneous adipose tissue were obtained at elective caesarean section. Serum were obtained from severe preeclampsia and gestational age-matched normal pregnancy(n=5 each) after written informed consent. Cytotrophoblast was separated with Percoll-based method, cultured on fibronectin for 24 hours and attached cells were cultured under 20%, 5%, and 0.1% oxygen condition for additional 24 hours. Cytotoxicity was observed with LDH assay. Adipose tissues were dissected, harvested in collagen gel and 10% preeclamptic/normal human serum-contained media for 24 hours after overnight starvation. Concentrations of M65 (marker of all cell death) and M30 (Apoptosis) proteins were measured with ELISA.
Results: Cytotoxicity observed in trophoblast was reduced in 5% oxygen condition, and reduced more in 0.1% severe hypoxia. Concentrations of M30 protein derived from adipose tissues showed no significant difference between preeclamptic and normal pregnant serum. However, M65 protein concentrations were significantly lower in preeclamptic culture than normal pregnant serum-added culture.
Conclusion: Hypoxia in placenta may lead protective effect in primary trophoblast from term placenta, which suggesting that the physiological oxygen concentration for trophoblast may be low and hypoxia is not a key trigger of cell death. Cell death on adipose tissue-culture was observed and apoptotic cell ratio (M30/M65) was high in preeclamptic serum. Exaggerated production of adipocytokines in preeclampsia may be related to this observation.

P2.15

REGULATION OF PLACENTAL CONNEXINS 43 AND 46 UPON LOW OXYGEN AND ITS IMPACT ON PREECLAMPSIA

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Objectives: While Cx43 regulates trophoblast fusion, Cx40 is needed for the proliferation of the extravillous trophoblast (EVT) which migrates into the maternal compartment. In preeclampsia trophoblast invasion is disturbed resulting in placental insufficiency and chronic hypoxia.
Methods and Results: Here we investigated whether the expression of placental connexins (Cxs) changes upon low oxygen. We found for the first time Cx46 expressed in the placenta, a connexin which is predominantly detected in lens and is important for the cell survival under hypoxia. First hints that these connexins could be regulated by oxygen came from data on early-onset preeclamptic placentas which revealed an increase in Cx43 and a decrease in Cx46. To figure out if hypoxia is responsible for this change invasive JAr trophoblast cells which express Cxs43, 40 and to a lesser extent Cx46 were cultivated under different O_2 concentrations. In contrast to Cx40 which is not changed, 1% O_2 levels increased Cx43 but decreased Cx46. Interestingly, upon hypoxia the localisation of Cx43 shifted from the cell membrane to the cytoplasm. This internalization was dependent on HIF-2α but not on HIF-1α. In contrast Cx46 protein translocated from nucleus adjacent areas to the cell membrane independent from HIF-1α but dependent from HIF-2α stabilization. Both hypoxia mediated translocations of the Cxs were reversible after reoxygenation. Though Cx46 shifted to the cell membrane cell coupling properties analysed by calcein transfer using flow cytometry showed a significant decrease in cell coupling under hypoxia which indicate the dominant function of Cx43. Thus though Cx43 expression levels in JAr cells upon hypoxia were upregulated cell coupling via connexin channels were decreased due to the translocation of Cx43 to the cytoplasm.
Conclusion: To summarize, this study showed that placental connexins Cx43 and Cx46 are hypoxia-regulated and are associated with loss in function which could contribute to the pathomechanisms observed in preeclampsia.

P2.16

MORPHOMETRIC ANALYSIS OF THE DIFFERENCES IN PLACENTAL ANGIOGENESIS DURING EARLY AND LATE-ONSET PRE-ECLAMPSIA

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Background: Early-onset pre-eclampsia rather than late-onset pre-eclampsia is commonly associated to high rate of adverse perinatal outcomes. Despite some controversies, causes have been associated with reduced feto-placental blood flow, related to placental vascular alteration such as infarct, calcification, ischemia, and reduced angiogenesis. These controversies could be associated with clinical parameters and methodological issues during placental histological analysis.
Aim: Investigate whether alteration in morphometric and histological analysis of placental vessels are related to pre-eclampsia onset in samples immunostained with CD31.
Methods: Placental samples from normal pregnant women (n=15), late-onset (n=5), and early-onset pre-eclampsia ($\leq$ 34 weeks, n=6) were obtained after Ethical Committee approbation. In each case, the entire clinical records were reviewed. Placentas were weighted and major and minor diameters were measured. Placental area was calculated considering an elliptical form. Full-depth columns of placental tissue were fixed in paraformaldehyde (4%) in phosphate buffer solution and maintained in paraffin. A histopathologist analyzed the slices of placental tissue stained by hematoxiline and eosine in a blind manner. Density of throphoblast nods, fibrin deposit, fibrosis, and calcification per visual camp were estimated. Morphometric analysis was performed in samples immunostained with CD31. Ten photos were taken for each sample in a randomized form and analyzed in a blind manner. Villus and vessel number, diameters and areas, as well as CD31 expression were analyzed digitally.
Results: Maternal demographic characteristics were similar in all studied women. According with clinical criteria gestation age (34 $\pm$ 1.4 weeks) was lower in early-onset pre-eclampsia compared to normal pregnancy (39 $\pm$ 0.3 weeks) or late-onset (38 $\pm$ 0.5 weeks). Early-onset was characterized reduced newborn weight and height, placental weight and area compared with normal pregnancy or late onset ($P<0.05$). There were not statistical differences in histological signs of fibrosis, fibrin deposits or calcification among the groups. Early (16 $\pm$ 2) and late-onset (18 $\pm$ 3) showed an increased number of vessels per visual camp, mainly located in terminal villi, compared to normal pregnancy (10 $\pm$ 2). Area of intermedia and terminal villi was higher in early-onset, as the area of vessels was in late-onset, compared to normal pregnancy. Accordingly, vessel density (area villi/area vessels) in terminal villi was increased in late-onset but not in early onset compared to normal pregnancy, a phenomenon associated to reduction of diffusion distance in late onset compared to normal or early onset pre-eclampsia (see figure). Digital and visual analysis showed a weak intensity of CD31 stained in all samples from early or late onset compared normal pregnancy ($P<0.05$).
Conclusion: early-onset pre-eclampsia is associated to structural changes in vessels and placental villi formation complicating substances interchange between mother and fetus. Whereas in late-onset pre-eclampsia a compensatory mechanism characterized by increased vessel formation located close to the diffusion area may ensure nutrient uptake toward the fetus under this maternal hypertensive disorder. Supported by FONDECYT 1100684, Conicyt Anillo ACT73.

P2.17

ACTIVATION OF TLR3 IN THE TROPHOBLAST IS ASSOCIATED WITH CHANGED EXPRESSION OF ANGIOGENIC FACTORS

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Objectives: Imbalance between pro-angiogenic (i.e., placental growth factor (PlGF)) and anti-angiogenic factors (i.e., soluble VEGF receptor-1 (sVEGFR-1, also referred to as sFlt1) and Endoglin) is involved in the pathophysiology of preeclampsia. Toll like receptor 3 (TLR3) is a receptor which recognizes viral double stranded RNA and triggers immune reactions upon viral infection. It has been shown that trophobalsts express TLR3, and TLR3 ligation results in productions of sFlt-1 in trophoblast cell line HTR8. In the present study, we showed that the expression of angiogenic factors is affected by TLR3 ligation in primary trophoblasts.
Methods: Under informed consents, human villi samples were obtained from cases of induced abortion in the first trimester. Trophoblasts were isolated and cultured. Swan 71, a first trimester trophoblast cell line was also used. Cells were stimulated with PIC (TLR3 specific ligand). sFlt-1, Endoglin, and PlGF mRNA and protein expression were evaluated by real time PCR and ELISA respectively.
Results: PIC (10 ug/ml) significantly induced sFlt-1, while suppressed PlGF expression in mRNA level in a time- dependent manner, which reached the highest effect 2 hs after stimulation ($P < 0.01$). The statistically significant difference disappeared 6 hs after stimulation. However, Endoglin mRNA was significantly suppressed 6 hr (up to 24 hr) after PIC stimulation. Concentration of sFlt-1 in the conditioned media of Swan cell line 24 hs up to 120hs after PIC stimulation was significantly higher than non-treatment control.
Conclusion: This study showed that anti-angiogenic sFlt-1 is induced, while pro-angiogenesis factor, PlGF is inhibited by TLR3 ligation in trophoblasts shortly after stimulation. Disturbance of angiogenesis factors during the first trimester of pregnancy upon viral infections may be involved in the pathophysiology of preeclampsia.

P2.18

CALCIUM SUPPLEMENTATION PREVENTS ENDOTHELIAL CELL ACTIVATION: POSSIBLE RELEVANCE TO PREECLAMPSIA

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Objectives: The deportation of trophoblast debris from the placenta has been hypothesised that deportation of necrotic trophoblast debris may contribute to maternal endothelial cell activation in preeclampsia. We have previously shown that IL-6 results in shedding of more necrotic trophoblast debris and that this debris when phagocytosed by endothelial cells results in activation of the endothelial cells. A number of studies suggest calcium supplementation may reduce the risk of developing preeclampsia by up to 50% but the protective mechanism of calcium supplementation is unclear. The aim of this study was to investigate whether calcium supplementation affects either the production of necrotic trophoblast debris from the placenta or influences endothelial cell activation.
Methods: First trimester placental explants were cultured with IL-6 in the presence or absence of calcium ($CaCl_2$) for 24hours. Trophoblastic debris was collected from the explants and then exposed to monolayers of endothelial cell for 24hours. In other experiments, endothelial cells were treated with IL-6 or necrotic trophoblastic debris in the presence of increasing concentrations of $CaCl_2$, ranging from 230 to 700μg/mL, or endothelial cells were treated with low concentration of $CaCl_2$, ranging from 0 to 230μg/mL, for 24hours. Endothelial cell activation was measured by quantifying cell-surface ICAM-1 levels by ELISA.
Results:

1) Increasing the concentration of $CaCl_2$ in the medium of placental explants treated with IL-6 did not significantly reduce the activation of endothelial cells induced by phagocytosis of the trophoblasts debris from these explants ($p>0.05$).
2) Increasing the concentration of $CaCl_2$ in the medium of endothelial cells treated with either IL-6 or necrotic trophoblastic debris significantly reversed the activating effects of IL-6 and necrotic trophoblast debris in a dose dependent fashion.
3) Reducing the amount of $CaCl_2$ in the culture of untreated endothelial cells caused a significant increase in endothelial cell activation as measured by ICAM-1 levels.

Conclusion: Our results demonstrate that calcium levels are important to endothelial cell activation and supplemental calcium may reverse the activation of the endothelium induced by proinflammatory mediators or necrotic trophoblastic debris. These results may in part help to explain the benefits of calcium supplementation in the reduction of risk for developing preeclampsia.

P2.19

EXPRESSION LEVEL OF THE RECEPTOR FOR ADVANCED GLYCATED END PRODUCTS (RAGE) IN THE SYNCYTIOTROPHOBLAST CORRELATES WITH THE SEVERITY OF PRE-ECLAMPSIA AS DEMONSTRATED BY A NOVEL METHOD FOR AUTOMATED IN-SITU QUANTIFICATION OF PROTEINS

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Objectives: The receptor for advanced glycated end-products (RAGE) contributes to tissue damage in many inflammatory diseases. Ligand-RAGE interaction activates a cell response that promotes inflammation. Pre-eclampsia (PE) is characterized by systemic inflammation. Available data suggest up-regulation of placental RAGE during PE and consequently involvement of the ligand-RAGE axis in the promotion of inflammation in PE. However, human-based approaches to quantify placental protein-expression remain semi-quantitative and prone to inter-observer differences. Tissue-Cytometry combines microscopic analysis of tissues with fast and reproducible computation of tissue-associated parameters. Automated image segmentation is essential for computer-based tissue evaluation, but often operates by cell identification via their nucleus preventing analysis of multinuclear or a-nuclear tissue elements, e.g. erythrocytes. To enable automated *in-situ* analysis of placental RAGE expression in health and PE, we developed and validated algorithms able to segment the multinuclear syncytiotrophoblast (STB) in the complex shaped placental villi.
Methods: Paraffin-sections of chorionic tissue from PE (n=14) and control (n=13) placentas were labeled with target-specific primary and fluorescent secondary antibodies. A composite image consisting of 81frames/placenta was acquired and digitalized with a software-driven motorized epifluorescence microscope (TissueGnostics GmbH). Automated identification of STB area by cytokeratin-7-expression or of auto-fluorescent erythrocytes by their shape was done combining classical digital image-processing and pattern recognition approaches with machine-learning techniques.
Results: The developed algorithms identified STB areas as well as erythrocytes *in-situ* as good as human experts. RAGE expression was quantified after *in-silico* subtraction of background fluorescence caused by erythrocytes. A positive correlation between RAGE-expression in the STB and severity of PE was demonstrated showing 2.5-fold increase of RAGE in severe cases of PE.
Conclusion: We established a novel approach for fast and reproducible automated analysis of immunofluorescent-labeled placental chorionic tissue, and demonstrated correlation of RAGE expression levels with severity of PE.

P2.20

HTRA3 AS AN EARLY MARKER FOR PREECLAMPSIA: SPECIFIC MONOCLONAL ANTIBODIES AND SENSITIVE HIGH-THROUGHPUT ASSAYS FOR SERUM SCREENING

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Objectives: Mammalian HtrA3 (high temperature requirement A3) is a serine protease of the HtrA family. It has two isoforms [long (HtrA3-L) and short (HtrA3-S)] and is important for placental development and cancer progression. Recently, HtrA3 was identified as a potential diagnostic marker for early detection (13-14 weeks gestation) of preeclampsia. However, there are no high-throughput assays available to detect HtrA3 in human serum. The aim of this study was to generate HtrA3 monoclonal antibodies (mAbs) and high-throughput assays.
Methods: HtrA3 mAbs were generated in mice against recombinant human HtrA3-L protein and a synthetic peptide. The clonal mAbs were epitope-mapped and validated on recombinant and endogenous HtrA3 by cell transfection, Western blotting, and immunohistochemistry. Amplified luminescent proximity homogeneous assays-linked immunosorbent assays (AlphaLISAs), were developed to detect HtrA3 isoforms in serum. Whether these mAbs could modulate (inhibit/stimulate) the proteolytic activity of HtrA3 was also tested.
Results: We cloned five HtrA3 mAbs, three recognised both HtrA3-L and HtrA3-S and the other two detected HtrA3-L only. All five mAbs were highly specific to HtrA3 and applicable in western blotting and immunohistochemical analysis of endogenous HtrA3 proteins in the mouse and human tissues. The newly developed HtrA3 AlphaLISAs detected HtrA3 protein isoforms in picomolar levels in human serum. Importantly, the HtrA3 AlphaLISA detected significantly higher serum levels of HtrA3 in women at 13-14 weeks of gestation who subsequently developed preeclampsia compared to gestation-matched controls. In addition, two of the mAbs modulated HtrA3 activity, one inhibiting and the other stimulating.
Conclusion: We produced a panel of highly specific mAbs recognizing different HtrA3 isoforms. These mAbs are valuable for the development of various immunoassays and characterisation of HtrA3 isoform-specific biology. The newly developed HtrA3 AlphaLISA assays are suitable for large scale screening of human serum. These mAbs also provide invaluable tools to modulate HtrA3 action.

P2.21

PRELIMINARY RESULTS OF THE MORE PREPARD STUDY (MICROPARTICLE ORIENTATED RISK EVALUATION IN THE PREDICTION OF PREECLAMPSIA AMONG RISK GRAVIDAS): A MULTICENTER PROSPECTIVE PROGNOSTIC MARKER STUDY

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Introduction: Preeclampsia (PE) is a potentially dangerous pregnancy pathology contributing to higher worldwide mortality and morbidity. The negative influence of syncytiotrophoblastic microparticles (STBMs) on the placenta and maternal endothelia is thought to play a key role in generating the inflammatory effects that lead to PE symptoms. Doppler sonography of the uterine arteries assists in identifying a risk population, however, the positive predictive value for this method is low.
Aim of this study is to evaluate whether STBMs can serve as an accessory marker to conventional Doppler sonography to better identify pregnant women who will actually develop PE.
Methods: Pregnant women between 19-21 gestational weeks (GW) with abnormal uterine perfusion were enrolled into this prospective study. Plasma samples were taken at inclusion (baseline) and at two further visits at 8 week intervals to follow STBM concentration alterations during pregnancy. The primary endpoint assessed is PE and/or hemolysis, elevated liver, low platelets (HELLP) syndrome. Other PE-associated pathologies (intrauterine growth retardation [IUGR], intrauterine fetal demise [IUFD], placental abruption, premature delivery) constitute the secondary endpoints. Maternal STBM concentrations were measured using a homemade Enzyme Linked Sorbent Assay (ELSA) which specifically measures STBMs. The receiver operating characteristics (ROC) for baseline measures are graphically displayed and area under curve (AUC) is estimated including 95% confidence levels.
Results: Of the 73 women included in the study, 16 developed PE (cases) and 56 did not (control). After analyses of mid-gestational probes, the ROC curve was in close proximity to the line of no-discrimination.
Conclusion: Our preliminary results indicate that the maternal STBM concentration at mid-gestation does not predict the development of PE or associated pregnancy pathologies. Further analysis is underway to assess whether STBM measurements at later gestational time points can predict PE shortly before onset of disease.

P2.22

PRO-COAGULANT CAPACITY OF SYNCYTIOTROPHOBLASTIC MICROPARTICLES (STBMS)

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Introduction: A characteristic of the severe pregnancy pathology, preeclampsia (PE), is endothelial dysfunction as seen through vasoconstriction and platelet activation. The release of syncytiotrophoblastic microparticles (STBM) is associated especially with severe, as opposed to mild, forms of PE. Classic STBM research has been geared to investigating their effects on the endothelial compartment, however, their thrombogenic potential is not well characterized.
Aim of the study was to investigate the pro-coagulant activity of STBMs.
Methods: STBMs were derived from placenta perfusates and, after staining with FITC-labeled annexin-V that binds to negatively charged phospholipids, quantified by flowcytometry. Pro-coagulant activity was determined on immobilized STBMs as prothrombinase activity in a plasma-free system or as the velocity of fibrin formation after addition of STBMs to normal plasma. ADP-induced aggregation of blood platelets in platelet-rich plasma (PRP) was measured in absence and presence of STBMs using PAP-4 aggregometer (mölab GmbH, Hilden).
Results: STBMs expose negatively charged phospholipids at their surface which can be used for flowcytometric quantification. Due to these phospholipids, STBMs exert a significant pro-coagulant activity indicated by their prothrombinase activity as well as by the accelerated fibrin formation. STBMs also significantly increase the rate of ADP-induces platelet aggregation.
Conclusion: STBMs have a pro-coagulant activity as well as a stimulating effect on platelet aggregation. Both effects may contribute the impaired microcirculation in PE. The prothrombotic effects of STBMs are at least partially related to the exposure of negatively charged phospholipids. However, also other factors such as exposure of tissue factor and receptors for interaction with platelets may contribute. Further research is underway to validate this hypothesis.

P2.23

A STUDY ON THE ASSOCIATION OF SFLT-1 WITH PLACENTAL OXIDATIVE STRESS AND PLACENTAL APOPTOSIS IN PREECLAMPSIA

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Introduction: Preeclampsia is a multi-systemic hypertensive disorder, characterized by new-onset of hypertension and proteinuria after 20 weeks of gestation. It occurs only in the presence of a placenta and is characterized by abnormal trophoblast invasion of the spiral arteries leading to placental ischemia. The hypoxic placenta may then induce the production of a circulating toxin sFlt-1 that functions as a decoy receptor during placental development and prevents the binding of pro-angiogenic factor (VEGF & PlGF) to its signaling receptor (VEGFR-1) leading to endothelial dysfunction. This may also lead to an exaggerated state of oxidative stress in the placenta which in turn may stimulate increased placental apoptosis. The linkage between the generation of circulating sFlt-1 and the placental oxidative stress and apoptosis remains unidentified.
Objectives: Therefore we aimed to study the association of circulating sFlt-1 with placental oxidative stress and apoptosis in preeclamptic patients.
Methods: 40 preeclamptic patients and 40 control pregnant women were selected and followed till their delivery. Blood samples were collected at the time of diagnosis and placentas were collected after the delivery. The serum levels of sFlt-1 were measured by enzyme-linked immunosorbent assay (ELISA) and the placental oxidative stress markers like MDA, GSH and SOD were estimated by biochemical estimation. The placental apoptosis was also studied by immunohistochemistry of various apoptotic markers like Bcl2, Bax, Fas, Fas L, M30 and TUNEL technique.
Results: The serum levels of sFlt-1 were significantly higher in preeclamptic women compared to the control. The levels of MDA were significantly higher and the levels of GSH and SOD were significantly lower in the placentas of preeclamptic patients as compared to the control group. This altered levels of oxidative stress markers proved that oxidative stress was significantly increased in preeclamptic placentas as compared to the control. The apoptotic index was significantly higher in preeclamptic placentas as compared to the control by various apoptotic markers. This also proved that apoptosis was significantly increased in placentas of preeclamptic patients as compared to the control.
Conclusion: Therefore it is concluded that higher levels of serum sFlt-1 may have an association with increased placental oxidative stress and placental apoptosis in preeclampsia.

P2.24

PLACENTAL GLUCOSE TRANSPORTER (GLUT)-1 EXPRESSION IN PREECLAMPSIA

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Objectives: Preeclampsia, a pregnancy-specific disease affecting up to 5% of all pregnancies worldwide, is a major cause of maternal and perinatal morbidity and mortality. Materno-fetal glucose transport is crucial for the fetal well-being since there is no de novo glucose synthesis in the fetus. Previously we have shown that placental glucose transporter (GLUT)-1 is up-regulated upon hypoxia. Preeclampsia is associated with hypoxia as well as intrauterine growth restriction. The aim of this study was to investigate placental (GLUT-1) expression in preeclampsia.
Methods: Placentae were obtained after elective caesarean sections following normal pregnancies (controls) and pregnancies complicated by preeclampsia. Syncytial basal membrane (BM) and apical microvillous-membrane (MVM) fractions were prepared using differential ultra-centrifugation and magnesium precipitation. Protein expression was assessed by Western blot analysis of syncytial BM and MVM fractions.
Results: In both preeclampsia and controls (GLUT)-1 protein expressions were significantly lower in BM than in MVM. In preeclampsia, (GLUT)-1 expression was significantly reduced in MVM but not altered in BM fractions when compared to controls.
Conclusions: (GLUT)-1 is asymmetrically expressed on syncytial basal and apical membranes of placentae following normal pregnancies and pregnancies compromised by preeclampsia. Compared to controls, the (GLUT)-1 protein expression pattern is substantially changed in preeclampsia. This altered (GLUT)-1 expression might have an impact on materno-fetal glucose transport, which in turn impairs fetal growth and development.

P2.25

IDENTIFICATION OF PREGNANCY-ASSOCIATED MICRORNAS AND THEIR CIRCULATING LEVELS IN PLASMA FROM NORMAL PREGNANT WOMEN AND PRE-ECLAMPTIC WOMEN

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Background: Recently, some of placental microRNAs (miRNA) were reported as pregnancy-associated molecules. However, the understanding of these novel molecules is still limited. In this study, we investigated the clinical significance of pregnancy-associated microRNAs in pre-eclampsia.
Methods: By microarray-based screening of 723 human microRNAs, we selected placenta-predominantly expressed microRNAs that exhibited signal intensities >100 times higher in placental tissues than in corresponding whole blood samples. Subsequently, by quantitative real-time RT-PCR, microRNAs that were predominantly expressed in the placenta and that showed significantly decreased levels in maternal plasma after delivery were identified as pregnancy-associated microRNAs. Association between the circulating levels of pregnancy-associated miRNA and the presence of pre-eclampsia was investigated.
Results: Eighty-two placenta-predominantly expressed microRNAs were selected and 24 out of them were identified as pregnancy-associated microRNAs. These included 16 microRNAs (66.7%; 16/24) clustered on 19q.13.42. The chromosome 19 miRNA cluster (C19MC) at 19q31.42 is specifically expressed in the placenta. As cell-free pregnancy-related miRNA markers, has-miR-515-3p and hsa-miR-519b-3p were selected from 3' and 5' region of C19MC. The plasma concentrations of CF C19MC-derived microRNAs (has-miR-515-3p and has-miR-519b-3p) increased significantly in plasma from pre-eclampsia than in that from normal pregnancy (Mann-Whitney's U test, P=0.0274 and 0.0019, respectively).
Conclusion: Most of pregnancy-associated microRNAs were clustered on 19q13.42, which are critical regions for placental development. Pregnancy-associated microRNAs may be useful molecular markers for monitoring the presence of pre-eclampsia.

P2.26

THE LIPID TRANSPORTERS ABCA1 AND ABCG1 ARE DIFFERENTIALLY EXPRESSED IN SYNCYTIOTROPHOBLASTS OF PREECLAMPTIC AND IUGR PLACENTAS

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Objectives: The ATP-binding cassette (ABC) transporter A1 (ABCA1) and ABCG1 are highly expressed in the placenta in various compartments, including the villous syncytiotrophoblast (V-STB) and foetal endothelial cells. Among other not yet characterized functions, they play a role in the foeto-maternal transport of cholesterol and other lipophilic molecules. In humans, preliminary data suggest expressional changes of ABCA1 and ABCG1 in pathologic gestation, particularly under hypoxic conditions, but a systematic expression analysis in common human pregnancy diseases has never been performed. Therefore, the aim of the present study was to characterize ABCA1 and ABCG1 expression in a large series of pathologic placentas, in particular from preeclampsia (PE) and intrauterine growth restriction (IUGR) which are associated with placental hypoxia.
Methods: Placentas from 152 pathological pregnancies, including PE and/or HELLP (n=24) and IUGR (n=21), and 20 normal control placentas were assessed for their ABCA1 and ABCG1 mRNA and protein expression with quantitative RT-PCR and semi-quantitative immunohistochemical analysis, respectively.
Results: *ABCA1* protein expression in the V-STB was significantly less extensive in PE compared with normal controls (<10% of V-STB stained for ABCA1 in 58% PE placentas vs. 25% controls; p=0.035). Conversely, it was significantly more widespread in IUGR (>75% of V-STB stained in 57% IUGR placentas vs. 15% controls; p=0.009). Moreover, there was an insignificant trend for increased ABCA1 expression in fetal endothelial cells of stem villi in PE (p=0.0588). ABCA1 staining levels in V-STB were significantly associated with placental histopathological features related with hypoxia: they were decreased in placentas exhibiting syncytial knotting (p=0.033) and decidual vasculopathy (p=0.0437) and increased in low weight placentas (p=0.015). The significant and specific alterations in ABCA1 protein expression found at a specific cellular level were not paralleled by changes in ABCA1 mRNA abundance of total placental tissue. *ABCG1* staining was universally extensive in the V-STB of normal placentas, always affecting more than 90% of V-STB surface. In comparison, ABCG1 staining of the V-STB was generally often reduced in pregnancy diseases. In particular, less than 90% of V-STB exhibited ABCG1 staining in 26% of PE placentas (p=0.022) and 35% of IUGR placentas (p=0.003). Similarly to ABCA1, ABCG1 mRNA expression in total placental tissue was not significantly different between controls and PE or IUGR.
Conclusion: ABCA1 and ABCG1 proteins are differentially expressed, with either down- or up-regulation, in the V-STB of placentas exhibiting features of chronic hypoxia, such as in PE and IUGR. This suggests that other factors in addition to hypoxia regulate the expression of placental lipid transporters. The specific changes on a cellular level were masked when only total tissue mRNA was analysed underlining the importance of cell specific expression analysis. The potential effects of decreased placental ABCA1 and ABCG1 expression on foetal nutrition and development remain to be elucidated.

P2.27

ASSOCIATION BETWEEN CARDIOTOCOGRAPH (CTG) MONITORING AND UMBILICAL CORD BLOOD ANALYSIS AT PARTURITION

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Objectives: Cardiotocograph (CTG) monitoring is useful to predict a fetal hypoxic stress during parturition. However, the diagnosis of neonatal hypoxia is not always easy only through CTG. The aim was to evaluate the association between CTG level and umbilical blood pH and leaking enzymes.
Methods: 101 term births at Juntendo hospital, Tokyo (from October 2011 to January 2012) were analysed on Apgar score, mode of delivery, umbilical blood pH and leaking enzymes (AST, LDH, CK, NSE) by CTG level based on the guideline 2009 of Japan Society of Obstetrics and Gynecology (JSOG). The pregnancies with maternal complication, chorioamnionitis and preterm birth were excluded.
Results: Apgar score (1 minute) of CTG level 4 was worse significantly than that of level 1 ($p<0.05$). With the deterioration of CTG level, umbilical blood pH declined with no significance ($p=0.06$). However, umbilical blood base excess (BE) significantly declined with the deterioration of CTG level ($p<0.05$). Moreover, umbilical blood AST significantly increased with the deterioration of CTG level ($p<0.05$). There were no significant differences in the other leaking enzymes. On mode of delivery, there was no significant differences of CTG level in cesarean section, however CTG level was significantly worse in forceps delivery compared with normal delivery ($p<0.0001$).
Conclusion: CTG level was closely related to umbilical blood pH, BE and leaking enzymes at parturition. These data might be important to consider the clinical management and justify the use of CTG level and umbilical blood analysis as an important predictor of neonatal condition.

P2.28

HYPOXIA INDUCIBLE FACTOR-1 (HIF-1)-DIRECTED DIVERSION OF CARBON FLUX IN MURINE TROPHOBLAST CELLS

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Objectives: Metabolic reprogramming is a reversible, HIF-1-regulated process by which cells under hypoxic stress transition into a hypometabolic state characterized by decreased oxidative and increased glycolytic metabolism. As part of this process it is proposed that HIF-1 increases the expression of pyruvate dehydrogenase kinase-1 (PDK1), which inhibits conversion of pyruvate to acetylCoA, and lactate dehydrogenase A (LDHA), which favours conversion of pyruvate to lactate. This results in the diversion of glucose carbon to lactate rather than to oxidation. In this study we investigated the role of HIF-1 in activating this response in murine trophoblast cells.
Methods: The murine SM9-1 trophoblast cell line was co-transduced with lentiviral particles containing a HIF-luciferase reporter and either shRNAmir for a non-silencing control (NSC) or three different HIF-1α shRNAmir (HIF1A52, HIF1A59, HIF1A93). The HIF-1 transcriptional response in cells expressing the shRNAmir was measured via luciferase activity following incubation with a hypoxia mimetic (dimethyloxalylglycine, DMOG, 0.5 mM; 24 hr). Western blotting for HIF-1α, PDK1 and LDHA was performed using extracts of NSC- or HIF1A-transduced SM9-1 cells obtained after incubation under normoxic (10% O_2) or hypoxic (1% O_2) conditions.
Results: Luciferase activity stimulated by HIF-1 following DMOG treatment was reduced to 0.72 ± 0.19, 0.67 ± 0.12 (NS) and 0.04 ± 0.12 ($p< 0.01$) of the NSC control by HIF1A52, HIF1A59 and HIF1A93 respectively ($n=3$). Expression of HIF-1a, PDK1 and LDHA protein was increased 2.00 ± 0.25, 1.78 ± 0.23 and 1.58 ± 0.07 fold ($p < 0.05$, $n=3$) in NSC-transduced cells exposed to hypoxia however no increase was observed in the HIF1A93-transduced cells (1.24 ± 0.29, 1.25 ± 0.13, 1.15 ± 0.14, NS).
Conclusions: HIF1A93 shRNAmir was able to inhibit HIF-1α up-regulation by DMOG and by 1% O_2. HIF1A93 also inhibited the hypoxic up-regulation of PDK1 and LDHA demonstrating that the hypoxia-stimulated increase in HIF-1 is responsible for the up-regulation of PDK1 and LDHA in murine trophoblast. *(Supported by NIH HD46982 to NPI).*

P2.29

DOES HYPOXIA ALTER THE PLACENTAL RENIN ANGIOTENSIN SYSTEM (RAS)?

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Objectives: We have previously shown an increase in the placental angiotensin type 1 receptor in pre-eclampsia compared to normotensive women[1] hypothesising that this is related to hypoxia during placental development. To test this, we studied placentae from low and high altitude locations.
Hypothesis: Placental RAS components will be increased in high altitude pregnancies.
Methods: Biopsies were collected from term normotensive women at sea-level (Cambridge and London, UK) and high altitude, 3100m (Leadville, Colorado) immediately after vaginal delivery or elective nonlaboured Caesarean section and with informed, written consent.
Immunohistochemistry was performed on paraffin-embedded serial sections using antibodies to angiotensinogen (AGT), prorenin, prorenin receptor (PRR), angiotensin type 1 (AT1R) and type 2 (AT2R) receptors. Protein expression was semi-quantitatively assessed (H-score) by a blinded observer and compared between groups (Mann Whitney U test).
Results: Expression of prorenin ($P = 0.032$), PRR ($P<0.0001$), AT1R ($P = 0.005$) and AT2R ($P = 0.004$) were all significantly higher in placentae from high altitude. Expression of AGT was not different ($P>0.5$). The ratio between prorenin and its receptor also rose significantly ($P = 0.012$), while that between the AT1R and AT2R was unchanged ($P>0.6$). The type of delivery did not influence expression of any of the components of the RAS ($P >0.2$ to >0.7).
Conclusion: Placental RAS is activated in high altitude pregnancies which may be hypoxia-related. The increased ratio of prorenin to its receptor may have functional effects. Binding of (pro)renin to PRR induces a 4-fold increase in its efficiency to generate angiotensin I (AngI) from AGT[2]. Changes in PRR expression, even without changes in AGT expression, can have a substantial effect on the generation of AngI, and hence AngII, affecting placental angiogenesis.

1. Mistry HD et al, (2012) abstract submitted to IFPA Japan 2012
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P2.30

HYPOXIA INCREASES UROKINASE-TYPE PLASMINOGEN ACTIVATOR RECEPTOR (UPAR) AND HYPOXIA INDUCED FACTOR (HIF) -1ALPHA IN EARLY HUMAN EXTRAVILLOUS TROPHOBLAST

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Objectives: Extravillous trophoblast cell (EVT) invasion in early human pregnancy progresses under low-oxygen environment. The urokinase-type plasminogen activator (uPA) system is regarded to be implicated in EVT invasion, since uPA binds to its receptor (uPAR) on cell surface has been clarified where it catalyses the conversion of plasminogen to plasmin. However, there have been few studies about the influence of low oxygen environment on HIF and uPA system of primary trophoblast in early first trimester. In this study, we investigated uPA, uPAR, PAI-1 and HIF-1alpha secretion by primary EVTs in normal or hypo/ hyperoxia condition, and examined whether uPA system contribute to the fair placental development in low-oxygen environment.
Methods: Placental samples (5-9 weeks gestation) were obtained from patients with artificial abortion after written informed consent. Cytotrophoblast was separated with Percoll-based method and cultured on MatrigelR for 24 hours to obtain invasive phenotype (EVT like cell). Caseinase activity in cytotrophoblast was studied by casein in situ zymography. Expression of uPA, uPAR, PAI-1 and HIF-1alpha protein on EVT was measured with Western Blot and ELISA. The culture was performed under 20% oxygen, 5%, and 5% that repeated three times of hypoxic stimulation for one hour (0.1%). All data were shown as a comparison of 20% or 0.1% with 5%.
Results: Stronger caseinase activity was observed under 5% oxygen than 20% oxygen in cytotrophoblast. The production of uPAR, PAI-1 and HIF-1alpha protein was increased under 0.1% oxygen stimulation and decreased in 20% oxygen condition.
Conclusion: Hypoxia altered casainase activity of cytotrophoblast and production of uPAR, PAI-1 and HIF-1alpha by EVT cells in early first trimester of pregnancy. These results indicated that up-regulation of uPAR by hypoxia may be mediated by HIF increase in early first trimester of human pregnancy. The regulation of uPA system by HIF-1alpha may contribute to fair trophoblast invasion.

P2.31

EFFECTS OF DIETARY OMEGA-3 FATTY ACIDS ON OXIDATIVE AND INFLAMMATORY STATUS OF THE RAT PLACENTA

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Objectives: Placental oxidative stress and inflammation play key roles in the pathophysiology of placental-related disorders. Protection from oxidative stress is provided by antioxidant enzymes which inactivate reactive oxygen species. Omega-3 (n3) polyunsaturated fatty acids (PUFAs) are proposed to have both anti-inflammatory and antioxidant properties. Here, we tested the hypothesis that dietary n3-PUFA intake reduces the oxidative and inflammatory status of the placenta.
Methods: Pregnant rats consumed a high n3-PUFA (Hn3) or control diet from day 1 of pregnancy. Fetuses and placentas were collected on days 17 and 22 (term = day 23), and placentas were dissected into junctional (JZ) and labyrinth (LZ) zones. Placental oxidative status was measured by F_2-isoprostane concentration, and placental gene expression of antioxidant enzymes Cat, Sod2, Txn1, Pxn1, Pxn5 and Gpx3, and of pro-inflammatory mediators Tnfα, IL-6, IL-1β, Cox1 and Cox2 were measured by qRT-PCR (males only).
Results: Hn3 consumption increased fetal ($P<0.05$) and placental ($P=0.05$) weights at day 22 (6.2% and 10.6%, respectively). The Hn3 diet decreased the concentration of F_2-isoprostanes in LZ on days 17 (28%; $P<0.001$) and 22 (8%, $P<0.05$), and in JZ at day 22 (25%; $P<0.05$). Hn3 intake increased placental expression of Cat in LZ at both days ($P<0.001$), but decreased Pxn5 in LZ ($P<0.05$) at day 17, and decreased Cat, Sod2 and Pxn1 in JZ at day 17 ($P<0.05$, $P<0.001$ and $P<0.05$, respectively). Surprisingly, LZ expression of IL-6 (2.7-fold, $P<0.05$) and IL-1β (1.7-fold, $P<0.05$) were both increased by Hn3 diet at day 22.
Conclusion: Dietary n3-PUFA supplementation increased fetal and placental growth, and this effect was associated with reduced placental oxidative status. The Hn3 diet clearly enhanced LZ expression of Cat, whereas it reduced JZ expression of Cat and other antioxidant enzymes. Pro-inflammatory cytokine gene expression was increased by the Hn3 diet at day 22, possibly linked to approaching parturition.

P2.32

THE EXPRESSION OF THE NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2) IN INVASIVE EXTRAVILLOUS TROPHOBLAST IN PREECLAMPSIA

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Objectives: Impaired trophoblast invasion into the uteroplacental arteries is accompanied with an evidence of oxidative stress in the extravillous trophoblast (EVT) in preeclampsia (PE) complicated with intra-uterine growth restriction (IUGR).
One of the major cellular defence mechanisms against oxidative stress is the activation of the Nuclear factor erythroid 2-related factor 2 (Nrf2). Recently, we have shown that PE is associated with an increased expression of Nrf2 in villous cytotrophoblast.
Furthermore, we found that VEGF could enhance *in vitro* the persistence of BeWo cells under oxidative stress conditions.
In this study the expression of both Nrf2 and VEGF was determined in the interstitial and intramural extravillous trophoblast in normal pregnancies and those complicated by early-onset preeclampsia and intra-uterine growth restriction (IUGR).
Methods: Full-thickness uterine tissues derived from caesarean hysterectomies performed in 6 healthy normotensive women delivering term infants and from 6 women with severe early-onset preeclampsia and IUGR (29-34 week's gestation). The interstitial and intramural EVT were studied by immunohistochemical analysis of paraffin sections stained with anti-VEGF, VEGFR-1/Flt-1, Nrf2, 4-HNE and cytokeratin-7.
Results: Cases suffering from preeclampsia with IUGR were characterised by reduced invasion of EVT into uteroplacental arteries in the endometrial and myometrial segments. In addition, these cells showed an increased expression of Nrf2 in the pathological sections. The increased expression of Nrf2 in cases of PE/IUGR was associated with decreased expression of VEGFR-1/Flt-1 in these cells compared to controls.
Conclusion: Our data suggests that besides villous cytotrophoblast, also the EVT is a source of Nrf2-dependent genes. VEGF deficiency may cause higher oxidative stress in extravillous trophoblast in cases with preeclampsia and IUGR. The resulting reduced basal defence against oxidative stress and the higher vulnerability to oxidative damage may play a role in the limited trophoblast invasion into spiral arteries in cases suffering from early-onset preeclampsia and IUGR.

P2.33

SYNCYTIOTROPHOBLASTIC TRANSCRIPTIONAL ACTIVITY AND OXIDATIVE STRESS IN COMPLICATIONS OF PREGNANCY

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Objectives: It has been shown that a proportion of nuclei in the syncytiotrophoblast of the normal human placenta are transcriptionally active at all stages of gestation (Fogarty et al 2011). It is currently unknown what stimulus renders nuclei inactive. We investigated transcriptional activity and oxidative DNA damage in the syncytiotrophoblast in cases of pre-eclampsia, with and without IUGR (PE, IUGR and PE+IUGR). These findings are correlated with an ex vivo model of induced oxidative stress.
Methods: Paraffin-embedded sections from placentas of PE, IUGR and PE+IUGR and gestational age-matched controls were selected (n=5, 30 weeks). RNA Pol II and 8-oxo-2'deoxyguanosine (8OHdG) were selected as markers of transcriptional activity and oxidative DNA damage. Immunohistochemistry was performed and proportions of active and damaged nuclei were calculated.
Villous explants from term Caesarean-delivered placentas were cultured in H_2O_2 (n=5, 0mM and 1000mM). Proportions of RNA Pol II and 8OHdG-positive nuclei were calculated at 0, 24 and 48 hr.
Results: IUGR and IUGR+PE had significantly reduced proportions of RNA Pol II-positive nuclei compared to normal (normal 60.7%; IUGR 47.6%, p=0.023; PE+IUGR 44.04%, p=0.012). In contrast, the proportion of nuclei staining for 8OHdG was significantly increased in PE, IUGR and IUGR+PE compared to normal (normal 39.5%; PE 59.34%, p=0.006; IUGR 63.08%, p=0.003; and PE+IUGR 63.09%, p=0.002).
In the ex vivo model an increase in 8OHdG staining was observed after 24hr, and a significant reduction in RNA Pol II-positive STB nuclei compared to control after 48hr (0mM 48hr 48.6%; 1000mM 48hr 67%, $p<0.05$).
Conclusion: PE, IUGR and IUGR+PE have significantly increased oxidative DNA damage. IUGR and IUGR+PE have significantly reduced percentages of transcriptionally active STB nuclei. In an ex vivo model of induced oxidative stress, the percentage of transcriptionally active STB nuclei is reduced. We hypothesise that increased oxidative stress results in a reduction in the transcriptional status of the syncytium in pathological pregnancies.
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P2.34

UTEROPLACENTAL CIRCULATION AND MATERNAL ABDOMINAL AORTIC STIFFNESS IN NORMAL AND COMPROMISED PREGNANCY

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Objective: to examine the elastic properties of the abdominal aorta (AA) in pregnant women with uteroplacental insufficiency.
Subject: A study of 82 normal pregnant women and 60 compromised pregnant women who were identified by uterine artery Doppler flow waveform systolic/diastolic ratio >95th percentile (increased peripheral resistance) was carried out to examine the elastic properties of the maternal abdominal aorta (AA).
Method: An aortic stiffness index (SI) was measured between 18 and 40 weeks at four weekly intervals with a phase-locked loop ultrasound technique to estimate the aortic systolic and diastolic diameters and their correlation with blood pressure.
Results: In the normal group, the aortic systolic and diastolic diameters, as well as the SI, increased with the maternal age. In the compromised group, aortic diameter and blood pressure were normal, but SI during the early second trimester was increased. Pregnancy outcome was examined in relation to the SI. Twenty-two women from the compromised group with an SI above 95th percentile for their age had a significantly higher prevalence of pre-eclampsia and intrauterine growth restriction in comparison with women with a normal SI ($p<0.001$). The aortic SI was significantly higher in severe than in mild pre-eclampsia.
Conclusion: This study has demonstrated that stiffness of the AA is increased in pregnant women with uteroplacental insufficiency and that a progressive increase of the SI on serial studies is associated with severity of the pregnancy outcome. Aberrant hemodynamic adaptation in pre-eclampsia seems to include increased stiffness of the larger artery beside high resistance in small peripheral arteries.

P2.35

FIFTEEN CASES OF CHRONIC ABRUPTION-OLIGOHYDRAMNIOS SEQUENCE (CAOS)

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Objectives: Recently it has been reported that chronic abruption with oligohydramnios induces premature labor and neonatal chronic lung disease. Chronic abruption-oligohydramnios sequence was defined by Elliot in 1998, by the following criteria: (1) clinically significant vaginal bleeding in the absence of placenta previa or other identifiable source of bleeding, (2) amniotic fluid volume initially documented as normal, and(3) oligohydramnios (amniotic fluid < or=5 cm) eventually developing without concurrent evidence of ruptured membranes.
Methods: We investigated the perinatal outcome of fifteen cases with CAOS compared with premature labor without CAOS.
Results: In the past 7 years, there were 30 cases that had been admitted to our hospital with recurrent vaginal bleeding from early gestational weeks. In the second trimester, 15 patients had oligohydramnios (CAOS group) and 15 patients had adequate amount of amniotic fluid (non CAOS group). In both groups, the first vaginal bleeding episode was seen in early gestational weeks. In the CAOS group, subchorionic hematoma (SCH) was detected in 12 cases. They had oligohydramnios without evidence of ruptured membranes. All cases resulted in preterm delivery before 31 weeks of gestation. Pathological analysis showed that chorioamnionitis (CAM). degenerative necrosis and diffuse chorioamniotic hemosiderosis were detected in their placentas. In non CAOS group, SCH was also detected, but amniotic fluid volume was adequate. Neonatal outcome was good even in patients with CAM in non CAOS group. Neonatal morbidity was high in patients with severe CAM in CAOS group.
Conclusion: The episode of recurrent vaginal bleeding followed by oligohydramnios is a risk factor for preterm delivery. Severe CAM and long term intrauterine inflammation lead to neonatal poor prognosis such as chronic lung disease. We need to pay attention to vaginal bleeding during the first trimester as one symptom of CAOS.

P2.36

EXPRESSION OF DECORIN AND ASSOCIATED EXTRA CELLULAR MATRIX (ECM) COMPONENTS DURING PLACENTATION IN AI AND SCNT PREGNANCIES IN COW

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Objectives: Based on our previous studies, we assumed that the composition of the extracellular matrix components (ECM) played a role during placentation and that misexpression of genes of the ECM were involved in placental pathologies observed in miscarriages of bovine cloned conceptuses. The aim of this study was to compare the expression pattern of Decorin (DCN), Collagen type1 alpha2 (Col1A2) and Fibronectin 1 (FN1) in pregnancies obtained by artificial insemination (AI) and produced by somatic cell nuclear transfer (SCNT) in cow.
Methods: Endometrial, extra-embryonic and placental tissues were obtained from bovine AI and SCNT pregnancies from day 18 of gestation to term. Tissue samples were processed for in situ hybridization (ISH), immunohistochemistry and RT-qPCR.
Results: In AI endometria, Decorin, Collagen1α2 and Fibronectin1 were expressed in the caruncular and intercaruncular stroma. In the maternal part of the placentomes, expression of all three genes decreased during pregnancy and became barely detectable at term. In the day 18- extra-embryonic tissues, FN1 was expressed in the primitive endoderm underlying the trophoblast. Later on during pregnancy, Decorin, Col1A2 and FN1 were expressed in the extra embryonic mesoderm cells, which constitute the allanto-chorion mesenchyme of the placental villi and the intercotyledonary chorion. In the core of the placental villi, however, a decreasing gradient of expression was observed from the chorionic plate to the deep end of the villi. In SCNT placentomes the levels of expression of Decorin and Col1A2 did not decrease during gestation and remain high at term.
Conclusion: These observations suggest that the expression of ECM components is altered in SCNT pregnancies and that these modifications might contribute to placental dysfunctions and term complications in bovine somatic cloned embryos.

P2.37

MATERNAL PLATELET RESPONSE TO THROMBIN REGULATES PLACENTAL DEVELOPMENT AND FETAL GROWTH IN A MURINE MODEL OF THROMBOPHILIA-ASSOCIATED PREGNANCY LOSS

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Objectives: Mechanisms by which thrombophilia alters placental function are unclear. In our previous work, we combined maternal and fetal thrombophilia mutations to generate a murine model of placental developmental failure and fetal death (PMID 17438064). We showed that platelets play a critical role in disease pathology. We examine the mechanism of platelet-mediated fetal loss in this model.
Methods: Breeding experiments were conducted to combine homozygous Arg504Gln mutation in blood clotting factor V (FVQ/Q) in the mother with thrombomodulin Glu387Pro mutation in the fetus (ThbdPro/Pro). Pharmacological and genetic tools were used to address the role of thrombin-mediated maternal platelet activation and the role of platelet receptors αIIbβ3 and GPVI in fetal loss.
Results: No live $Thbd^{Pro/Pro}$ embryos were observed when $FV^{Q/Q}Thbd^{Pro/+}$ females were mated to $Thbd^{Pro/+}$ males and analyzed at 12.5 dpc or later. In contrast, all genotypes were present at normal Mendelian ratio in the reverse genetic cross. The combination of $FV^{Q/Q}Thbd^{Pro/+}$ mother and $Thbd^{Pro/Pro}$ embryo resulted in placental developmental failure. Genetic absence of thrombin receptor Par3 in the mother rescued 50% of all $Thbd^{Pro/Pro}$ embryos. These had smaller placentae and were growth retarded by 15.5 dpc. Histological analysis of growth retarded placentae did not reveal evidence of increased thrombosis. In contrast to Par3, when the mother lacked thrombin receptor Par4 the placental and embryonic sizes of $Thbd^{Pro/Pro}$ embryos were comparable to littermate controls. In ongoing studies with the genetic absence of αIIb in the mother or pharmacological inhibition of GPVI we observe occasional live $Thbd^{Pro/Pro}$ embryos, but the number of abortions continue to be high. We will present genetic data and statistical analysis from these studies at the meeting.
Conclusion: Even a small decrease in EC50 of maternal platelet response to thrombin overcomes placental developmental block, but results in suboptimal development through mechanisms that are independent of placental thrombosis.

P2.38

ANTIPHOSPHOLIPID ANTIBODIES AND DYSREGULATED CLOTTING FACTORS DIFFERENTIALLY AFFECT VILLOUS TROPHOBLAST IN FETAL GROWTH RESTRICTION

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Objectives: Antiphospholipid antibodies (aPLs) and dysregulated clotting factors (CF) are known to be associated with pregnancy pathologies including preeclampsia and fetal growth restriction. Here we investigated the effect of aPLs and dysregulated CF on the biology of villous trophoblast.
Methods: Placental samples were collected from normal controls (NC; n=8, 25-38w) and cases (all with an SGA infant) having aPLs/APS (APS; n=10, 25-36w) or dysregulated CF (CF; n=9, 27-35w). Placentas were stained for Ki-67 and cytokeratin 7 to identify proliferating villous cytotrophoblasts. Images were systematically randomly selected and Ki67 positive cytotrophoblasts and fibrin depositions were counted.
Results: There was no significant difference regarding gestational age at delivery, maternal age, and BMI. Fetal weight was significantly different between NC versus APS, and NC versus CF (NC; 2291 ± 833g, APS; 1160 ± 413g, CF; 1182 ± 516g). In APS compared to CF, placental weight showed a tendency to be decreased and the ratio of P/F to be increased. The ratio of Ki-67 positive cytotrophoblasts in NC gradually decreased in accordance with gestational age, while this was not apparent in APS and CF. The relative number of Ki-67 positive cells was significantly reduced in APL (12.1 ± 7.1%) and CF (12.7 ± 5.8%) compared to NC (22.6 ± 6.4%). The total number of cytotrophoblasts was reduced in APS (578 ± 209) compared to CF (830 ± 258) and NC (780 ± 149). Regarding fibrin deposition, there were no significant differences in total fibrin deposition and intravillous fibrin deposition. However, perivillous fibrin deposition significantly increased in CF (3.7 ± 1.5%) compared to APS (1.9 ± 1.1%).
Conclusion: We suggest that aPLs downregulate cytotrophoblast proliferation throughout pregnancy, while in the CF group, an increasing amount of fibrin deposition finally leads to a decrease in trophoblast proliferation but does not affect total cell count.

P2.39

PLACENTAL MESENCHYMAL DYSPLASIA: CLINICAL FEATURES, HISTOPATHOLOGICAL FINDINGS, AND DIAGNOSIS

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Objectives: Placental mesenchymal dysplasia (PMD) is an unusual condition characterized by an enlarged, hydropic placenta. Its diagnostic criteria have not been defined. In this study, we investigated the clinical features, histopathological findings, and diagnosis of PMD in Japanese women.
Methods: We accumulated twenty-four cases reported or identified as PMD-complicating pregnancies between 2000 and 2010 in Japan. Attending physicians were asked to complete a questionnaire that helped us gather clinical details for each case and to provide us with paraffin-embedded placenta specimens. Histopathological findings and the P57kip2 immunohistochemistry of these samples were examined.
Results: Of 24 cases, artificial abortion was performed in 1 because of a suspected hydatidiform mole. In the subjects, PMD was strongly associated with female infants, small-for-date babies, preterm births, and increased incidences of intrauterine fetal demise, but not with increased maternal age or assisted reproductive technology. In 17 of 23 cases, placental gross weight was greater than mean+2SD of each gestational week; the placental/birth weight ratio exceeded the mean+2SD in 19 cases. Histopathological findings for the placentas of 12 cases were as enlarged edematous stem villi, dilated thick-walled chorionic plate vessels with fibromuscular hyperplasia, and fresh or organized thromboi. These findings were consistent with PMD. The immunohistochemistry of P57kip2 revealed that 9 of 12 cases were stained as PMD patterns, but other 3 showed the coexistence of both normal and PMD patterns.
Conclusions: PMD is strongly associated with adverse pregnancy outcomes. Although placentomegary has been reported as a common feature of PMD, enlarged placentas were not always found because of premature birth. P57kip2 immunohistochemistry is utilized as a diagnostic marker of PMD. Further research is required to understand PMD's clinical features and to establish its diagnostic criteria.

P2.40

EARLY DEXAMETHASONE TREATMENT RESULTS IN STRUCTURAL ABNORMALITIES IN SHEEP PLACENTOMES

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Objectives: We have shown that maternal dexamethasone (DEX) administration early in gestation resulted in a decrease in fetal weight, crown rump length and head circumference in female fetuses at 100dG, which did not persist until term. Presently, we aimed to identify microstructural changes in placenta tissue that might explain this transient reduction in birth weight.
Methods: Pregnant ewes carrying singleton female fetuses of known gestational age were randomized to control (n=11) or DEX (n=5) treatment (0.14mg/kg ewe body weight) consisting of four intramuscular injections at 12-hourly intervals over 48 hours on days of gestation (dG) 40-41. Animals were euthanized 100dG. Major fetal organs were removed, weighed and collected for use in other studies. Placentomes were dissected from the uterus, classified according to their gross morphology into A, B, C and D-subtypes, weighed and fixed. Placentome subtype as well as total placental morphometry was performed with a modified cycloid grid in cytokeratin and Von Willebrand Factor double stained sections. Vascular endothelial growth factor (VEGF) mRNA expression was quantified with RT-PCR.
Results: DEX treatment increased B and C placentome weight and increased fetal and maternal placentome surface area in A, B and C types as well as total placental surface area compared to controls. DEX did not affect surface density or the ratio of maternal to fetal surface area. Maternal vessel density was significantly increased in DEX C-types, whereas VEGF mRNA expression was significantly reduced in DEX C-types compared to controls.
Conclusions: Maternal DEX administration increased placentome surface area as well as total placental surface area; suggesting a change in the nutrient and gaseous exchange area between materno-fetal circulations without changing placental surface density (a marker of the degree of interdigitation of maternal/ fetal tissue). Further evaluations of placental vessel diameter and length are needed to understand DEX-induced changes in placental vascularity.

P2.41

PLACENTAL CHARACTERISTICS IN A CASE OF TWIN ANEMIA-POLYCYTHEMIA SEQUENCE (TAPS)

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Twin anemia–polycythemia sequence (TAPS) is a rare variant of twin–twin transfusion syndrome (TTTS), which does not show the characteristic twin oligohydramnios–polyhydramnios sequence. We report a case of TAPS based on placental findings.

A 34-year old primiparous woman was diagnosed in a maternity clinic as having monochorionic–diamniotic (MD) twins at 8 weeks of gestation. Periodic ultrasonography revealed no abnormal findings including amniotic fluid volume. At 30 weeks, the patient was referred to a general hospital because of pleural effusion in one fetus. Two days later, a testicular hydrocele and ascites were observed in the fetus and the patient was referred to our hospital, a tertiary care center of Hiroshima city.

There were no intertwin discordancies in amniotic fluid volume, nor in the fetal weight estimated by ultrasonography. A Doppler ultrasound measurement of the middle cerebral artery peak systolic velocity (MCA–PSV), which reflects fetal anemia or polycythemia, was within normal range in both fetuses. At birth by Cesarian section, the fetus with hydrops was pale and weighed 1,644 g; the other twin was plethoric and weighed 1,754 g. Their hemoglobin concentrations were 4.1 mg/dL and 24.5 mg/dL, respectively. Three findings were noted in the placenta. First, the maternal side was pale in the area corresponding to the anemic fetus and congested in that relating to the polycythemic fetus; second, there was intense vascularization on the fetal side of the polycythemic fetus, with poor vascularization in the anemic fetus; finally, there was a velamentous insertion of the umbilical cord in the anemic fetus.

It is not clear why the donor twin in cases of TAPS does not develop oligohydramnios and why the recipient does not develop a polyhydramnios condition, such as TTTS. However, we should be aware of this unique potential condition in managing MD twins.

P2.42

MORPHOLOGICAL COMPARISON OF PLACENTAL STEM VILLI ARTERIES BETWEEN TERM AGA AND SGA NEWBORNS

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Objectives: The purpose of this study was to compare morphology of placental stem villi arteries between small-for gestational age (SGA) and appropriate for gestational age (AGA) newborns among full-term singleton pregnancy.

Methods: We studied the placentas from 45 singleton pregnancies delivered at Hamamatsu University Hospital from 2005 to 2011. Placentas were classified into 2 groups, SGA newborns (n=25) and AGA group (n=20). All cases had no complications such as pre-eclampsia. SGA newborns were defined as those whose birth weight was below the 10^{th} percentile for that gestational age. The control group comprised the placentas of term pregnancies elective cesarean section and those newborns birth weight were appropriate for gestational age. The tissue section was stained with elastic tissue van Gieson stains. The vessel wall thickness, luminal area and obstruction ratio were measured. Semi-quantitive measurement of collagen fiber in the vessel wall and immunohistochemistry were performed.

Results: Stem villi morphometry revealed a reduction of stem vessels lumina concomitant with increased intima and media in SGA newborns placenta. Obstruction ratios were 6.4% and 20.8% in SGA and AGA respectively. The ratios of the collagen to total vessel area were significantly lower in SGA placenta than AGA placenta. Immunohistochemistry revealed strong staining for 8-hydroxy-2'-deoxyguanosine(8-OHdG)、4-hydroxy-2-nonenal(4 HNE) as oxidative stress marker in endothelial cell of stem villous vessels in SGA placenta compared to AGA.

Conclusion: these significant differences between SGA and AGA placenta suggest possibility involved in the pathogenesis for SGA newborns.

P2.43

VILLOUS FIBROSIS FOLLOWING CHRONIC VILLITIS IS THE MAIN CAUSE OF FETAL GROWTH RESTRICTION

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Objectives: Chronic villitis is known to be associated with fetal growth restriction (FGR). However, chronic villitis is also seen in placenta of normal pregnancy. Chronic villitis shows various pathological features such as inflammation, vasculopathy and villous fibrosis. It has not been clarified what is essential for pathogenesis of FGR. In this study we investigated the distinctive pathological feature of chronic villitis of FGR.
Methods: 21 placenta from FGR ($<10^{th}$ percentile) cases and 19 placenta from non-FGR cases were pathologically analyzed retrospectively. This study was conducted in national center for child and development, Japan from April 2007 to December 2011. Below the 22nd week of gestation, multiple, TORCH syndrome or fetal anomalies were excluded. Grade and localization of inflammation, vasculopathy and villous fibrosis were analyzed. T cell infiltration was determined immunohistochemical staining for cCD3. Severity of inflammation were graded into four groups; low and focal, low and multi focal, high and patchy, high and diffuse. Expansion of inflammation was divided into four levels ; stem villous level, intermediate villous level, distal villous level and anchoring villous level.
Results: There were no statistical differences between the groups in respect to maternal age, previous pregnancy history or delivery week. Inflammation was more severe and spread to proximal in FGR group than in non-FGR group. Villous fibrosis and vasculopathy were both found more frequently in FGR group than in non-FGR group (vasculopathy: FGR vs. non-FGR = 100% vs. 57.1% ($p < .05$). fibrosis: FGR vs. non-FGR = 90.5% vs. 31.6 %($p < .05$)).
Conclusion: Villous inflammation is more severe in FGR cases. Also, fetal growth was definitely influenced by the existence of villous fibrosis and vasculopathy of villitis.

P2.44

CHARACTERIZATION OF THE PLACENTAL DEVELOPMENT IN THE INTRA-UTERINE GROWTH RETARDED PIGLET

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Objectives: In pig production, the selection of hyperprolific animals has lead to an increased rate of intrauterine growth retarded (IUGR) piglets in litters. This is an important economic issue as these piglets display higher mortality rates, lower growth capacity and lower quality carcasses. The purpose of this work was to investigate placental morphology and function during early (45d), mid (71d) and late gestation (112d) in pairs of control and IUGR siblings.
Methods: Altogether, 18 pairs were used (N=5, 6 and 7 at 45, 71 and 112 days, respectively), each pair, but one, being of the same sex. The IUGR piglets were 25%, 33% and 41% lighter than controls. Relative uterine and placental proportions of blood vessels, connective tissue and trophoblast were measured by stereology. Placental expression of genes involved in fetal growth (IGF2), angiogenesis and vascularization (eNOS, VEGFa, FLT1, KDR), nutrient transport (GLUT3, SNAT2, LPL) and oxidative stress (SOD1, SOD2) was measured by RT-qPCR. Data were analyzed using Mann-Whitney test (morphometry) and ANOVA (gene expression).
Results & Conclusion: Morphometric analysis did not reveal any significant difference according to gestational age nor group. IGF2 expression was significantly increased in IUGR placenta at 71d of gestation (x 1.48, $p<0.05$) but there was no other significant difference in gene expression. The methylation of the IGF2 promoter was not significantly different between groups. Since IGF2 induces fetal and placental growth, its increased expression in IUGR could be an early compensatory mechanism to meet the fetal nutrient demand.

P2.45

A CASE OF PLACENTA FENESTRATA WITH FETAL GROWTH RESTRICTION AND A NON-REASSURING FETAL STATUS AT 29 WEEKS GESTATION

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We report a case of placental malformation that became an emergency cesarean section due to apparent fetal growth restriction and non-reassuring fetal status. A 25 year-old woman at first pregnancy had no past history or complications. Although she went through her first trimester uneventfully, an ultrasonographic exam demonstrated fetal growth restriction (-2.1SD) at 29 weeks of gestation. She had no diabetes, thyroid gland dysfunction, antiphospholipid syndrome or pregnancy-induced hypertension syndrome.
There were no infectious diseases to cause the observed fetal growth retardation. The ultrasound findings revealed a velamentous insertion of the cord, and no deformity of fetus. There was no abnormal arterial umbilical blood flow at 29 weeks 3 days'-gestation. Amniotic fluid volume had decreased (liquor amnion pocket 1.5 cm), and a reflux of the arterial umbilical blood flow was observed by using ultrasound at 29 weeks 4 days'- gestation. The fetal heart rate monitoring revealed mild prolonged bradycardia, and frequent variable deceleration. Because of non-reassuring fetal status, and fetal growth restriction, we performed an emergency cesarean section at 29 weeks 4 days gestation. The male baby was delivered at 840 g in weight, 33.5 cm tall. The Apgar score was 8 at 1 min and 9 at 5 min, umbilical cord arterial blood pH 7.336, and no anomalies noted. The placenta was 250 g in weight (14*12.5*1.0 cm). The real part of the insertion of the umbilical cord site in the placenta center suffered a loss, and presented placenta fenestrata. The histopathological examination of the placenta indicated that the chorioamnionitis and the funiculitis were absent. It is suggested that intrauterine growth restriction and fetal dysfunction occurred due to placental dysfunction by the velamentous insertion with placenta fenestrata.

P2.46

A CASE OF A RARE PLACENTA WHICH SHOWED NO INDEPENDENT UMBILICAL CIRCULATION IN THE SMALLER TWIN OF MONOCHORIONIC TWIN

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Objectives: We report a case of monochorionic(MC) twin with selective intrauterine growth restriction(sIUGR) presenting intermittent absent or reversed end-diastolic flow (iAREDF) doppler pattern in the umbilical artery.
Methods: A 31-years-old woman, gravid 0, para 0, visited our hospital at 7 weeks of gestation because of MC twin pregnancy. At 21 weeks of gestation, sIUGR and iAREDF were pointed out. The twins were delivered by cesarean section at 34 weeks of gestation because of polyhyramnios of the smaller twin. The larger baby was 1780g and Apgar score was 7 at 1 minute. The smaller baby was 1490g and Apgar score was 7 at 1 minute. The placenta was examined by dye injection.
Results: Examination of placenta revealed that the distance between the points of cord insertion was close. There were one large AA anastomosis, 38 artery to vein anastomoses, and no vein to vein anastomoses. All umbilical arteries(UA) of the smaller twin circulated to the umbilical veins(UV) of the larger twin, and all UVs of the smaller twin circulated to the UAs of the larger twin. No independent circulation of the smaller twin was found. The smaller twin was speculated to share 15% of whole placental volume.
Conclusion: It is reported that iAREDF indicates the presence of large AA anastomoses. It is also reported that poor outcome is often associated with these pregnancies because of acute fetofetal transfusion through the large AA anastomoses. However, AA anastomosis was essential for sIUGR survival in this case since the smaller twin had no own placental circulation. It is not certain whether this particular vascular anatomy is the reason for the good prognosis or not.

P2.47

THROMBUS IN THE DECIDUA LEADING TO THE STILLBIRTH AND INTRAUTERINE FETAL GROWTH RESTRICTION (FGR)

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Purpose: There is a group which does not show coagulation disorder hematologically in repeated stillbirth or intrauterine Fetal Growth Restriction (FGR). Thrombus in a large number of decidua, infarction, and villous ischemic change is common in this group which does not show coagulation disorder hematologically in repeated stillbirth or FGR. Without evidence of coagulation disorder, I made pathological examination of the placenta looking for evidence pertaining to the group which does not show coagulation disorder in repeated stillbirth or FGR. I estimated the grading of placenta ischemic change as a result of unknown coagulation disorder, because if there is ischemic change it can possible lead to stillbirth and FGR. In these cases, anticoagulation therapy was successfully performed.
Materials, method: I diagnosed villous ischemia in 70 patients out of 125 FGR patients. In the Grading of placental ischemic lesions, Grade 1 shows villous downsizing, fibrosis, and the collapse of the villous blood vessel. Grade 2 is not only villous downsizing, fibrosis, the collapse of the villous blood vessel, but also necrosis coheres, and small microscopic infarction. Grade 3 is a massive thrombus, infarction, and extensive villous collapse occurring diffusely.
Results: Many Grade 3 cases led to early delivery, and there was large number of Grade 3, in which the degree of FGR was high.
Discussion: We were initially subjective, but the grading of the ischemic change enabled us to become objective and clinical.

P2.48

FETAL AND PLACENTAL GROWTH RESTRICTION IN MATERNAL OBESITY IS NOT ASSOCIATED WITH INCREASED PLACENTAL INFLAMMATION

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Objectives: Maternal obesity induces fetal growth restriction in several pregnancy models. This may be due to impaired placental function, possibly because of increased placental inflammation or endoplasmic reticulum (ER) stress. This project investigated the placental mRNA expression of pro-inflammatory factors and markers for ER stress within the context of maternal obesity.
Methods: 8 week old female Wistar rats were raised on a cafeteria (CAF) diet consisting of ad libitum access to rodent chow and 4 snack food items rotated daily to maintain novelty. Control animals had access to chow only. After 8 weeks of diet exposure, animals were mated and maintained on their respective diets throughout pregnancy. Fetal and placental weights were recorded and placental tissues were collected on day 21 of gestation. Placental mRNA expression of inflammatory and ER stress markers were measured in male labyrinth zones by RT-qPCR.
Results: CAF-fed animals were significantly heavier than controls from week 3 (13%) to week 8 (24%) of diet exposure. During pregnancy, CAF-fed animals gained 16% more weight than controls. Maternal obesity in the CAF group resulted in growth restriction in CAF fetuses (6% males, 10% females) and whole placentas (15% males, 16% females). This corresponded to a reduction in both JZ (19% males, 14% females) and LZ (13% males, 21% females) weight.
Despite effects on fetal and placental growth, diet had no effect on male labyrinth zone expression of inflammatory genes Tnfα, Il-6, Il-1β, Tlr2 orTlr4. Similarly, expression of ER-stress related genes Hsf2, Hsp90 and Chop10 were also unaffected.
Conclusion: Despite fetal and placental growth restriction in a maternal obesity model, this study noted no changes in mRNA expression of inflammatory and ER stress related genes in male labyrinth zone tissue. Further studies are required to determine the cause of the reduced fetal and placental growth.

P2.49

DECREASED EXPRESSION OF PROGESTERONE RECEPTOR (PGR) IS ASSOCIATED WITH HUMAN IDIOPATHIC FETAL GROWTH RESTRICTION

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Objectives: Progesterone is critical for the establishment and for the maintenance of pregnancy. The effects of progesterone depend on the availability of progesterone receptors (PGR) that act as nuclear transcription factor and modulates cell signaling pathways in a variety of reproductive processes. However, the role of PGR in human placental pathologies including idiopathic fetal growth restriction (FGR) is unknown. The aim of this study was to determine the expression of PGR in idiopathic FGR-affected placentae compared with gestation-matched controls (GMC) and to identify the functional role of PGR in trophoblasts.
Methods: Placentae (n=25) from third trimester idiopathic FGR and GMC were collected. PGR mRNA was determined by real-time PCR and protein quantitation by immunoblotting. The effect of siRNA-mediated PGR gene-inactivation on trophoblast function was determined in trophoblast-derived cell line, BeWo. Trophoblast differentiation and apoptosis was determined by real-time PCR using markers of differentiation such as βhCG, 3βHSD, syncytin and markers of apoptosis such as caspase 3 and p53.
Results: PGR mRNA was significantly decreased in FGR-affected placentae compared with GMC [0.53 ±0.09, FGR (n=25) vs. 1.10±0.31, GMC (n=25), t-test, $p<0.05$]. Immunoblotting revealed reduced PGR protein (86 kDa) in FGR compared with GMC. Following PGR inactivation, mRNA expression for βhCG, 3BHSD, syncytin, caspase 3 and p53 were significantly increased in siRNA-treated BeWo cells compared with untreated control (n=6, $p<0.05$).
Conclusion: Decreased PGR expression observed in FGR may contribute to increased trophoblast differentiation and apoptosis in human FGR.

P2.50

EVALUATION OF THE MECHANISM OF PRE-TERM DELIVERY INDUCED BY IL-1β

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Objectives: Preterm birth is known to be characterized by increased levels of pro-inflammatory cytokines (e.g. interleukin(IL)-1β) in the fetal membranes, amniotic fluid, and in the lower segment of the uterus.
On the other hand, progesterone (P4) is thought to be key factor for the maintenance of pregnancy.
Recently, senescence-associated growth restriction with increased levels of p21 in decidua cells have reported to participate in the processes of preterm labor. In this study, we investigate the effects of supernatant of a trophoblast cell line (TCL-1 cells) as well as these cytokines on the levels of PTGS2(COX2) and PTGFR(FP), and induction of senescence in the primary human decidual cells.
Methods: Decidua tissues were obtained for primary cell culture following elective cesarean section.
We modeled the pre-term labor by decidual cells treated with IL-1β in vitro .
Results:

1) In the decidual primary cultured cells treated with IL-1β, the levels of PTGS-2 and FPmRNA were increased comparing with vehicle.
2) The proportion of SA-β gal-positive cells was higher in the cells treated with IL-1β than the cells treated with vehicle.
3) The level of PTGS2-mRNA was not controlled by PTGFR siRNA.
4) Up-regulated genes treated with P4+ IL-1β in decidua cell were completely different from which treated with IL-1β alone.

Conclusion: We showed involvement of cell senescence induction by IL-1β in the process of pre-term labor. These results suggest that some signals derived from chorion trophoblast are associated with suppression of induction of pre-term labor. And we check signal transmission to examine FPsiRNA, PTGFR did not exist up stream. The differences in up-regulated genes between P4+ IL-1β and IL-1β may suggest that P4 makes changes in the suppression of genes toward anti-inflammation.

P2.51

CHORIOAMNIONITIS AND FUNISITIS AFFECT THE SURVIVAL RATES OF PRETERM INFANTS BORN AT 22 AND 23 WEEKS OF GESTATION

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Objectives: At present, preterm infants born at 22 or 23 weeks of gestation are intensively managed in most of NICU units in Japan. We studied factors that affect the survival rates of such preterm infants born in our hospital.
Methods: Thirty-tree singleton infants born alive at 22 and 23 weeks of gestation between 2001 and 2011 were subjects of the study. We studied obstetrical backgrounds of preterm birth, method of delivery, existence of pathological chorioamnionitis (CAM) or funisitis and prognosis of the infants.
Results: Ten infants was born at 22 weeks, and 23 infants was born at 23 weeks. Of these, premature rupture of membranes was observed in 14 cases (42.4 %). Caesarean section was done in 20 cases (60.6 %) due to mainly a breech presentation. All the infants born at 22 weeks were discharged alive. Of the 23 infants born at 23 weeks, however, 6 infants (26.1 %) died in NICU. The main cause of neonatal death was; immaturity (1), pneumothorax (3), sepsis (1) and cardiovascular failure (1). In the 27 survivors, high-grade (grade III) CAM and funisitis (with CAM) were observed in 16 cases (59.3 %) and 9 cases (33.3 %), respectively. In contrast, in the 6 infants died in NICU, grade III CAM and funisitis (with CAM) were observed in 5 cases (83.3 %) and 4 cases (66.7 %), respectively.
Conclusion: The surviving rates of the infants born at 22 and 23 weeks of gestation in our hospital was 81.8 % (27/33). Although the number of present cases is not enough to be analyzed statistically, higher incidents of CAM and funisitis were observed among the dead infants.

P2.52

RESIDUAL VASCULAR COMMUNICATIONS AFTER FETOSCOPIC LASER SURGERY IN TWIN-TWIN TRANSFUSION SYNDROME: FREQUENCY AND OUTCOME

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Objectives: In recent years, fetoscopic laser surgery (FLS) has been used to treat twin-twin transfusion syndrome (TTTS) by coagulating placental vascular anastomoses. It is known to improve the outcome of TTTS and has gained worldwide recognition as the first line of treatment. Postpartum injection studies of the placenta can reveal if any residual vascular communications (RVC) remain after FLS. The aim of this study was to determine the incidence of RVC after FLS and to evaluate the perinatal outcomes of cases complicated by RVC.
Methods: Patients who underwent FLS for TTTS at our institution between March 2003 and December 2010 were included in this study. After birth, placentas were inspected grossly and histologically, and the presence of RVC was studied by placental resin injection. Placentas were divided into those with and without RVC. The charts of patients treated with FLS were reviewed to retrieve the preoperative, perioperative, and postoperative obstetrical data.
Results: During the study period, 230 women with TTTS underwent FLS. Intact placentas of 115 cases with dual survivors or double demise were analyzed. Of these, thirteen placentas had RVC (11%). Arterio-arterial anastomoses were recognized in 8 RVC cases, veno-venous anastomoses in 7 cases and arterio-venous anastomoses in 9 cases. There were no statistically significant differences in maternal background, Quintero stage, operative findings and location of the placenta between cases with and without RVC. Survival to birth (77% vs. 96%, $p<0.05$), neonatal survival (77% vs. 95%, $p<0.05$), and gestational age at delivery (28.5 ± 4.7w vs. 33.0 ± 4.0w, $p<0.05$) were significantly lower in patients with RVC. Twin anemia polycythemia sequence was diagnosed in patients with RVC, and the incidence of recurrent TTTS was significantly higher in patients with RVC (4/13 vs. 0/102, $p<0.05$).
Conclusion: Outcomes in patients with RVC were poor, so it is important to find prepartum indicators for RVC.

P2.53

FOXM1 IS DECREASED WITH HUMAN PRETERM LABOUR AND REGULATES PRO-LABOUR CYTOKINES IN PRIMARY AMNION CELLS

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Objectives: The most important complication contributing to neonatal mortality and morbidity is infection/inflammation-induced preterm birth. While approximately one third of preterm births are indicated, the remainder follow spontaneous labour (with intact membranes) or prelabour rupture of membranes (PROM). In non-gestational tissues, Forkhead Box M1 (FOXM1) is a transcription factor that regulates inflammation and proliferation of tumour cells. The aims of this study were to determine the effect of (1) human preterm labour on FOXM1 expression in human gestational tissue, and (2) the effect of FOXM1 inhibition on pro-inflammatory and pro-labour cytokines in primary amnion cells.
Methods: FOXM1 mRNA expression was determined by qRT-PCR on fetal membranes from women grouped as (1) preterm no labour: Caesarean section with no labour and (2) preterm labour: after spontaneous labour and normal vaginal delivery. In human primary amnion cells, FOXM1 knockdown was achieved using siRNA. After treatment with IL-1β, pro-labour mediators were assayed.
Results: FOXM1 mRNA expression in fetal membranes was significantly decreased after preterm spontaneous labour. In primary amnion cells, FOXM1 inhibition by siRNA increased IL-1β-induced cytokine mRNA expression and release (IL-6, IL-8). Current experiments will determine the effect of FOXM1 knockdown on the cyclooxygenase-prostaglandin pathway.
Conclusion: We found that FOXM1 expression was down-regulated in preterm labour fetal membranes, and knockdown of FOXM1 in primary amnion cells showed an increase in IL-1β-induced pro-inflammatory cytokines. With its anti-inflammatory actions in human pregnancy, more studies are required to determine if FOXM1 can be used in the management of infection-induced preterm labour.

P2.54

MASSIVE INTERVILLOUS FIBRIN DEPOSITION IN WOMEN WITH RECURRENT PREGNANCY LOSS

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Objective: Pathological examination of villi and placenta is performed for women with recurrent pregnancy loss(RPL) in Osaka Medical Center for Maternal and Child Health. The patients with recurrent massive intervillous fibrin deposition(MIFD) have a poor prognosis. Then in order to find effective treatments, we investigated the RPL patients with MIFD.
Methods: We examined retrospectively the background previous pregnancy, inspection data, therapy and prognosis of 18 RPL patients with MIFD who visited Osaka Medical center for Maternal and Child Health from 1998 to 2011.
Results: Pregnancy success rate of cases with once MIFD was 66%(6/9).Two of them had not received treatment was successful. Pregnancy success rate of cases with recurrent MIFD was 33%(3/9). In these cases, the treatment was difficult and the prognosis was poor. The successful three cases with recurrent MIFD treated with low dose aspirin therapy (LDA), heparin and steroids or LDA and steroids.
Conclusion: RPL patients with recurrent MIFD had a poor prognosis. But our study has suggested that adding steroids to LDA or heparin probably be effective for RPL patients with recurrent MIFD.

P2.55

GSK3β IS RESPONSIBLE FOR ALTERED MCL-1 EXPRESSION IN IUGR PLACENTAE

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Objectives: Pro-survival Myeloid cell Leukaemia Factor 1(Mcl-1), a critical regulator of trophoblast cell fate, is a short-lived protein whose stability is tightly regulated through post-translational modification events including caspase cleavage and phosphorylation leading to proteosomal degradation. Glycogen Synthase Kinase-3β (GSK3β) is central to Mcl-1 phosphorylation (P-Mcl-1). Notably, when phosphorylated at Serine 9, GSK3β becomes inactive, while its phosphorylation at Tyrosine 216 residue renders it functional. We reported that different mechanisms involving caspase-mediated cleavage and MULE-dependent ubiquitination are responsible for decreased Mcl-1 levels in preeclampsia (PE) and IUGR respectively. However; Mcl-1phosphorylation status and its regulation in placental pathologies remains unknown. Herein, we examined the role of GSK3β in Mcl-1 phosphorylation in PE and IUGR placentae.
Methods: Placentae from pregnancies complicated by PE (n=17), IUGR (n=20) and age-matched-controls (AMC) (n=16) were used. P-Mcl-1, total and phospho-GSK3β(p-S9-GSK3β and p-Tyr216-GSK3β) protein levels were measured by immunoblotting using specific antibodies. Mcl-1/GSK3β associations were assessed by immunoprecipitation. The effect of GSK-3β on Mcl-1 phosphorylation was evaluated by treating Jeg3 choriocarcinoma cells with GSK-3β inhibitor IX.
Results: GSK3β inhibitor significantly reduced P-Mcl-1 levels in Jeg3 cells implicating a role for GSK3β in regulating Mcl-1 stability in trophoblast cells. P-Mcl-1 and total GSK3β protein levels significantly increased in IUGR while, in stark contrast, their expression decreased in PE. Accordingly, despite Mcl-1 low levels, Mcl-1/GSK3β association increased in IUGR whereas in PE this interaction was disrupted. Importantly, in IUGR, increased levels of p-Tyr216-GSK3β associated with decreased p-S9-GSK3β indicating that GSK3β is active in this pathology. Conversely in PE, GSK3β was inactivated as p-Tyr216-GSK3β expression decreased while p-S9-GSK3β levels increased.
Conclusion: In IUGR, decreased levels of pro-survival Mcl-1 are in part due to activation of GSK3β pathway that in turn is liable for Mcl-1 phosphorylation thereby contributing to altered trophoblast cell death found in this pathology (Supported by CHIR and Canada-Hope Fellowship).

P2.56

IDENTIFICATION OF COMPLETE HYDATIDIFORM MOLE-RELATED MICRORNA

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Objective: Recently, circulating placental microRNAs in plasma from pregnant women are expected to be biomarker for pregnancy-related diseases. In this study, we tried to identify the microRNA marker of the complete hydatidiform moles (CHMs) in plasma.
Methods: By next generation sequencing, top three out of microRNAs, which expression level was higher in CHMs tissues than in normal villi tissues, but no expression in blood cells, were selected as candidate CHMs-associated microRNAs. Subsequently, the expression levels of candidate CHMs-associated microRNAs were confirmed by quantitative real-time reverse-transcription PCR, and the microRNAs predominantly expressed in the CHMs tissues were identified as CMHs-associated microRNAs. Finally, plasma concentrations of cell-free CHMs-associated microRNA were measured before and after evacuation.
Results: The hsa-miR-520f, 520c-3p and 520b were selected as candidate CHMs-associated microRNAs. The median (minimum-maximum) of each multiple of median (MOM) values in control and CHMs were 1.00 (0.01-1.85) and 1.89 (0.02-4.23) for 520f, 1.00 (0.01-1.84) and 1.31 (0.01-2.95) for 520c-3p, and 1.00 (0.01-2.47) and 1.51 (0.02-2.54) for hsa-miR-520b, respectively (Mann-Whitney's U test, $p<0.05$). The median plasma concentration of cell-free hsa-miR-520f in CHMs pregnancy was significantly higher than in normal pregnancy (27,194 copies/mL vs 13,118 copies/mL, Mann-Whitney U test, $P=0.002$), and decreased significantly after evacuation (4,149 copies/mL, Wilcoxon signed rank test, $P = 0.002$). However, plasma concentrations of cell-free hsa-miR-520c-3p and -520b in CHMs pregnancy did not show significantly difference comparing with those in normal pregnancy.
Conclusions: The hsa-miR-520f is CHMs-related microRNA and its plasma concentration may be possible molecular marker of complete hydatidiform mole.

P2.57

MOLECULAR GENETIC DIAGNOSIS OF TWO RARE CASES OF GESTATIONAL TROPHOBLASTIC NEOPLASIA

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Background: Gestational trophoblastic neoplasia (GTN) during normal pregnancy is very rare. The causative pregnancy of this type of GTN has the potential to be antecedent pregnancy or current pregnancy. Genetic diagnosis has proven important in the management of patients with GTN. The objective of this study is to identify the causative pregnancy through molecular genetic analysis.
Methods: DNA was prepared from cheek swab samples from the patient and her partner. DNA from GTN and normal placental tissue was prepared by using laser-capture microdissection technique. Using these DNA, PCR amplification and microsatellite genotyping were performed. The genetic contributions to the GTN were determined by comparing the genotypes of the GTN and those of the couples and normal placenta.

Case1: Intraplacental choriocarcinoma in a term placenta with maternal metastases.

A 31-year-old woman, gravida 7, para 4, underwent an emergency cesarean section at 38 weeks of gestation because of repeated melena which were treated with blood transfusion frequently. At the laparotomy, a jejunum tumor was seen and resected. This tumor was confirmed as choriocarcinoma pathologically and CT scan showed multiple metastases of brain, lungs, liver, and colon. Detailed examination of the placenta provided the final pathological diagnosis which was term placenta with focal intraplacental choriocarcinoma.

Case2: Placental site trophoblastic tumor (PSTT) diagnosed in the first trimester.

A 37-year-old woman, gravida 8, para 6, was pregnant for the 9^{th} time. Abdominal total hysterectomy was performed at the 11^{th} week gestation for artificial abortion and treatment for a huge myoma. Pathological examination of the uterus revealed a PSTT besides a leiomyoma and normal villi.
Results: Both cases were gestational in origin and the causative pregnancies were identified as current pregnancies.
Conclusion: Intraplacental choriocarcinoma and PSTT with normal pregnancy are extremely rare variants of GTN. Both of GTN are highly malignant and the causative pregnancy is controversial. Therefore, it is reasonable to identify the causative pregnancy by its genetic origin. We can determine the causative pregnancy of GTN accurately by microsatellite polymorphism analysis.

P2.58

CLINICAL FINDINGS OF MULTIPLE PREGNANCY WITH A COMPLETE HYDATIDIFORM MOLE AND COEXISTING FETUS

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Objective: Twin pregnancies with a complete hydatidiform mole and coexisting fetus (CHMCF) represent an obstetric challenge. The aim of this report is to characterize the clinical features of this very rare disorder and to discuss the diagnostic and therapeutic implications in light of the literature.
Methods: Data from three women who were diagnosed as CHMCF at our institution between 2007 and 2012 were reviewed. Gestational ages at diagnosis, symptoms, serum beta-human chorionic gonadotropin levels, immunohistochemical results, complications, route of delivery, pregnancy outcome, and disease course were assessed.
Results: A diagnosis of CHMCF was made by ultrasound in two cases at around 18 weeks' gestation and in the other case at 13 weeks' gestation. One patient had been diagnosed as a large hematoma before referral to our unit. All the cases underwent termination of pregnancy before 21 weeks' gestation due to complications such as heavy bleeding and rupture of the membranes. No one presented with preeclampsia and hyperthyroidism. In all the cases, the in utero diagnosis of CHMCF was confirmed pathologically and genetically. One patient developed metastasis to the lung and needed chemotherapy.
Conclusions: Our three cases with complications resulted in termination of pregnancy. However, reports in the literature indicate that the option of pregnancy continuation can be offered when maternal complications are absent or controllable. Nevertheless, patients should also be informed that twin pregnancies with a hydatidiform mole carry an increased risk of persistent and/or metastatic gestational trophoblastic disease compared with single molar gestations, as demonstrated in our experience, and of an unfavorable outcome for the fetus.

P2.59

A RARE CASE OF PARTIAL MOLE AND CO-EXISTING NORMAL FETUS ORIGINATED FROM ONE EMBRYO SHOWS PREECLAMPSIA-LIKE SYMPTOMS AT 19 WEEKS GESTATION: ANGIOGENIC IMBALANCES IN MOLAR PLACENTA LEADING TO HYPERTENSION, PROTEINURIA AND PLEURAL EFFUSION

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Case Report: We present a very rare case of single embryo-derived partial mole with placental triploidy (69, XXX) and a 46, XX fetus. A 30-year-old Japanese woman, gravida 0 para 0, was transferred to our hospital with hypertension, proteinuria, weight gain, and respiratory discomfort at 19 weeks. A detailed ultrasonography depicted a large multicystic placenta without dilated vessels around the cyst. Labor was induced at 20 week because of clinical deterioration, followed by a vaginal delivery of a normal female fetus and a molar placenta. After delivery, the patient's blood pressure rapidly normalised and the proteinuria resolved. An expelled placenta macroscopically demonstrated villi with multiple hydropic cysts and vesicles. The nuclei of the villous stroma and cytotrophoblastic cells were positive for $p57^{kip2}$ and cell culture showed placental triploidy (69, XXX). A female fetus had normal karyotype (46, XX). The genetic profiles using fifteen polymorphic DNA markers demonstrated that the fetus and the cord consisted of monospermic paternal allele and one maternal allele, and that all examined genotypes of the placenta were consistent with those of the fetus, showing that partial mole was originated from monospermic fertilization and first in the world both the partial mole and the fetus have their origin in a single embryo. The maternal serum level of sFlt-1 (14,393 pg/mL), sEndoglin (127 ng/mL), and the sFlt-1: PlGF ratio (476) were extremely high that have been reported. The levels of sFlt-1 and sEndoglin in the cystic fluid from placental mole were also extremely high (104,000 pg/mL and 54 ng/mL, respectively). This report confirmed the hypothesis that preeclamptic symptoms are mediated by circulating factors of placental origin. This is a first report that showed paternal isodisomy in partial mole might contribute to angiogenic imbalances in the placental tissue even in the absence of fetal triploidy.

P2.60

A CASE OF EXTRAUTERINECHORIOCARCINOMA AFTER DELIVERY OF INTRAUTERINE FETAL DEATH IN THE FIRST PREGNANCY

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Objectives: We would report a rare case of extrauterinechoriocarcinoma immediately after stillbirth at preterm in the first pregnancy.
Case: The patient was 26 years old and had the first pregnancy. The pregnancy progress was normal until pregnancy 30 weeks but the fetus died suddenly at preg.30w+4d. She delivered by oxytocin induction at preg.30w+6d and the fetus was a girl at 1,330g. Then, the patient had sever bradycardia on the 4th day after delivery and was introduced to our hospital. We found multiple permeation shadows in chest X-rays and the serum hCG was measured to be 409,700mIU/ml. Also, the findings of cerebral infarctions and subarachnoid hemorrhage were observed in brain CT, suggesting the presence of brain metastasis. However, no lesion of choriocarcinoma was found in the uterus by CT, MRI and curettage of endometrium. We sampled the tissue of the lung lesion by VATS (Video-Assisted Thoracic Surgery) and diagnosed as metastatic choriocarcinoma. She was diagnosed as extrauterinechoriocarcinoma after stillbirth. Immediately, we carried out chemotherapy, two courses of MEA (Methotrexate, Etoposide, Actinomycin-D) and eight courses of MTX (Methotrexate 5 days). The serum hCG was decreased to be negative after the eighth MTX treatment but small pulmonary shadows remains in the lung.
Discussion: Unfortunately, the stillborn baby and placenta were already cremated when she was admitted in our hospital. No macroscopic abnormal findings in the placenta and no lesion of hydatidiform mole was reported when she delivered in the previous clinic. Also, no evidence of hydatidiform mole was observed by ultrasonic tomography during pregnancy. Choriocarcinoma in the lung was identified on seven days after delivery, supposing that the choriocarcinoma might already develop during pregnancy. Though it is hard to prove that this pregnancy is the first but it should be possible that the extrauterinechoriocarcinoma is originated from normal placenta.

P2.61

THE POLYMORPHISM OF FOLATE METABOLIC ENZYME GENES MAY PREDICT THE OUTCOME OF METHOTREXATE THERAPY FOR LOW-RISK GESTATIONAL TROPHOBLASTIC NEOPLASIA

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Objectives: The single nucleotide polymorphisms (SNPs) of folate metabolic enzyme genes have been reported to be associated with toxicity and efficacy of methotrexate (MTX) in a variety of diseases. Especially, methylenetetrahydrofolate reductase (MTHFR), key enzyme in folate metabolism, has been widely analyzed. We investigated the association between the functional SNPs of MTHFR C677T and the outcome of MTX treatment for low-risk gestational trophoblastic neoplasia (LR-GTN).
Methods: Twenty-four cases of LR-GTN primarily treated with MTX (20mg/body /day, day1-5, 14 days cycle) were included in this study. In both patient's blood and molar tissue of antecedent pregnancy, we determined the genotype of MTHFR C677T by HybProbe assay and high-resolution melting method with LightCycler®, or by restriction fragment length polymorphism analysis with gel electrophoresis. We evaluated the resistant rate and the toxicity rate between CC and CT/TT genotype on C677T.
Results: Thirteen patients were primarily remitted (PR group; 13/24), whereas eleven patients needed second line regimen (non-PR group; 11/24). Seven patients of non-PR group discontinued treatment with MTX toxicity (AD grope; 7/24), while the other four patients of them were changed into another regimen because of MTX resistant (Resist group; 4/24). All patients in non-PR group achieved remission with second line regimens. The MTX resistant rate in CT/TT genotypes of MTHFR C677T in molar tissues (3/4) was higher than that in CC genotype (1/11) ($P = 0.03$ Fisher's exact test), which was the only significant association among all combinations of genotypes and clinical outcome in both patient's blood and molar tissue.
Conclusion: The CC genotype of MTHFR C677T in molar tissues of antecedent pregnancy may predict favorable results of 5-days MTX therapy for LR-GTN.

P2.62

THREE CASES OF INVASIVE MOLE ARISING FROM COMPLETE MOLE WITHIN VERY SHORT PERIODS

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Objective: Hydatidiform mole is an abnormal gestation identified by trophoblastic proliferation and hydropic change in the chorionic villi. Pathological diagnosis is clinically important to distinguish complete mole from partial mole because the majority of post-molar trophoblastic diseases arise from complete moles.
Methods: We show three cases of invasive mole arising within 3weeks from complete moles. Immunohistochemistry was performed with monoclonal antibodies against p57kip2.
Results:

Case1: A 21-year-old woman suspected molar pregnancy underwent dilution and curettage (D&C). The initial pathological diagnosis was a partial mole. Only three weeks later, her serum hCG was increased. Immunohistochemistry was performed with p57kip2, which shows negative p57kip2 expression in the villous cytotrophoblasts and mesenchyme. Reviewing the histological specimen and performing MRI, CT and trans-vaginal ultrasonography, we made a diagnosis of complete mole developing invasive mole.
Case2: A 28-year-old woman underwent D&C at 9 weeks' gestation. The pathological diagnosis was complete mole with negative p57kip2 staining. Three weeks later, her serum hCG was increased. She was performed MRI, CT, and trans-vaginal ultrasonography, which diagnosed invasive mole.
Case3: A multipara 29-year-old woman was diagnosed a ectopic pregnancy. She underwent D&C and right salpingectomy. They diagnosed a partial mole. Two weeks later, her serum hCG was increased and she was started systemic chemotherapy. Then she was referred to our hospital. She underwent a total abdominal hysterectomy with a pathological diagnosis of invasive mole. Immunohistochemistry was performed with the result of negative p57kip2 staining. We diagnosed invasive mole arising from complete mole at interstitial tube.

Conclusion: Chemotherapy was performed and complete remission was acquired in all three cases. Serial serum hCG measurements every 1-2weeks are needed within 4weeks following evacuation of complete mole for detection of early onset of invasive mole. Since the pathological diagnosis of complete mole is sometimes difficult, the immunohistochemical detection with p57kip2 may be useful.

P2.63

MORPHOLOGIC PECULIARITIES OF PAPILLAR THYROID CARCINOMA METASTASING INTO PLACENTA

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Objectives: to find histologic and hystochemical peculiarities of placentas from women operated for thyroid cancer during pregnancy
Materials: The 17 placentas from women of gestation age 38-40 weeks operated due to thyroid papillary carcinoma. 16 cases was incapsulated lesion (T1N0M0) and 1 case with invasion into the thyroid capsule and distal metastasis (T3N0M1). Diagnosis was verified by professor T.Bogdanova (Ukrainian institute of endocrinology) and international experts of thyroid cancer data bank. Control group was placentas from 40 healthy women with gestation age 38-40 weeks.
Methods: Histological: hematoxilin-eosinum stain, picrofuxin Van-Gison stain; Immunohistochemical: Anti-human antigen Ki-67 (Clone MIB-1 Dako) with calculation of proliferation index, anti-human CEA (Dako); electron microscopy of syncytiotrophoblast villi (epon, contrast by Reinolds), electronic microscope SELMI.
Results: Macroscopic findings were similar in both groups. Atypical syncytium proliferation, picnosis of nucleus, eosinophilia of cytoplasm of syncytium villi was found in 2 cases from 17. Apoptosis of syncytium was detected by electron microscopy.
Marked reaction of Ki-67 in nucleus of cyto- and syncytiotrophoblast with significant increase of proliferation index in main group was found. In 25% of cases in main group was found high expression of CEA in stroma cells cytoplasm and vascular endothelium.
In one placenta from woman operated in 22nd week of gestation for invasive papillary thyroid carcinoma was found metastasis in intervillous space with positive Ki-67 reaction.
Conclusion: In placentas of women operated for thyroid carcinoma during pregnancy were found metastasis of papillary carcinoma in 1 case; proliferative changes; high expression of Ki-67 in 50% of cases and CEA in 25% of cases.

P2.64

PLACENTAL IMMUNE RESPONSE WAS INCREASED IN PREGNANT WOMEN WITH HEPATITIS B VIRUS WITH PRECORE G1986A MUTANT

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Introduction: Hepatitis B virus (HBV) infection is endemic in China [1]. Vertical transmission of the infection mainly occurs in perinatal period and is the major cause of HBV infection in China. Traditionally intrauterine transmission of HBV infection to infants is believed to come from HBeAg-positive mothers. However, recent studies suggest that intrauterine transmission of HBV infection also occurs from HBeAg- negative mothers and may be due to a precore mutant (G1896A) of the HBV genome. Placental hepatitis B infection is a major risk factor for intrauterine transmission. This study aimed to investigate the placental immune response in pregnant women with hepatitis B virus with the precore G1986A mutant.
Methods: Placentae fromHBeAg- negative pregnant women with (n=32) or without (n=29) precore G1896A mutant were collected after delivery and the expression of HBsAg and HbcAg and numbers of $CD3^+$ T cell and $CD68^+$ macrophages were measured. Levels of IL-2 expression were measured by immunuohistochemistry.
Results:

1. HBsAg or HBcAg positivity was significantly higher in HBeAg-negative pregnant women with the precore G1896A mutant (19 out of 32 women, 59.38%), in comparison to HBeAg-negative pregnant women without the precore G1896A mutant (9 out of 29 women, 31.03%)($p<0.05$).
2. Immunohistochemical analysis showed that the numbers of $CD3^+$ T cell and $CD68^+$ macrophage were significantly increased in HBeAg- negative pregnant women with precore G1896A mutant, compared to HBeAg- negative pregnant women without precore G1896A mutant ($p<0.01$).
3. The positive rate of IL-2 was significantly increased in HBeAg-negative pregnant women with precore G1896A mutant (25 of 32 pregnant women, 78.12%), compared to HBeAg- negative pregnant women without precore G1896A mutant (4 of 29 pregnant women, 13.79%). Immunohistochemical images also showed that the levels of IL-2 expression were higher in trophoblasts.

Conclusion: These dates suggest that the T lymphocyte mediated immune response was increased in intrauterine transmission of HBV infection from HBeAg- negative pregnant women with the precore G1896A mutant.

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P2.65

INFLUENCE OF LAMINARIA TENT INSERTION FOR PREMATURE RUPTURE OF MEMBRANES AT TERM ON CHORIOAMNIONITIS

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Objectives: There is no consensus as yet about the necessity for mechanical cervical ripening in women with premature rupture of membranes (PROM). We elucidated the influence of mechanical cervical ripening on the mother and fetus, to establish proper management criteria for PROM.
Methods: The study subjects were divided into Group A (singleton pregnant women with PROM at 37-41 weeks of gestation hospitalized to our center until October 2010, whose deliveries were managed by insertion of laminaria tents for unfavorable cervical ripening; n = 486) and Group B (pregnant women with PROM hospitalized to our center after November 2010, whose deliveries were managed without insertion of laminaria tents, n = 296). The patient backgrounds, complications, and perinatal outcomes were retrospectively compared between the groups.
Results: No significant differences in the patient backgrounds were observed between the groups. There were also no differences in the duration of labor, time from PROM to delivery, rate of cesarean section, rate of non-reassuring fetal status, or meconium staining frequency between the groups. No significant differences were observed in the newborn outcomes between the groups. Chorioamnionitis (CAM) was diagnosed in 27 patients in Group A (grade I in 10, II in 7, and III in 10 patients) and 15 patients in Group B (grade I in 8, II in 3, and III in 4 patients). The CAM incidence did not differ either histopathologically or clinically between the groups managed with and without laminaria tent insertion.
Conclusion: Although laminaria tent insertion was not associated with any significant increase in the CAM incidence, it did not appear to have any significant influence in reducing the time from PROM to delivery either. Because laminaria tent insertion is highly invasive for pregnant women, our results suggest that mechanical cervical ripening is unnecessary for pregnant women with PROM at term.

P2.66

INCIDENCE AND MATERNAL FACTORS ASSOCIATED WITH PLACENTAL HEPATITIS B VIRUS INFECTION IN HEPATITIS B SURFACE ANTIGEN CARRIERS

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Objectives: Placental infection with hepatitis B virus (HBV) is found in women screened positive for hepatitis B surface antigen (HBsAg), but the related factors remain unclear. We examined the incidence of and maternal factors associated with placental HBV infection in this study.
Methods: Gravidae with positive antenatal screening for hepatitis B surface antigen (HBsAg) were recruited at the antenatal clinic for the assessment of maternal HBV e antigen (HBeAg), viral DNA in maternal blood and placenta (TaqMan real-time polymerase chain reaction), hemoglobin, and alanine transaminase (ALT) in the second and third trimesters. Cases with and without placental HBV DNA were compared.
Results: 33 of the 94 cases studied (35.1%) had HBV DNA detected in placenta. While there was no significant difference in maternal characteristics, this group had high incidence of HBeAg (OR 122.2, 95% CI 14.5-1026.8), and higher HBV DNA level in the second and third trimester, together with higher ALT in the third trimester.

	Placental HBV DNA		*P* value
	Presence	Absence	
Age (years)	31.0±3.9	32.0±4.7	0.301
≥35 years 9%)	18.2	32.8	0.131
Multiparous (%)	53.1	62.7	0.374
Height (cm)	158.1±5.7	158.9±5.3	0.485
<152 cm (%)	6.1	5.0	0.828
Booking weight (kg)	56.9±11.5	54.7±8.5	0.303
Booking body mass index (kg/m^2)	22.6±3.8	21.7±3.2	0.189
≥25 kg/m^2	27.3	16.7	0.225
Hepatitis e antigen (%)	69.0	1.8	<0.001
Hemoglobin (g/dL) – 2nd trimester	11.3±0.9	11.4±0.7	0.622
Hemoglobin (g/dL) – 3rd trimester	11.3±1.0	11.3±1.1	0.740
ALT (mmol/L) 2nd trimester	69.2±245.4	17.9±6.9	0.278
ALT (mmol/L) 3rd trimester	32.8±41.2	16.7±5.7	0.042
HBV DNA ($\log_{10}$ copies/ml) – 2nd trimester	6.923±2.554	1.556±1.684	<0.001
HBV DNA ($\log_{10}$ copies/ml) – 3rd trimester	6.505±2.545	1.620±1.552	<0.001

Conclusion: A third of HBsAg carriers had placental infection, which was associated with higher incidence of HBeAg, ALT level, and circulating HBV DNA, which was likely to be the underlying reason for the placental infection.

P2.67

IMPLICATIONS OF PLACENTAL HEPATITIS B VIRUS INFECTION IN MATERNAL HEPATITIS B SURFACE ANTIGEN CARRIERS ON PREGNANCY OUTCOME

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Introduction: The mechanism of vertical transmission of hepatitis B virus (HBV) infection in high endemicity populations is uncertain. We suspect that this operates through HBV infection of the placenta before the fetus becomes infected.
Methods: Gravidae screened positive for hepatitis B surface antigen (HBsAg) were recruited at the antenatal clinic. Cord blood and placental tissue were collected at delivery for the extraction and measurement of HBV DNA (TaqMan real-time polymerase chain reaction). Pregnancy outcome, newborn acid-base status, and presence of HBV DNA in cord blood, were compared between cases with and without placental HBV DNA detected.
Results: 33 of the 94 cases studied (35.1%) had HBV DNA in placenta. This group has fewer antepartum hemorrhage and more gestational diabetes, but these did not reach statistical significance (Table). In this group, cord arterial pCO_2 was significantly lower, and incidence of HBV DNA in cord blood was higher (OR 25.78, 95% CI 2.83-234.82).

	Placental HBV DNA		*P* value
	Presence	Absence	
Antepartum hemorrhage (%)	6.3	11.5	0.418
Preterm labor (%)	3.0	1.6	0.656
Pregnancy hypertension (%)	3.0	3.3	0.948
Gestational diabetes (%)	18.2	9.8	0.247
Placental insufficiency (%)	21.2	18.0	0.708
Vaginal delivery (%)	81.8	85.2	0.665
Cord arterial pH	7.255±0.068	7.234±0.067	0.161
Cord arterial base deficit (mmol/L)	7.21±3.11	6.66±3.46	0.469
Cord arterial pO_2	2.74±0.93	2.52±0.70	0.290
Cord arterial pCO_2	6.18±1.40	6.89±1.19	0.015
Cord blood HBV DNA +ve (%)	47.1	3.3	<0.001

Conclusion: In HBsAg carriers, HBV infection of the placenta is likely to be the main event preceding fetal infection and plays the major role in vertical transmission. This may also account for the higher incidence of pregnancy complications reported previously.

P2.68

VIPERIN, AN ANTIVIRAL PROTEIN, IS INDUCED BY TOLL LIKE RECEPTOR 3 (TLR3) LIGATION IN HUMAN TROPHOBLASTS

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Objectives: Viperin is a cytoplasmic antiviral protein, named for the characteristics of virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible. Toll like receptor 3 (TLR3) is a receptor which recognizes viral double stranded RNA and triggers immune reactions upon viral infection. It has been shown that trophobalsts express TLR3, and TLR3 ligation results in trophoblasts productions of chemokines and anti-viral molecules. In the present study, we showed that the expression of viperin in human trophoblasts and its induction by TLR3 ligation.
Methods: Under informed consents, human villi samples were obtained from cases of induced abortion in the first trimester. The expression of viperin was evaluated by immunohistochemistry. Trophoblasts were isolated and cultured. Swan 71, a first trimester trophoblast cell line was also used. Cells were stimulated with PIC (TLR3 specific ligand,10 μg/ml). Viperin mRNA and protein expression were evaluated by real time PCR and Western Blotting respectively. To examine whether IFN-β was involved in PIC-induced viperin expression, a neutralizing antibody to human IFN-β was added before PIC treatment.
Results: Viperin localized in the cytoplasm of trophoblasts. PIC significantly induced viperin expression in mRNA ($P < 0.01$) and protein ($P < 0.01$) levels in a dose- and time- dependent manner. Pretreatment trophoblasts with neutralizing antibody to IFN-β (20 μg/ml) significantly reduced PIC-induced viperin mRNA compared with non-immune rabbit IgG ($P < 0.001$).
Conclusion: This study showed that viperin is expressed by trophoblasts for the first time. It is also demonstrated that viperin is induced by TLR3 ligation, and this is mediated by IFN-β. Induction of viperin is one of the immune responses provoked by trophoblasts upon viral infections.

P2.69

UP-REGULATION OF GLUTAREDOXIN GENE EXPRESSION UPON PLACENTAL HEPATITIS B INFECTION

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Objective: To investigate the effect of placental Hepatitis B virus (HBV) infection on the glutaredoxin (GLRX) gene expression level and its correlation with placental HBV DNA level.
Methods: HBsAg positive mothers, identified by antenatal screening, were recruited in the antenatal clinic. After delivery, fresh placental tissues were collected for DNA/RNA extraction (QIAamp DNA min-kit, Qiagen). HBV DNA and GLRX expression level were detected by quantitative real-time PCR. The GLRX expression level was normalized to GAPDH for comparison between the HBsAg/HBV DNA positive (N=20) and HBsAg negative control groups (N=30), and the placental GLRX expression level was correlated with the placental HBV DNA quantity, maternal age, maternal body mass index (BMI), birthweight and Apgar score (1 minute) of the newborn.
Results: HBsAg/HBV DNA positive placenta showed a significant up-regulation of the GLRX gene expression (P=0.006), but in this group of pregnancies, there was no difference in maternal age (32.1±3.1 vs 30.1±4.5 years; P=0.094), birthweight (3226±386 vs 3165±428 grams; P=0.609) and Apgar score (9.1±0.2 vs 8.9±1.1; P=0.385), and only maternal BMI was higher (23.8±4.0 vs 20.8±2.1; P=0.005). Moreover, placental GLRX expression was correlated with the quantity of the HBV DNA detected in the placenta (Rho=0.395, P=0.005) and maternal BMI (Rho=0.409, P=0.003) but not for maternal age (Rho=0.241, P=0.091), birthweight (Rho=0.090, P=0.534), or Apgar score (Rho=0.028, P=0.848).
Conclusion: Placental infection with HBV was associated with significantly increased expression of placental GLRX gene, which was correlated with the quantity of HBV DNA in the placenta, as well as maternal BMI. This may reflect increased oxidative stress in these placentas. Increased oxidative stress in these pregnancies could be one of the mechanisms for the observed increase in pregnancy complications.

P2.70

EXPRESSION OF PLACENTAL TOLL-LIKE RECEPTORS-3, -7 AND -8 UPON PLACENTAL HEPATITIS B INFECTION

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Objective: To investigate the placental Toll-like receptors (TLRs)-3, -7 and -8 mRNA/protein expression level in the presence of Hepatitis B virus (HBV) infection.
Methods: HBsAg seropositive mothers were recruited during antenatal visit. After delivery, fresh placental tissues were collected for DNA/RNA extraction (QIAamp DNA min-kit, Qiagen). HBV DNA and TLR-3, -7, and -8 mRNA expression levels were quantified by real-time PCR. The expression levels were normalized to GAPDH for comparison between the HBsAg/HBV DNA positive (N=20) and HBsAg negative control groups (N=30). Immuno-histochemical staining was performed for the semi-quantification of the TLRs protein level between groups.
Results:

	Placental HBV DNA		Difference
	Positive (n=20)	Negative (n=30)	
Maternal age (yrs)	32.1 ± 3.1	30.1 ± 4.5	NS
BMI (Kg/m^2)	23.8 ± 4.0	20.8 ± 2.1	P=0.005
Birthweight (grams)	3226 ± 386	3165 ± 428	NS
Apgar Score	9.1 ± 0.2	8.9 ± 1.1	NS

Results are expressed in mean ± SD.HBsAg/HBV DNA positive placentas showed a significant up-regulation of TLR7 (P=0.009) and TLR8 (P=0.012), but not TLR3 (P=0.303), gene expression, and Immunohistochemical staining confirmed increased activation of TLR7 and TLR8, but not TLR3, in these placentas. Significant correlation was found between placental TLR7 with TLR8 expression (Rho=0.824, P<0.001), but not between TLR3 with TLR7 (Rho=-0.121, P=0.408) or with TLR8 (Rho=-0.184, P=0.205) expression. Placental HBV DNA level was correlated with TLR8 (Rho=0.304, P=0.034), showed a borderline correlation with TLR7 (Rho=0.260, P=0.071), but not with TLR3 (Rho=0.138, P=0.343), expression.
Conclusion: Increased expression of TLR7 and TLR8 in the HBsAg/HBV DNA positive placentas, which was correlated with HBV DNA levels, suggest that this is probably a consequence of HBV infection. Further studies on the response of the innate immune system and TLR pathways to maternal viral infections could help to elucidate the mechanisms of in-utero HBV infection.

P2.71

EFFECTS OF CYTOMEGALOVIRUS INFECTION ON PLACENTA IN A GUINEA PIG MODEL

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Objectives: Human cytomegalovirus (HCMV) is the most common cause of congenital virus infection. Congenital infection occurs in ~0.3% of all births in Japan, and causes birth defects and developmental abnormalities, including sensorineural hearing loss and developmental delay (Koyano et al., 2011). Since guinea pig CMV (GPCMV) crosses the placenta and causes infection in utero, guinea pig models are valuable for understanding pathogenesis as well as for developing therapeutics for congenital infection. We demonstrated congenital CMV-associated labyrinthitis in a guinea pig model in which vertical infection by GPCMV occurred through the placenta (Katano et al., 2007). In this study, to understand effects of GPCMV infection on the placenta in guinea pigs, we performed pathological and virological analyses of infected placentas and characterized changes of cellular gene expression in the placentas.
Methods: Guinea pigs (strain Hartley, Japan SLC) 2-, 3- or 4-week after conception were inoculated subcutaneously with GPCMV. Blood, placenta, fetus and other organs were collected at various time points after the inoculation. Immunohistochemistry was done by using monoclonal antibodies against GPCMV. Viral loads were determined as genome copy numbers by real-time PCR. Total RNA samples were isolated from placenta tissues, and used for microarray analyses with custom array-slides (Agilent Technologies).
Results: Our findings are as follows. 1) Animals in early and mid-term pregnancies were more susceptible to infection, which frequently resulted in IUGR of fetus. 2) Histologically there were no apparent changes in infected placentas. 3) Infection in placentas was detected in limited areas. 4) Expression of several genes, mainly those relating to cell differentiation and metabolisms but not to inflammation, were affected by infection.
Conclusion: The lack of apparent impairment of placentas in histology and the significant effects of infection on expression of particular genes suggest that CMV impairs functions of the placenta, which results in IUGR.

P2.72

DO MALARIA PARASITES BIND TO THE PLACENTA EARLY IN PREGNANCY? EVIDENCE FROM A RELEVANT PLACENTAL ADHESION ASSAY

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Objectives: Placental malaria, defined by the accumulation of malaria-infected erythrocytes (IE) in the intervillous spaces, impairs placental function. Yet, we have an incomplete understanding of the interaction between placenta and IE. At term, IE adhere to chondroitin sulphate A (CSA) on the syncytiotrophoblast microvillous membrane, but little is known about the dynamics of IE placental adhesion during the course of pregnancy. Therefore, we studied the placental binding characteristics of IE derived from malaria isolates collected at various gestational ages.
Methods: We used a high throughput flow cytometry-based IE placental adhesion assay [1] to determine the binding characteristics of IEs collected from eight Papua New Guinean women between 19 and 36 weeks gestation as compared to a CSA binding control parasite line.
Microvillous plasma membrane (MVM) vesicles were isolated from term placentae from uninfected pregnancies, stained with a fluorescent lipid dye, and incubated with ethidium bromide-stained IE. The IE bound to MVM vesicles, and the ability of soluble CSA to inhibit this adhesion, was used to characterize IE placental adhesion.
Results: IE from malaria isolates showed a similar level of MVM adhesion as compared to the control parasite line (27±13% vs. 25±4%, P=0.9). Placental adhesion of IE was dynamic across gestation and increased 2.5 fold from 19 to 28 weeks (P=0.08). In a pilot experiment, we found that IE collected before mid-gestation were more likely to bind to MVM in a CSA-independent manner, compared with isolates infecting women after mid gestation.
Conclusion: The adhesion assay can be applied to study placental adhesion characteristics of IE in malarial pregnancy from the second trimester onwards. Further studies are required to determine whether a shift from CSA-independent to CSA-dependent placental adhesion occurs over pregnancy, in order inform whether the hypothetical CSA-adhesion based malaria vaccine would protect women from malaria over the duration of pregnancy.

[1] Boeuf P, Hasang W, Hanssen E, Glazier JD, Rogerson SJ. Relevant assay to study the adhesion of Plasmodium falciparum-infected erythrocytes to the placental epithelium. PLoS One. 2011, 6(6): e21126.

P2.73

PLASMODIUM FALCIPARUM MALARIA ELICITS INFLAMMATORY RESPONSES THAT IMPAIR PLACENTAL AMINO ACID TRANSPORT

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Placental malaria can lead to poor neonatal outcomes, including low birthweight due to fetal growth restriction (FGR), especially when associated with local inflammation (intervillositis). The pathogenesis of placental malaria-associated FGR is largely unknown but, in idiopathic FGR, impaired transplacental amino acid transport, especially through the group of amino acid transporters system A, has been implicated.
Objectives: We hypothesized that placental malaria-associated FGR could result from impairment of transplacental amino acid transport triggered by intervillositis.
Methods: Malawian women with uninfected placentas or with placental malaria with or without intervillositis were recruited together with their infants. We used laser capture microdissection and real-time quantitative RT-PCR to measure transcript levels of system A isoforms in the syncytiotrophoblast. Na^+-dependent MeAIB uptake into vesicles of the microvillous plasma membrane of the syncytiotrophoblast was used as a measure of system A activity. To gain insight into the mechanisms of placental malaria-associated FGR, we developed an *in vitro* model of placental malaria with intervillositis using BeWo cells and monocyte/malaria conditioned medium. Amino acid concentrations in paired maternal and cord plasmas were quantified by reversed phase ultra performance liquid chromatography.
Results: System A transcript levels and activity were reduced in placental malaria, especially when associated with intervillositis, compared to uninfected placentas. BeWo cells exposed to monocyte/malaria conditioned medium showed decreased system A activity. Amino acid analysis revealed specific alterations of amino acid transport by placental malaria, especially with intervillositis.
Conclusion: Our data suggest that the fetoplacental unit responds to placental malaria by altering its placental amino acid transport to maintain adequate fetal growth. However, intervillositis more profoundly compromises placental amino acid transport function, leading to FGR. Our study offers the first pathogenetic explanation for FGR in placental malaria.

P2.74

SEVERE INTRAUTERINE HERPES SIMPLEX VIRUS INFECTION

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Introduction: Neonatal herpes simplex virus (HSV) infection is usually acquired at birth. Intrauterine HSV infection is rarely documented and the prognosis is generally considered poor. We report a case of an intrauterine HSV infection.
Case Report: A 27-year-old primigravida was referred to our department at 26 + 5 weeks of gestation due to pregnancy induced hypertension and fetal growth restriction (FGR). Her past medical history was unremarkable. She had no history of a genital HSV-infection. His husband was suffered from facial palsy after a month of her last menstrual period. Her blood pressure was 177/97mmHg and 1.98g/day of urinary protein was detected. Our ultrasound scan showed severe FGR (460g, -3.9SD), without other structural abnormalities. The amniotic fluid index was decreased. The umbilical artery end-diastolic velocity was absent. The placenta was thick and small. Due to progressive rise in her blood pressure and fetal growth arrest, C-section was selected at 27+0 weeks of gestation. A female baby weighing 458g was delivered with Apgar score of 5(1')/5(5'). The placenta was small, measuring 13.0×10.0cm, and weighing 152g. At delivery, the infant had a tendency to bleed and the ultrasonography demonstrated hepatic bleeding. Although hemostatic surgery was done on the 3rd day of life, she lost her life on the 5th day. On later, the placental pathology showed that several necrotic abscesses in the intervillous spaces and immunoreactive HSV-1 and HSV-2 were tested positive in the chorionic cells and the trophoblast.
Conclusion: The case of an intrauterine HSV infection is presented. She had no history of a genital lesion, the time of onset of fetal infection was uncertain. Although intrauterine HSV infection is rare, clinicians, particularly when the patient complicate with severe FGR, should be alert to the fact that severe invasive HSV disease can occur without history of a genital HSV infection.

P2.75

THE ASSOCIATION BETWEEN GRADE THREE FUNISITIS AND FETAL INFLAMMATORY RESPONSE SYNDROME (FIRS)

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Objective: Fetal inflammatory response syndrome (FIRS), as characterized by elevated levels of fetal plasma inflammatory cytokines (i.e. interleukin-6 et al.), is an independent risk factor for the occurrence of severe neonatal morbidity. It was proposed that high levels of fetal plasma inflammatory cytokines could cause grade three funisitis, especially subacute necrotizing funisitis (SNF); however, its specificity or sensitivity to FIRS is still controversial. The aim of present study was to investigate the association between grade three funisitis, including SNF, and onset of FIRS.
Methods: 551 singleton deliveries in Hamamatsu University Hospital from April 2010 to March 2011 were retrospectively examined from medical records of both mothers and neonates. All placentas and umbilical cords were histologically examined.
Results: There were 17 cases of grade three funisitis among 551 umbilical cords (3.1%), which included 2 cases of SNF (0.36%). The rate of SNF in grade three funisitis was 11.8% (2/17). There were two cases of FIRS. One case of FIRS was characterized by bronchopulmonary dysplasia, in which chorioamnionitis grade 3 and SNF were observed. Another case of FIRS was manifested by encephalomalacia, in which chorioamnionitis grade 3 and funisitis grade 3, but not SNF, were observed. There was one normal uncomplicated neonate, in which chorioamnionitis grade 3 and SNF were observed.
Conclusion: It was suggested that SNF or grade three funisitis may be necessary, but not sufficient, condition of the onset of FIRS. Since SNF was observed in an intact neonate, further investigation is necessary to clarify the pathological association between FIRS and severe funisitis.

P2.76

VESICLE-BASED CURCUMIN FORMULATION FOR THE TOPICAL APPLICATION TARGETED TO VAGINAL INFLAMMATION DURING PREGNANCY

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Objectives: Vaginal inflammation during pregnancy is linked to miscarriage, preterm labour, placental dysfunction and perinatal complications. Despite the availability of several anti-inflammatory drugs in the market, there is no general consensus regarding safe formulation especially for use in early pregnancy. Turmeric (powdered rhizome of *Curcuma longa*) has been traditionally used as an anti-inflammatory remedy and considered as safe. One of its widely investigated pharmacologically active components is curcumin (>14544 citations in SciFinder) which has confirmed strong anti-oxidant and anti-inflammatory properties. However, due to its poor solubility and low bioavailability, its clinical application remains challenging. Our aim was to develop a non-teratogenic and non-toxic substance which can be used in early pregnancy for the treatment of vaginal inflammation.
Methods: Curmumin/curcuminoids (curcumin analogues) were standardized by UV-VIS/HPLC/MS/NMR. Liposomal curcumin/curcuminoids were characterized through their stability, size and entrapment efficiency. Antioxidant activities were evaluated by DPPH/$ABTS^{+}$/O_2^{-} and SOD activities. Anti-inflammatory activities were evaluated by measuring LPS-induced NO and pro-inflammatory cytokines such as IL-1β, TNF-α, IL-8 in macrophages (J774.1) and LPG-induced cytokines IL-8 in human vaginal cells lines (End1/E6E7, Ect1/E6E7, VK2/E6E7). Antioxidant and anti-inflammatory activities of curcumin and their corresponding vesicle-based formulation were compared.
Results: Nanoparticles (approx. 200nm), phospholipid-based vesicles were found to be stable and incorporating high amount of curcumin/curcuminoids. Liposomal curcumin was found to be 2-6 folds more potent than curcumin in inhibiting NO production and pro-inflammatory cytokines in macrophages. IL-8 was inhibited up to 67% by the liposomal curcumin in human vaginal cell lines (End1/E6E7, Ect1/E6E7, VK2/E6E7) compared to curcumin. A mixture of curcuminoids was found to be more soluble and more potent than individual compounds in respect to their antioxidant and anti-inflammatory activities.
Conclusion: Standardized liposomal curcuminoids might be an appropriate, effective and safe topical formulation for the treatment of vaginal inflammation in early pregnancy.

P2.77

THE INVOLVEMENT OF ENZYMES WHICH ACTIVATE AND INACTIVATE ENDOTHELIN-1 (ET-1) IN HUMAN LABOR ONSET

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Objectives: The mechanisms of human labor onset remain unclear, but its interpretation is important to define the pathology of preterm labor. We previously reported that S1P, which is involved in inflammation, play a role in human labor onset via induction of cox-2 and MMP-9. Others have reported that endothelin (ET) -1, an uterotonin, is increased during labor. The aim of the present study was to determine the role of ET-1 on human labor the relationship of S1P and ET-1.
Methods: Amnions were obtained with informed consent from normal pregnancies following caesarean (labor (-)) and vaginal deliveries (labor (+)). The expression of endothelin-converting enzyme (ECE), which synthesizes ET-1, and neutral endopeptidase (NEP), which inactivates ET-1, were investigated in amniotic tissues in labor +/- by Western blotting. The ET-1 levels were also examined by ELISA. The expression of ECE-1 in cultured human amnion epithelial cells (HAECs) or mesenchymal cells (HAMCs) with or without inflammatory cytokines, oxytocin and S1P treatment was investigated by Western blotting. In the mouse model of the intra-abdominal administration of lipopolysaccharide, the expressions in placenta were examined by Western blotting.
Results: The expressions of ECE-1 were increased, but that of NEP was decreased in amnion of labor (+) *vs*. labor (-). Then ET-1 was significantly elevated in amnion of labor (+). HAECs and HAMCs primarily expressed ECE-1 and NEP, respectively. The expression of ECE-1 in HAECs was significantly induced by treatment with TNF-α, IL-1β and S1P, while NEP levels in HAMCs were decreased. However, oxytocin had no effect on those expressions. In addition, NEP was decreased in the placentas with administration of lipopolysaccharide in comparisons to those with saline.
Conclusion: Those results suggest that ET-1 was increased by inflammation via the metabolizing enzymes. S1P also induced ET-1 and these molecules might play a role in human labor co-coordinately.

P2.78

THERAPEUTIC EFFECT OF MATERNAL HYDROGEN WATER ADMINISTRATION IN A MOUSE MODEL OF FETAL BRAIN DAMAGE

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Objectives: Oxidative stress caused by inflammatory cytokines and reactive oxygen species has been reported to be involved in the onset of the perinatal brain damage at intra-amniotic infection. In addition, by using an in utero infection model mouse with the LPS (lipopolysaccharide) intraperitoneal administration, it has been reported that antioxidizers are useful for the prevention of the embryo brain disorder. However, there remain few approaches reaching the clinical application. In this study, the protective effect of hydorogen water, which has been already reported to have both antioxidant and anti-inflammatory action, were investigated in an embryo brain disorder model. Hydrogen water is clinical applied saturation water in other fields now.
Methods: Water or hydrogen water was given to pregnant mice at 14days in free drinking style for 24 hours. Then they were treated with LPS 5μg/body or PBS intraperitoneally. Samples were collected 24 hours later and examined as below mentioned.

1) The expressions of inflammatory cytokines (IL-6, IL-1β, TNF-α) in fetal brains and placentas by qRT-PCR.
2) The expression of activated caspase-3, which was a marker of apoptosis, in fetal brains by immunohistochemsitry.
3) The expression of cox-2 in placentas by Western blotting

Results:

1) By the LPS dosage, IL-6 expression was remarkably increased in embryo brain, where this induction was completely suppressed in the LPS - hydrogen water administrated group ($P<0.01$).
2) Activated caspase-3 expression was increased in LPS dosage, but it was reduced by the hydrogen water dosage ($P<0.01$).
3) Cox-2 expression was increased in LPS dosage, but it was partially suppressed by the hydrogen water dosage ($P<0.01$).

Conclusion: In the perinatal brain damage model by LPS treatment, IL-6 might be a key molecule. The mother's body hydrogen water dosage suppressed IL-6 in fetal brain, might have a protective role on perinatal brain damage by inflammation, but further examination is needed.

P2.79

MATERNAL CHARACTERISTICS AND PERINATAL OUTCOMES OF WOMEN WITH HISTOLOGIC CHORIOAMNIONITIS AT THE NATIONAL CENTER FOR GLOBAL HEALTH AND MEDICINE

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Objective: To evaluate the maternal characteristics and perinatal outcomes of women who were histologically diagnosed with chorioamnionitis at the National Center for Global Health and Medicine.
Methods: We conducted a retrospective study in which we enrolled all singleton pregnant women at our hospital whose placentas had been pathologically examined upon delivery between January 2005 and August 2010. We compared the maternal characteristics and perinatal outcomes of women with histologic chorioamnionitis with those of women without any histological findings. We also evaluated prenatal outcomes and the incidence of funisitis among the cases of chorioamnionitis, according to Blanc's stages of placental inflammation.
Results: A total of 146 women with and 146 women without histologic chorioamnionitis were enrolled. There were no differences in the mean maternal age, the incidence of preterm delivery, the mode of delivery and the incidence of low birth weight between the groups. The women with histologic chorioamnionitis were significantly more likely to be primiparous, less likely to have sought prenatal care and more likely have delivered post term. In a multiple logistic regression analysis, the failure to seek prenatal care was an independent factor in predicting the risk for developing histologic chorioamnionitis (odds ratio, 0.13; 95% confidence interval, 0.03-0.62). Among the 146 women with histologic chorioamnionitis, 45 cases were identified as showing Blanc's placental inflammatory stage 1 on histology, 81 cases were identified as showing stage 2, and 19 cases were identified as showing stage 3 inflammation. The women who were diagnosed with Blanc stages 2 and 3 chorioamnionitis had a significantly higher incidence of histologic funisitis compared with the Blanc stage 1 women.
Conclusions: Our findings show that the risk for chorioamnionitis increases with inadequate prenatal care and postterm delivery and could be mitigated by the proper provision of prenatal care or the appropriate perinatal interventions.

P2.80

ABERRANT INFLAMMATION IN PREGNANT RATS INDUCES RENAL ALTERATIONS, PROTEINURIA AND UTERO-PLACENTAL OXIDATIVE STRESS

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Objectives: Glomerular endotheliosis (GEN) is a PE-specific renal alteration characterised by glomerular endothelial hypertrophy, capillary occlusion and sub-endothelial fibrin deposition. Hypercellularity of the glomerulus, occlusion of glomerular fenestrations, pedicel effacement and thickening of the glomerular basement membrane (GBM) have also been reported in association with confirmed cases of PE. Additionally, proteinuria is a hallmark feature of PE-associated renal dysfunction. Using a recently developed inflammation-mediated rat model of IUGR, this study determined whether aberrant inflammation is causally linked to the development of PE-associated renal pathologies.
Methods: To induce aberrant maternal inflammation, dams received intraperitoneal injections of lipopolysaccharide (LPS; 10-40 μg/kg) on gestational days (GD) 13.5-16.5. To elucidate the role of tumour necrosis factor-α (TNF-α; a pro-inflammatory molecule) in the pathophysiology of GEN, LPS-treated rats were administered Enbrel® (TNF-α inhibitor; 10 mg/kg). Additionally, to investigate whether deficient nitric oxide (NO) signalling is important to the development of GEN, a transdermal nitroglycerin patch (GTN; 25 μg/h; NO-mimetic) was administered to LPS-treated rats.
Results: Kidneys from LPS-treated animals exhibited alterations characteristic of GEN. Transmission electron microscopy revealed LPS-induced thickening of the GBM and pedicel effacement. While Enbrel® treatment inhibited GEN it did not prevent GBM thickening. Administration of GTN, however, attenuated all inflammation-induced renal alterations. Proteinuria was associated with abnormal inflammation and was prevented with either Enbrel® or GTN. Renal alterations were found to be pregnancy specific; thus, placentas from LPS-treated animals were examined for evidence of oxidative stress. Immunohistochemistry against nitrotyrosine revealed that Enbrel® or GTN prevented LPS-induced utero-placental oxidative stress.
Conclusions: These results provide evidence that aberrant inflammation during pregnancy is causally linked to the development of renal alterations possibly by a mechanism associated with deficient NO-signalling. These findings support the potential use of TNF-α inhibitors and NO-mimetics in the treatment of renal alterations associated with PE/IUGR.

P2.81

SREBP-1C AND HCHOP-C/EBPα INVOLVEMENT IN INSULIN MODULATION OF HENT2 ACTIVITY IN HUMAN PLACENTA MICROVASCULAR ENDOTHELIUM FROM GESTATIONAL DIABETES

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Objectives: Insulin restores gestational diabetes mellitus (GDM)-reduced hENT2 activity and expression (including *SLC29A2* transcriptional activity) in human placenta microvascular endothelial cells (hPMEC). Nitric oxide (NO) synthesis is modulated by insulin and activated by the transcriptional factors hCHOP-C/EBPα and SREBP-1c, but nothing is known in hPMEC. We studied insulin effect on hCHOP-C/EBPα and SREBP-1c activity in hPMEC from GDM.
Methods: hPMEC were isolated from full-term normal or GDM pregnancies and cultured under standard conditions. hENT2-mediated adenosine transport (10 μM adenosine, 2 μCi/ml [^{3}H]adenosine, 22oC), protein abundance and mRNA were determined in absence or presence of insulin (1 nM, 8 hours) by Western blot and Q-PCR. Transcriptional activity of two fragments of the *SLC29A2* promoter region (pGL3-hENT2^{-1483}, pGL3-hENT2^{-593}) was measured by luciferase assay. hCHOP-C/EBPα and SREBP-1c protein abundance and activity was assayed by western blot and chromatin immuneprecipitation, respectively.
Results: Insulin increased hENT2-mediated adenosine transport by ~10 and ~1.7 fold in hPMEC from GDM and normal pregnancies, respectively. This effect was paralleled by the recovery of hENT2 expression (protein and mRNA). Insulin also increased the promoter activity of pGL3-hENT2^{-1491}, but not pGL3-hENT2^{-602} in cells from normal (1.8 ± 0.1 fold) and GDM (2.5 ± 0.3 fold) pregnancies. hCHOP-C/EBPα protein abundance and activity were reduced by insulin in GDM cells (2.9 ± 0.4 fold), but increased (2.3 ± 0.3 fold) in cells from normal pregnancies. Insulin increased SREBP-1c protein abundance (~2.6 fold) and activity in cells from GDM or normal pregnancies.
Conclusion: hCHOP-C/EBPα and SREBP-1c could play a role in the modulation of *SLC29A2* expression and hENT2 activity in the fetoplacental microvascular endothelium in GDM.
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P2.82

PLACENTAL FINDINGS IN OBSTRUCTIVE SLEEP APNEA

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Objectives: Sleep disordered breathing during pregnancy has been associated with adverse maternal outcomes. We reported previously an increased incidence of hypertensive disorders and gestational diabetes mellitus (GDM) associated with sleep disordered breathing. This study further investigated the placental findings in OSA.
Methods: This was a retrospective case-control study of pregnant women with obstructive sleep apnea (OSA). The control group consisted of women with a negative screen for OSA. Gestational age at delivery, placental weight percentile, findings of gross and microscopic examinations of placenta were collected. Placental findings were further classified into four categories. Frequency of pathological findings in OSA and control groups was compared by Fisher's exact test with $P<0.05$ as significant.
Results: Fifteen placentas (10 term and 5 preterm) were available from women with OSA. There was one preterm stillbirth due to uteroplacental insufficiency. Forty-five placentas (32 term and 13 preterm) were randomly selected from the control group. There was one preterm stillbirth with no cause of death determined. Significant pathological findings in placentas from women with OSA included decidual vasculopathy (7/15 vs 8/45, $P<0.05$), abruption (3/15 vs 1/45, $P<0.05$), increased syncytial knots (8/15 vs 8/45, $P<0.05$). Significantly more "delayed villous maturity" was identified in term placentas with maternal OSA (3/10 vs 1/32, $P<0.05$). All above findings are related to maternal underperfusion. There were no significant differences in small-for-gestational age placentas or preterm birth.
Conclusions: Significant changes related to maternal underperfusion were found in the placentas with maternal OSA. Decidual vasculopathy is often seen in hypertensive disorders and GDM and is regarded as underlying causes leading to infarct and abruption. Delayed villous maturity in term placenta is often seen with maternal GDM. These findings are consistent with clinical outcome findings and suggested a potential relationship between altered sleep patterns and placental underperfusion and reaction.

P2.83

PLACENTAL FINDINGS IN PREGNANCIES WITH MATERNAL OBESITY

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Objectives: Obesity has become a worldwide epidemic. In the United States, close to 20% of pregnant women are obese. Obesity during pregnancy leads to a wide spectrum of maternal, fetal and neonatal complications. The aim of this study was to examine the relationship between maternal obesity and placental histopathology.
Methods: This was a retrospective case-control study of placental pathology. Cases (BMI>30) and controls (BMI<25) were randomly selected. Placental weight, gross and microscopic pathology, and clinical parameters were extracted from medical records. Placental weight range was defined as small-for-gestational-age (SGA) if $< 10^{th}$ percentile and large-for-gestational-age (LGA) if $>90^{th}$ percentile. Placental pathology was further classified into maternal underperfusion, fetal circulation obstruction, infection/inflammation or others. The prevalence in the two groups was compared with Fisher's exact test ($P<0.05$ = significant).
Results: Median age was 29 in the obese group (n=50, 18-40) and 30 in the control group (n=46, 19-40). Mean BMI was 35.98 +/- 4.89 in the obese group and 21.90 +/- 2.33 in controls. There was significantly more gestational diabetes (GDM) in the obese group (13/50 vs 2/46, P=0.004). In placentas of obese mother, histopathology related to underperfusion was commonly observed (71.4%) and 12.5% related to infection/inflammation, without significant differences seen between the 2 groups. There were significantly more LGA placentas in the obese versus non-obese (13/50 vs 2/46, P=0.003; even after adjusting for GDM). More marginal insertion of the umbilical cord was also noted in the obese group (11/50 vs 2/46, P<0.05).
Conclusions: This study provides a preliminary description of the placental pathology in pregnancy with maternal obesity. LGA placentas and marginal insertion of cord are more common in obese women, even after adjusting for GDM. These results suggested that maternal obesity may have effects on both placental implantation and growth.

P2.84

IMPAIRED GLUCOSE TOLERANCE MAKES AN INFLUENCE ON THE PLACENTAL PATHOLOGY

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Objectives: The purpose of this study is to investigate pathologic differences of the placenta in pregnancies complicated by gestational diabetes (GDM/DM).
Methods: fifteen pregnancies complicated by gestational diabetes were obtained from January 2010 to December 2011at Hamamatsu University Hospital. We reviewed all histological samples of the placentas. The histological assessment for villous immaturity, villous fibrinoid necrosis and abnormalities of the vascular lesions were carried out.
Results: thirteen cases of gestational diabetes group were treated with Insulin or diet therapy (improved group), and another two cases were untreated (fetal death group). In improved cases, the presence of degenerative lesions such as fibrinoid necrosis was apparent. Villous edema or reduction of vasculo-syncytial membranes in terminal villi were found in 60% cases. Villous immaturity, the presence of syncytial knots as an indication of chronic fetal hypoxia and fibrosis in the stem villous vessels were significantly increased. In fetal death group, histological findings show extensive infarction, fibrinoid necrosis or villous immaturity and narrowed or obstructed change in stem vessels.
Conclusion: A histological abnormality, that is, villous immaturity or vascular pathological change such as obstructed vessels in stem villi, were observed in the diabetic placentas. Impaired glucose tolerance makes an influence on feto-placental circulation and placental development. These findings support the hypothesis that impaired placental function is one of the main reasons for the increased frequency of fetal complications in diabetic pregnancies

P2.85

EFFECT OF OBESITY AND FETAL GENDER ON EXPRESSION OF MITOCHONDRIAL ELECTRON TRANSPORT COMPLEXES AND BIOENERGETICS IN HUMAN PLACENTA

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OBJECTIVES, METHODS, RESULTS, CONCLUSION

Objectives: Obesity is associated with chronic inflammation and mitochondrial dysfunction in many tissues. We determined the effect of increasing maternal BMI and of fetal sex on ATP generation and gene expression of mitochondrial electron transport chain subunits in villous tissue and on trophoblast mitochondrial respiratory function *in vitro*.
Methods: Villous tissue was collected following c-section (no labor) at term. cDNA was prepared from placentas of both genders from lean, overweight and obese women (n=4/group) and expression of 84 genes involved in oxidative phosphorylation determined using the Mitochondrial Energy PCR Array (SaBiosciences). Villous ATP levels were measured with chemiluminesence. Primary cytotrophoblasts were isolated from women of BMI range 18.5 to 45 (n=12/gender), syncytialized over 72hr and mitochondrial bioenergetic parameters measured using a XF24 analyzer (Seahorse Bioscience).
Results: With increasing BMI, placentae of male fetuses showed 1.2-1.7 fold ($p<0.05$) downregulation of genes in complexes I, IV and V, suggesting adaptation/dysfunction. In contrast, with increasing BMI, placentae from a female fetus showed increased gene expression of a smaller number of subunits particularly catalytic subunits from complexes II and V. Comparisons between genders revealed that placentae from a female fetus of lean women displayed a 1.5x higher expression of complex V assembly factor OXA1L and of LHPP phosphatase whereas increased expression of catalytic complex I and V subunits, NDUFS7 and ATP6V1E2 was seen in obese women. Villous ATP levels decreased significantly with increasing BMI in males but not females, agreeing with energetic array data. In syncytiotrophoblast basal and maximal respiration, spare capacity, and non-mitochondrial respiration but not ATP coupled respiration or proton leak, showed a significant inverse correlation with increasing maternal BMI. The effect was more marked in trophoblast from males.
Conclusions: A sexually dimorphic effect of increasing maternal adiposity on placental mitochondrial function is seen. Placental energetic capacity is compromised to a greater extent in the male fetus.

P2.86

RELATIONSHIP BETWEEN SERUM LEVELS OF D-DIMER AND WEIGHT OF PLACENTA IN THE LATE PREGNANCY

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Objective: It is well known that coagulation is enhanced during pregnancy and serum levels of D-dimer increase gradually toward the late stage of gestation. The main cause of this increase is thought to be local degeneration of fibrin in the uteroplacental circulation. However, there is no report that proves D-dimer is generated in the placenta. We investigated whether serum levels of D-dimer correlated with placental weight.
Method: We enrolled 59 women with singleton pregnancies who had measurements of platelet counts, hematocrit, and serum levels of FDP and D-dimer at 35 or 36 weeks of gestation and delivered normal birth weight babies within 14 days after these measurements. The mean value and standard deviation of each parameter were evaluated retrospectively. Relationships between these values and placental weight, and the other background data (age, parous number, height, body mass index, weight gain, birth weight) were examined.
Results: Platelet counts, hematocrit, FDP, D-dimer, and placental weight were $231 \pm 97 \times 10^3$/ml, $33.4 \pm 3.0\%$, 5.0 ± 3.1 μg/ml, 2.4 ± 1.5 μg/ml, and 578 ± 91 g, respectively. The serum levels of D-dimer correlated with placental weight ($R = 0.400$, $p = 0.0015$), FDP ($R = 0.801$, $p < 0.0001$), and hematocrit ($R = -0.311$, $p = 0.0162$), but did not with birth weight. Simple linear regression formula between serum levels of D-dimer and placental weight was evaluated: [D-dimer (μg/ml)]= $-1.316 + 6.5 \times$ [placental weight (g)] $\times 10^{-3}$; $R^2 = 0.160$.
Conclusions: That the serum levels of D-dimer in the late pregnancy correlated with weight of placenta suggests that D-dimer is generated in the placenta. The regression formula of D-dimer according to placental weight might be available in investigating hypercoagulability during the third trimester.

P2.87

HUMAN MACROPHAGES REGULATE CHORIONIC GONADOTROPIN WITH TRUNCATED LH/CG RECEPTOR

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Objectives: The major role of human chorionic gonadotropin (hCG) in early pregnancy is to maintain the corpus luteum for persistent progesterone production. Maternal hCG also enters fetal plasma via the mesenchyme of the placental villi and promotes male sexual differentiation; therefore, excess fetal hCG induce aberrant genital differentiation. We have hypothesized that hCG is regulated by macrophages in the stroma of placental villi, known as Hofbauer cells. The present study analyzed the expression of luteinizing hormone (LH)/CG receptor with a deletion of exon 9 (LH/CG-R Δ9) in human macrophages and assessed whether LH/CG-R Δ9 was specifically involved in the degradation of hCG.
Methods: Phorbol 12-myristate 13-acetate (PMA)-treated THP-1 cells were exposed to 1,000 mIU/ml of hCG, LH or follicle stimulation hormone (FSH). The incorporation of each gonadotropin into the cells was evaluated by immunocytochemistry. The expressions of cytoplasmic LH/CG receptor and α-tublin were analyzed by western immunoblotting. Their time-dependent morphological changes were also examined.
Results: Immunocytochemistry showed that PMA-treated THP-1 cells selectively took up hCG. Western blotting analyses demonstrated that PMA-treated THP-1 cells expressed a 60-kDa protein designated endogenous LH/CG-R Δ9 and that hCG induced transient reduction of the LH/CG-R Δ9. hCG also induced transient reduction of α-tublin in PMA-treated THP-1 cells. Vacuoles formation mimicking the structure of Hofbauer cells was transiently induced by hCG in PMA-treated THP-1 cells.
Conclusions: Our results suggest that the LH/CG-R Δ9 is specifically involved in the degradation of incorporated hCG. hCG-LH/CG-R Δ9 complex may be trafficked into lysosome by the cytoskeletal remodeling with microtubules. The degradation may lead to change the structure of PMA-treated THP-1 cells to the vacuolated form mimicking human placental Hofbauer cells, which may degrade hCG with cytoplasmic LH/CG-R Δ9 to protect the fetus from exposure to excess maternal hCG during pregnancy.

P2.88

MECHANICAL MODELLING OF SYNCYTIAL NUCLEAR AGGREGATES; CAN THEY PASS THROUGH SMALL PULMONARY VESSELS?

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Objectives: Syncytial nuclear aggregates (SNAs) are large (average diameter of 66 μm) multinucleated cell bodies that are shed from the syncytiotrophoblast into the maternal blood. Due to their large size, most SNAs are trapped in the small vessels of the maternal lungs but some SNAs pass through the pulmonary capillaries into the maternal peripheral blood. This preliminary study examined the mechanical behaviour of SNAs to determine why some SNAs can pass through the pulmonary capillaries.
Methods: Forty-four SNAs were harvested from fourteen first trimester placentae (8.0–12.6 weeks) as published, and individually aspirated into glass micropipettes using pressure-calibrated micromanipulators. The deformation and entry/exit flow of the SNAs into ~20 μm micropipettes at pressures up to 3,450 Pa was recorded. Apparent viscosity (η, force required to move one layer of fluid in relation to another), cortical tension (T_0, surface tension which resists surface area increase), dissipation ratio (μ, loss rate of energy) and critical pressure at aspiration were calculated.
Results: SNAs could be divided into two groups, those that did recover their shape after aspiration into the micropipette (n = 15, 34%) and those that did not (n = 29, 66%). The SNAs that did recover their shape had mean apparent viscosity of 88.35 (SE ±10.42) Pas, cortical tension of 11.07 (SE ±4.98) 10-3 N/m, dissipation ratio of 1.47 (SE ±0.22) and critical pressure of 800.09 (SE ±148.72) Pa.
Conclusion: 34% of SNAs behaved as Newtonian liquid droplets, deforming into the pipette then regaining their shape when the pressure was released. The force required to deform the SNAs that behaved as Newtonian droplets was less than the pressure that would induce damage to a pulmonary capillary. The ability to deform and recover their shape could explain how some SNAs pass through the maternal pulmonary vessels to enter the peripheral circulation.

P2.89

WHAT PERCENTAGE OF THE VILLOUS SURFACE IS REALLY COVERED WITH CYTOTROPHOBLASTS IN THE FULL-TERM PLACENTA?

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Objectives: As a feto-maternal interface, the thickness of trophoblast layers has significant influence over molecular exchange and the barrier function of placentas. It has been long accepted that the cytotrophoblast (CT) layer either becomes interrupted or disappears during the course of gestation. General textbooks describe that the ratio of villous surface covered by CT is less than 27.5%. In 2007, however, there was a report that CT cells occupy 80% of villous surface at term, using a cell membrane marker of villous CT; HAI-1 (Hepatocyte Growth Factor Activator Inhibitor Type 1). Contrarily, Jones et al. (2008) reported that the term CT coverage ratio was only 44%, using electron microscopy. In this study, we reappraised the distribution of CT in the full-term human placental villi by immunohistochemistry.
Methods: Nine normal human placentas were obtained according to a protocol approved by the Ethical Committee of Tokyo Women's Medical University. They were fixed with 4% paraformaldehyde and frozen in liquid nitrogen. Cryosections were made and stained immunohistochemically with antibodies against various markers including HAI-1.
Results: The confocal laser scanning microscopic observations of thin sections revealed the distinct continuity of HAI-1 positive area immediately on the basement membranes surrounding villous core. This was also confirmed on EPON embedded semithin sections. In three dimensional examinations of thick sections, CTs had prominent and sometimes long processes, and this apparent CT coverage ratio at term villous surface was about 45%.
Conclusion: The discrepancy in ratios between HAI-1 positive areas and CT coverage led us to further investigate to elucidate the HAI-1 positive structures in non-CT areas at the electron microscopic level.

P2.90

CONJUGATED LINOLEIC ACID STIMULATES EXPRESSION OF ANGIOPOETIN LIKE-4 (ANGPTL4) IN THE PLACENTAL EXTRAVILLOUS TROPHOBLAST CELLS

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Objective: Conjugated linoleic acid (CLA) concentration in human body is 0.1% of the total fatty acid composition however its roles in feto-placental growth and development are not well known. We therefore examined the effects of two CLA isomers (*cis-9, trans-11-CLA* (*c9t11*)-CLA and *trans-10 cis-12* CLA (*t10c12*)-CLA) on angiogenic factors such as VEGF, ANGPTL4, relevant lipid metabolic genes, and tube formation (as a measure of angiogenesis) in first trimester placental trophoblast cells, HTR-8/SVneo.
Methods: HTR-8/SVneo cells were incubated with CLA isomers for 24h and then expression of genes involved in angiogenesis, lipid metabolism and transport was investigated. In addition, their effect on tube formation (as a measure of angiogenesis) was examined. The mRNA expression was analyzed using quantitative real-time RT-PCR (Applied Biosystems). Fold change gene expression was calculated according to the ΔΔCt method. Tube formation in these cells was measured.
Results: *c9t11*-CLA stimulated the expression of mRNA and protein of ANGPTL4 accompanied with increased tube formation in HTR-8/SVneo cells whereas *trans-10 cis-12* (*t10c12*)-CLA had no such effects. *c9t11*-CLA however did not stimulate expression of VEGF in these cells. Silencing ANGPTL4 in these cells significantly reduced the stimulatory effect of *c9t11*-CLA on tube formation, indicating that involvement of ANGPTL4. In addition, *c9t11*-CLA altered mRNA expression of several angiogenesis modulating factors such as fatty acid binding protein-4(FABP4), FABP3, cyclooxygenase-2 (COX-2) and adipose differentiation-related protein (ADRP) in HTR-8/SVneo cells. *c9t11*-CLA also induced the uptake of docosahexaenoic acid,22:6n-3 (DHA), a stimulator of tube formation in these cells. Triacsin C, an acylCoA synthetase inhibitor, attenuated *9c11t*-CLA induced DHA uptake, tube formation and cellular proliferation in HTR8/SVneo cells.
Conclusion: *c9t11*-CLA isomer may help in early placentation processes via increased expression of ANGPTL4 and other modulating factors such as FABP4, FABP3, COX-2 and ADRP with concomitant increase in the uptake of DHA in these cells.

P2.91

PREPARATION METHOD FOR ESSENCE EXTRACTED FROM PIG PLACENTA

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Objectives: We intend to verify the applicability of the Sepa-sigma's pore diffusion technology to purify the extract essence from pig placenta while removing bacteria to the level of LTV≧6, and to confirm the product contain the physiological active components such as ILs, TNF, EGF, FGF and other proteins without loss of their activity. METHODS,
The cryo-placenta of pig was prepared through the rapid refrigeration to -60°C. After a cut-trimming the cryo., the cells in the cryo. Were destroyed by the repetition of melt-freeze. The drip components of the cell were pre-purified using a centrifuge. The solution part was connected with the pore diffusion instrument installed with the regenerated cellulose membrane.
Results: The molecules with less than 30 million of molecular weight could pass through the membrane having the mean pore size between 10 nm and 500 nm. The diffusion coefficient decreased with an increase in their molecular weight. The plugging phenomena did not occur. As for pig placenta drip, the membrane having mean pore size of 500 nm gave the solution free from germs indicating the removing level of LRV > 6. the treating speed was 5 little/㎡hr and the plugging appeared slightly after the stationary state. The solution showed the high level of bio-active concentration such as IL-1 of 80 pg/ml, IL-10 of 200 pg/ml, TNFα of 70 pg/ml, EFG of 70 pg/ml and FGF-2 of 1200 pg/ml.
Conclusion: We developed the new technology Sepa-Sigma's pore diffusion system of purification of the extract essence from pig placenta while removing bacteria to the level of LRV > 6. The extract essence contained most of components in the extra- and intra-cellular liquid without loss of their activation.

P2.92

EFFECT OF ADENOVIRAL-MEDIATED IGF-1 ON HUMAN PLACENTA MICROVASCULAR ENDOTHELIAL CELLS

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Objectives: Insulin-like growth factor axis including IGF-1 has an important role in feto-placental growth and development throughout gestation. Recent studies demonstrated that adenoviral-mediated-IGF-1(Ad-hIGF-1) significantly increased placenta growth1 and altered placental labyrinth volume and fetal capillary density. We hypothesized that Ad-h**IGF-1 treatment may promote the proliferation of human placenta microvascular endothelial cells (HPMVECs) and prevent the apoptosis of HPMVECs.**
Methods: We isolated HPMVECs and cultured in vitro to investigate the direct effect of Ad-hIGF-1 on HPMVECs. Using a novel HPMVECs co-culture system with BeWo cells, we investigated the indirect effect of Ad-hIGF-1 on HPMVECs. Ad-hIGF-1 treated BeWo cells were seeded with non-treated HPMVECs, and non-treated BeWo cells were treated with Ad-hIGF-1 treated HPMVECs. Crystal violet assay and immunocytochemistry study were performed to examine the proliferation(Ki67) and apoptosis(caspase-3) of HPMVECs. Statistical analyses were performed with student's t-test. P values <0.05 were considered statistically significant.
Results: Direct Ad-hIGF-1 treatment on HPMVECs significantly increased the proliferation of HPMVECs($N=4$, $p<0.001$) and the apoptosis of HPMVECs was reduced($N=4$, $p<0.001$). The proliferation of HPMVECs increased after Ad-hIGF-1 treated BeWo cells were seeded with non-treated HPMVECs, whereas apoptosis was decreased. When Ad-hIGF-1 treated HPMVECs were seeded with untreated BeWo cells, proliferation in BeWo cells was increased and apoptosis was decreased. Direct Ad-hIGF-1 treatment to HPMVEC showed more effect than following indirect with BeWo cells. Both direct and indirect Ad-hIGF-1 treatments result in same effect on proliferation and apoptosis of BeWo cells.
Conclusion: Direct Ad-hIGF-1 treatment may enhance human placental microvascular endothelial cells growth more effectively than via treatment of the trophoblast.

Reference:

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P2.93

A MATERNAL LIPID AND CHOLESTEROL-ENRICHED DIET DISRUPTS FETAL DEVELOPMENT AND PLACENTAL FUNCTION IN A RABBIT MODEL

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Objectives: We have shown that maternal administration of a lipid (8%) and cholesterol (0.2%) enriched diet (HH diet) in a rabbit model leads to IUGR and increased offspring susceptibility to excess body fat, overweight and hypertension once adults. To examine the link between the fetal development and metabolic consequences in later life, placental development has been explored.
Methods: Female rabbits were fed with a control (C) or HH diet from 10 weeks of age and throughout gestation. At 28 days of gestation, dams were anesthetized and a laparotomy was performed to collect placenta and plasma.
Results: Fetal weight in HH group was significantly reduced compared to C. Total cholesterol and triglycerides concentrations in HH fetuses were significantly increased by 1.2- and 2.3-fold respectively, compared to C. The structural analysis of HH placentas revealed an abnormal accumulation of light vesicles, identified as lipid droplets in the trophoblast layer. Total content of cholesterol esters was also significantly increased in HH placentas. The expression of genes implicated in placental growth, vascularization and nutrient transfer has been studied. HH placentas were characterized by a significantly decrease in *LDL-receptor, CD36, LXR-alpha, ABC-G1* and *SLC38A1* transcripts. The down-regulation of *LXR-alpha* mRNA was correlated with a decrease in protein expression.
Conclusion: These data demonstrate that maternal HH diet reduced cholesterol transport through the placenta as evidenced by placental gene expression and cholesterol esters accumulation. In contrast, fatty acids transport was not regulated, which could explain the excess of body fat in adults.

P2.94

ALTERED ENDOTHELIAL FUNCTION ASSOCIATED WITH GESTATIONAL DIABETES BEGINS *IN UTERO*

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Objectives: The 'Developmental Origin of Health and Disease' paradigm proposes environmental factors in pregnancy act *in utero* to program the risk for adverse health outcomes. Despite its short duration, gestational diabetes mellitus (GDM) has long term consequences for the offspring and confers an increased risk for endothelial dysfunction.
We hypothesized that primary human arterial endothelial cells (AEC) isolated from term placentas after healthy pregnancies (normal AEC) and pregnancies complicated with GDM (diabetic AEC) would differ in their intrinsic biological program. We focused on proliferation and angiogenesis as key endothelial processes.
Methods: Normal and diabetic AEC were cultured for 24, 48 and 96h. Viable and dead cells were counted to determine proliferation. *In vitro* angiogenesis (2-D network formation) was studied in media containing 2% normal or diabetic cord blood serum (CBS). The global DNA methylation profile was determined by 450k methylation arrays.
Results: Diabetic AEC proliferated less (ANOVA $p<0.003$) with $36\pm10\%$ fewer viable and $33\pm8\%$ fewer dead cells after 96h culture than normal AEC. In the presence of normal CBS total tube length ($+ 45\pm10\%$), number of branching points ($+ 311\pm28\%$) and number of meshes ($+ 163\pm50\%$) were increased in diabetic vs. normal AEC (ANOVA $p<0.001$). Diabetic CBS did not influence network formation neither in normal nor diabetic AEC. Thus the difference in proliferation and tube formation is the result of an intrinsic cell program. Principal component analysis revealed differences in the global methylation pattern between normal and diabetic AEC.
Conclusion: The altered proliferation and network formation potential of diabetic placental AEC even when cultured under identical conditions as normal AEC argues for changes in the cells, which are independent of the acute environmental conditions. These changes appear to reflect an intrinsic program to which epigenetic modifications contribute. Thus GDM-associated programming of endothelial cells of the offspring may begin *in utero*.

P2.95

PATHOLOGICAL CHANGES IN ABORTED TISSUE FROM RSA (RECURRENT SPONTANEOUS ABORTION) PATIENTS WITH ANTI-PHOSPHOLIPID ANTIBODIES INDUCED BY PATERNAL LYMPHOCYTES IMMUNIZATION

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Introduction: The therapy with paternal lymphocytes for immunization of patients with recurrent spontaneous abortion (RSA) has been reported to stimulate maternal immune-response and conduct blocking-antibodies, therefore contribute to the maintenance of pregnancy. However, this treatment may induce auto-antibodies including anti-phospholipid antibodies (aPLs), and these antibodies may be unfavorable for pregnancy, a topic not elucidated so far.
Materials and Methods: 71 patients with RSA of unknown causes with negative auto-antibodies including aPLs were immunized with paternal lymphocytes at the Jikei University Hospital from April 2003 to December 2011. APLs were analyzed after treatment. The therapeutic outcome was compared between aPLs-induced patients (PC group) and non-induced patients (NC group). We investigated pathological changes in aborted tissues from patients with miscarriage after treatment.
Results: From 71 patients, 15 showed aPLs induction (PC group, 21.1%), while 56 patients remained non-induced (NC group, 78.9%). In the PC group anti-cardiolipin IgG antibodies were observed in 14 cases. The ratio of a successful pregnancy was 7/11 (63.6%) in the PC group and 27/40 (67.5%) in the NC group. In the PC group, anti-coagulant therapy was introduced to 7 cases, and 6 of them (85.7%) successfully maintained pregnancy, while in 4 cases without this therapy, only 1 successfully maintained pregnancy (25%).
Investigation of pathological changes in aborted tissues with normal karyotype revealed characteristic changes in 4/7 cases in patients with aPLs, while no changes were observed in patients without aPLs (0/6). Changes included abortive vessels, pink-amorphous depositions and lymphocytes infiltrations.
Conclusion: After immunization with paternal lymphocytes it is recommended to examine for possible induction of aPLs. A subsequent anti-coagulant therapy may prevent abortion even under these adverse conditions.

P2.96

EFFECTS OF ANTI β2-GPI ANTIBODIES ON CYTOKINE PRODUCTION IN NORMAL TROPHOBLAST CELLS

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Objectives: The antiβ2-GPI antibody was detected in recurrent fetal loss with strong pathogenic activity. The pathogenic activities of anti-phospholipid antibodies consist of thrombosis and trophoblastic damage. The anti β2-GPI antibody increases NFκB via TLR-4 on the endothelial cells. In order to investigate the effects of anti β2-GPI antibody for the placenta, the effects of anti β2-GPI antibody on the cytokine production in first trimester trophoblast cells were evaluated.
Methods: The first trimester trophoblast cells were cultured in 24-well tissue culture plate.
The cells after incubation with purified β2-GPI were cultured with the IgGs taken from anti β2-GPI antibody positive and negative serum.
Cytokines such as IL-4, IL-6, IL-8, IL-10, TNF-alpha, IFN-gamma and GM-CSF in cultured supernatant were measured by using the suspension array system Bio-Plex® (BIO-RAD) and IL-17 and IL-23 were measured by using ELISA.
Results: Cytokine production such as IL-6 and IL-8 by anti β2-GPI antibody positive IgG increased much more than that by negative IgG in the first trimester trophoblast cells.
Conclusion: We suspect that the anti β2-GPI antibodies may bind to the β2-GPI/phosphatidylserine complex on the surface of first trimester trophoblast cells and that the anti β2-GPI antibodies may increase the cytokine production in these cells.
The increased cytokine production by anti β2-GPI antibody may play a role of increased inflammatory response in placenta.

P2.97

HEAT SHOCK PROTEIN 70 OF PLACENTA AND SERUM BETWEEN PREECLAMPTIC PATIENTS AND NORMOTENSIVE PREGNANT WOMEN

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Objective: The purpose of this study was to measure placental and serum protein 70 in mothers with preeclampsia and to evaluate whether it can be applied as an useful predictor for preeclampsia.
Methods: In a matched pair study, placental and serum levels of hsp70 were measured in 36 patients with mild and severe preeclampsia, and in 36 normotensive controls.
After obtaining informed consent, placental and serum samples were collected from all participants to measure hsp70 levels. The levels of placental hsp70 were measured using Western blotting and serum hsp70 were measured using enzyme-linked immunosorbent assay. Placental hsp70 were stained with Immunohistochemistry.
Results: Measurement of placental and serum hsp70 levels showed statistically higher values among preeclamptic patients compared to control groups. The placental hsp70 levels in normotensive pregnant women and preeclamptic patients were 0.877 and 5.012 volume intensity mm^2 (p=0.000). And the serum hsp70 levels in normotensive pregnant women and preeclamptic patients were 2.161 and 4.272 ng/mL (p=0.057) respectively. The difference of hsp70 on birth before and after 34 weeks in preeclampsia is not significant. Mild and sever preeclampsia are not significant. There are not significant in preeclampsia with IUGR or without IUGR, either. But if we had more samples, the placental hsp70 levels could be significant values in preeclamptic patients between without IUGR and with IUGR.
There are not significant in correlation coefficients between clinical characteristics and laboratory parameters of normotensive pregnant women and preeclamptic patients. There are significant in Immunohistochemistry staining for placental hsp70 in normotensive pregnant women and preeclamptic patients.
Conclusion: Placental hsp70 levels are elevated in pre-eclamptic women and but, serum hsp70 levels are not significant. However, further studies are needed to understand the underlying mechanism for this elevation in the pathogenesis of preeclampsia.

P2.98

AMNIOTIC LAMELLAR BODY COUNTS AND CONGENITAL DIAPHRAGMATIC HERNIA IN HUMANS AND RATS

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Objectives: We examined the extent of fetal lung hypoplasia and lung maturation using amniotic lamellar body count (LBC) in congenital diaphragmatic hernia (CDH).
Methods: We obtained 30 amniotic fluid samples at Cesarean section in human CDH. In normal and nitrofen-induced CDH rat, we collected amniotic fluid and lung tissue of the newborn at E21.
Results: In human CDH, LBC was significantly higher in alive group than in death group ($P < .01$). In rat, LBC was significantly higher in controls than in CDH ($P < .01$). Then, LBC per unit lung weight in controls was similar to that in CDH ($P = .544$) and the expression of ABCA3 was not significantly different in lung tissue ($P = .551$).
Conclusion: LBC can be a useful predictor for hypoplastic lung in human CDH after 35 weeks. Fetal lung maturation does not delay in CDH comparing with controls from human and rat CDH.

P2.99

WHOLE GENOME AMPLIFICATION OF SYNCYTIAL NUCLEAR AGGREGATE DNA - POTENTIAL FOR MINIMALLY-INVASIVE PRENATAL DIAGNOSTICS

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Objectives: Syncytial nuclear aggregates (SNAs) are clusters of syncytiotrophoblast nuclei shed from the placenta and into the maternal blood, and are a potential source of fetal/placental DNA for minimally-invasive prenatal diagnostics. We conducted this investigation to determine if DNA of sufficient quantity and quality for downstream genetic testing procedures was present in SNAs.
Methods: Four first trimester placentae (gestations 12.2, 11.0, 9.2 and 8.4 weeks) were cultured and SNAs harvested using our published *in vitro* model. Nine SNAs from each placenta were individually collected with micromanipulators and subjected to whole genome amplification ($n = 36$ SNA). Post-amplification double stranded DNA quantity was measured with a fluorometer, and the amplification product was subjected to gel electrophoresis to determine DNA quality.
Results: We found a mean of 135 (SE $\pm$120) ng/μL of DNA from single SNAs, with a range from none to a maximum of 372 ng/μL per SNA. Qualitative variation in DNA degradation (as measured by gel electrophoresis) was also observed between individual SNAs. Twenty-five SNA (69%) were considered to have DNA of sufficient quality and quantity for downstream applications (DNA quantity above 50 ng/μL and fragments over 1,000 base pairs), with 13 of the 25 having DNA of high quality (DNA quantity above 150 ng/μL and fragments over 2,000 base pairs).
Conclusion: These results show that a high proportion of SNAs could be a source of fetal genetic material to be applied in genetic procedures such as DNA microarray, digital PCR or massive parallel sequencing; and suggest that SNAs have potential for use in minimally-invasive prenatal diagnostics based on a maternal blood sample.

P2.100

A CASE REPORT OF PLACENTAL HEMANGIOMA

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Placental hemangioma is observed in approximately 1 % of histopathologically examined placentas, but prenatal diagnosis via imaging is extremely rare. When the diameter of the tumor is 5 cm or more, it is known to cause complications including threatened premature labor due to polyhydramnios, or fetal cardiac failure - or hydrops fetalis - due to intratumoral shunting. This report describes a case of suspected placental hemangioma at 30 weeks gestation, for which a Cesarean section was performed at 37 weeks due to polyhydramnios. This procedure resulted in the birth of a healthy infant.
The patient was a 35-year-old pregnant woman with no notable family or previous medical history.
A placental tumor was suspected during the prenatal examination at 30-weeks and the patient was referred to our hospital for consultation.
Ultrasound examination at our hospital revealed a solid tumor with a diameter exceeding 6 cm peripheral to the normal fetus which included the umbilical cord. Color Doppler revealed abundant internal blood flow.
Plain magnetic resonance imaging also suggested placental hemangioma.
Due to an amniotic fluid index of 32cm, polyhydramnios was suspected.
While no subsequent abnormal signs were observed in the fetus or the placenta, the patient was admitted due to advancing polyhydramnios.
Following admission, the patient was administered ritodrine hydrochloride, underwent weekly amniocentesis and was put under observation as she showed signs of a threatened premature labor.
Breech presentation led to the decision to deliver via Cesarean section at 37 weeks.
The infant was a female weighing 2746 g and with no apparent abnormalities.
A histopathological examination of the expelled placenta resulted in diagnosis of a capillary angioma.
As placental hemangioma is by no means a rare disease, it is essential to pay sufficient attention to the shape of the placenta from the early stages of pregnancy.

P2.101

EMBRYO EUTHANASIA AND AMNIOTIC FLUID LEAKAGE INDUCES UNK CELLS CYTOTOXIC ACTIVITY

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Objectives: Human and mice uNK cells express immunoglobulin-like receptor (KIR-human and Ly49-mouse) and c-lectin-like receptor (NKG2-human and mouse) activating receptors with high affinity to MHC-I molecules and also cytoplasmic granules containing cytolytic proteins that predict their innate immune type cytotoxic response. The present work used the mouse in utero-embryo euthanasia and intra-uterine amniotic fluid inoculation to evaluate the triggering of uNK cell cytotoxic activity.
Methods: Twenty percent of embryos in the pregnant mice uteri at gestational days (gd) 9 and 12 were surgically euthanized and after 0.5, 1 and 6hr, uterine samples from both surgical embryo euthanized (SEE) and non-manipulated were collected for morphological and immunocytochemical analysis. Tissue homogenates from embryo tissue free mesometrium were collected and processed for protein and RNA extractions. Amniotic fluid (AF) were collected from normal embryo developing sites at gd12 and inoculated (10μL) into the inter-implantation sites of pregnant uterus at gd 9 and12 and the uterine samples collected in the same way of SEE.
Results: Both SEE and non-manipulated sites showed decreasing of uNK cell perforin, granzymeA contents by immunostain, as soon as 30 min after SEE with loosing of secretory-lysosome granules contents at ultrastructural analysis, suggesting the releasing of cytolytic contents from the granules. Similar time-dependent uNK cell morphological changes and granule contents loosing were confirmed after AF inoculation confirming AF soluble contents, rather than the embryo death or trophoblast driven factors triggers the uNK cell. PCR analysis showed increasing of TNFα, IFNγ and IL18 pro-inflammatory cytokines, and perforin and granzymeA cytolytic proteins transcripts, all of them produced by uNK cells.
Conclusions: Taken all together, uNK cell is a sensitive sentinel of maternal-fetal interface ready responsive cytotoxic effectors cells that can be triggered in abnormal pregnancy. Which component from the AF is capable to stimulate such a response of uNK cells is the next challenge.

P2.102

A HIGH THROUGHPUT *IN VITRO* MODEL OF HUMAN EMBRYO ATTACHMENT

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Objectives: Currently used *in vitro* models of human embryo attachment involve co-culture of a monolayer of human endometrial epithelial cells (to mimic the uterine epithelium) and trophoblast spheroids (to mimic blastocysts). However, these are technically demanding and time consuming as spheroids are selected microscopically and their attachment is quantified by manual counting. These assays are also highly variable and not suitable for high throughput studies. The aim of this study was to establish a high throughput attachment assay that is simple and can be performed in most laboratories.
Methods: We made the following significant modifications to the existing model: label trophoblast spheroids with green fluorescence, select spheroids of size similar to implanting blastocysts using cell strainers, and assess spheroid attachment using an automated microplate reader.
Results: Human trophoblast BeWo spheroids were prepared by rocking cell suspension for 24h. They were then labeled with Calcein and filtered sequentially through cell strainers (100 um and 70 um) to select spheroids with sizes similar to implanting human blastocysts. The uniformly-sized and fluorescently-labeled spheroids were then incubated with a monolayer of endometrial epithelial cells. The fluorescence signals from spheroids that were initially seeded and from those that attached following the incubation were quantified respectively by a microplate reader, and the percentage of attachment was calculated. The assay was validated by testing human endometrial epithelial cells of different receptivity (receptive: RL95-2; nonreceptive: AN3-CA), and by applying a known attachment inhibitor (to RL95-2) or enhancer (to AN3-CA). Our results demonstrate that this high throughput assay is efficient and reproducible to assess attachment under different experimental conditions.
Conclusion: We describe a new and high throughput assay for the study of human embryo attachment. This assay will also permit high throughput screening of factors/drugs affecting embryo attachment.

P2.103

EVTS STIMULATE AND ENHANCE THE DECIDUALIZATION OF HUMAN ENDOMETRIAL STROMAL CELLS

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Inadequate implantation and placentation during the establishment of pregnancy is thought to lead to obstetric complications. To create the placental blood supply, extravillous trophoblast (EVT) invade through the decidua to engraft and remodel uterine spiral arteries. Impaired decidualization is associated with recurrent miscarriage, preeclampsia and placenta accrete.

Objectives: To determine whether EVT secreted factors induce or enhance human endometrial stromal cell (HESC) decidualization.
Methods: Isolated primary 1st trimester EVT (pooled n=5), HTR8SVneo (HTR8, EVT cell line) and HEK293 (control cell line) cells were cultured on growth-factor reduced matrigel. Conditioned media (CM) from the 3 groups was collected every 24h. Isolated human endometrial stromal cells were decidualized by treatment with estradiol (E, $5x10^{-9}$M), or E+medroxyprogesterone acetate (MPA, $5x10^{-8}$M) for 7 days after which CM from either of the 3 groups was added for a further 6 days (n=3). Prolactin secretion (decidualization marker) by HESC was measured every 48h. EVT CM was assayed for progesterone and used for proteomic analysis.
Results: HESCs treated with E alone, or E+HTR8 or HEK293 CM did not secrete detectable levels of prolactin. HESC treated with EVT CM induced prolactin secretion (0.037+0.002 mIU/mg) demonstrating it stimulated decidualization. By comparison HESC treated with E+MPA (decidualization control) secreted prolactin at 0.026+0.004mIU/mg. HESC treated with EVT CM and E+MPA enhanced prolactin secretion 3-fold compared to decidualization control E+MPA alone and HEK293+E+MPA: (EVT+E+MPA: 0.09+0.02 vs E+MPA: 0.026+0.004 mIU/mg; vs HEK293 0.028+0.019mIU/mg, $p<0.05$) and 2-fold compared to HTR8 (0.042+0.018mIU/mg, $p<0.05$). EVTs secreted low levels of progesterone. Proteomics analysis identified 10 factors secreted by EVTs which have previously been associated with decidualization.
Conclusion: The data demonstrated that EVT secreted factors induced and enhanced decidualization and suggested EVT secreted factors that synergised with progesterone to promote decidualization. We identified EVT proteins that are associated with decidualization and studies are underway to investigate their functional role.

P2.104

PC6 IS CRITICAL FOR ENDOMETRIAL RECEPTIVITY IN WOMEN: MODULATING SCAFFOLDING PROTEINS AND MEMBRANE-CYTOSKELETAL INTERACTIONS

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Objectives: Establishment of endometrial receptivity involves dramatic structural changes in the plasma membrane and cytoskeleton. However, the molecular regulations governing fundamental cellular changes for receptivity are not well understood. Proprotein convertase 5/6 (PC6), a serine protease of the proprotein convertase (PC) family, is up-regulated in the human endometrium specifically at the time of receptivity. The current study aimed to address the importance and mechanisms of PC6 action in regulating endometrial receptivity in women.
Methods: Endometrial epithelial PC6 levels were determined by immunohistochemistry in fertile and infertile women. An ex vivo model of embryo attachment, in combination with siRNA knockdown, was applied to establish the importance of PC6 in receptivity. Proteomics was used to identify the mechanisms of PC6 action.
Results: PC6 was dys-regulated in the endometrial epithelium during the window of implantation in infertile women of three demographically different cohorts. The critical role of PC6 in receptivity was evidenced by a significant reduction in mouse blastocyst attachment of endometrial epithelial cells following PC6 knockdown by siRNA. Using a proteomic approach, we discovered that PC6 cleaved the key scaffolding protein EBP50. This cleavage profoundly affected the cytoskeleton-membrane interactions causing significant cytoskeletal reorganization: following PC6 cleavage, EBP50 dissociates from its binding protein ezrin (a key protein bridging actin filaments and plasma membrane), triggering membrane de-localization of EBP50/ezrin and cytoskeleton-membrane dissociation. We further validated this novel PC6 regulation of endometrial receptivity in fertile vs infertile women.
Conclusion: Our study demonstrates that PC6 plays a critical role for embryo implantation in women. PC6 cleaves a key scaffolding protein EBP50 thereby regulating fundamental cellular remodelling processes required for receptivity. Our discovery of PC6 cleavage of EBP50 to profoundly regulate membrane-cytoskeletal reorganization, greatly extends the current knowledge and provides substantial new mechanistic insight into the fields of reproduction, basic cellular biology and PC biochemistry.

P2.105

THE ROLE OF CAMP-MEDIATED EPAC SIGNALLING PATHWAY IN DECIDUALIZATION OF HUMAN ENDOMETRIAL STROMAL CELLS

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Objectives: Decidualization of human endometrial stromal cells (ESCs) is accompanied by production of decidual markers, IGF binding protein-1 (IGFBP-1) and prolactin (PRL) as well as morphological changes. The protein kinase A (PKA)-mediated cAMP signalling is well known to be crucial for decidualization. However, the involvement of Exchange protein directly activated cAMP (Epac)-mediated cAMP signalling in decidualization has not been so far elucidated. We examined here the spatiotemporal expression of Epac in endometrium during the menstrual cycle and the role of Epac in decidualization of ESCs.
Methods: Epac1 and Epac2 expressions in human endometrium were examined by immunohistochemistry. Cultured ESCs were treated with Epac-selective agonist (8-CPT-2-OMe-cAMP:CPT) in the presence of PKA-selective agonist (N^6-Phe-cAMP:Phe) or both progesterone and estradiol (P4/E2). The expression of IGFBP-1 or PRL was analyzed by real-time RT-PCR and ELISA. The activation of Rap1, a putative mediator of Epac signalling, were detected by pull-down assay. Effects of siRNA-based knockdown of Epac1, Epac2 or Rap1 on decidualization were examined.
Results: Epac1 and Epac2 were expressed in glandular epithelium and stroma in the endometrium of the proliferative and secretory phases. CPT alone affected neither IGFBP-1 nor PRL expression in ESCs. However, CPT enhanced Phe-induced IGFBP-1 or PRL expression. CPT activated Rap1 in Phe-treated decidualizing ESCs. Knocking down Epac1, Epac2 or Rap1 inhibited the cAMP analog-induced IGFBP-1 and PRL expressions. Furthermore, CPT stimulated P4/E2-induced IGFBP-1 expression and increased the number of large- and round-shaped ESCs, whereas the knockdown of Epac1, Epac2 or Rap1 repressed these changes.
Conclusion: These results suggest that Epac/Rap1 signalling may be involved in ovarian steroids-induced decidualization of endometrial stromal cells in human endometrium.

P2.106

A CASE OF WOLF-HIRSCHHORN SYNDROME PRENATALLY CONFIRMED BY FLUORESCENCE IN SITU HYBRIDIZATION

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Wolf-Hirschhorn syndrome (WHS) is defined by a collection of core characteristics that include mental retardation, epilepsy, growth delay, and craniofacial dysgenesis. The disorder is caused by a hemizygous deletion of the short arm of chromosome 4, called the WHS critical region (WHSC). And the WHSC1 gene is located in this region, and its loss is believed to be responsible for a number of WHS characteristics.
Case Report: A 36-year-old gravida five, para two woman was referred to the hospital at 11 weeks of gestation for aminocentesis because of nuchal translucency thickness of 3.9mm. She has already had three times abortions. Amniocentesis was performed at 17 weeks of gestation, and cytogenetic studies showed that the fetal karyotype was 46,XY,add(4) (p15.2). Furthermore, fluorescence in situ hybridization study was added, and it showed the deletion of the 4p subtelomeric region(GS-36P21) and WHSC1 region. After a genetic counseling, the parents decided to terminate the pregnancy. A 256g fetus was delivered with growth delay and its appearance was normal. Cytogenetic studies were done to examine whether the parents had a translocation. The maternal karyotype was 46,XX,t(1;4)(p36.3;p15.3). The paternal karyotype was normal. Thus, we could know that the final fetal karyotype was 46,XY,der(4) t(1;4)(p36.3;p15.3)mat. ish der(4) t(1;4)(p36.3;p15.3)(GS-36P21-,WHSC1-).
Conclusion: We report a case of WHS that it is prenatally confirmed by fluorescence in situ hybridization in amniocentesis.

P2.107

TWO DIFFICULT CASES FOR GENETIC COUNSELING IN AMNIOCENTESIS

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Case Report: We recently encountered two cases where two different types of mosaics were found on amniocentesis, causing difficulties in genetic counseling. In a first case, the patient was a 38-year-old woman. Because of advanced maternal age, she underwent an amniocentesis at 16 weeks of gestation. The test revealed mosaic (46,XX/47,XX,+18). Precisely described, one of the 3 flasks used for cell culture was rated as showing level II mosaic (normal karyotype 46,XX, for 21 of the 47 cells, and karyotype 47,XX,+18, for 26 cells). Because this result highly probably represented an artifact, the test was conducted again. In the re-test with *in situ* method, all colonies were rated as having normal karyotype. The final judgment on amniocentesis results was normal karyotype 46,XX. In a second case, the patient was a 41-year-old woman. Because of advanced maternal age, she underwent an amniocentesis at 16 weeks of gestation. The test revealed mosaic 45,X/46,XX (a karyotype 45,X, for 15 of the 66 cells, and normal karyotype 46,XX, for 51 cells). In this case, it was considered that the child's true karyotype would be 45X, 45,X/46,XX, or 46,XX, and the phenotype would be different by each karyotype. The born child was 2780g of weights, and the Apgar score was 9, and then, no abnormality was found at the phenotype. Also, the exam of echocardiography was normal. The result of child's karyotype test was mosaic 45,X/46,XX (a karyotype 45,X, for 7 of the 20 cells, and normal karyotype 46,XX, for 13 cells).
Conclusion: Each cases, we needed several times of genetic counseling to get understanding about the results. So, in such mosaic cases, genetic counseling before and after amniocentesis is quite important.

P2.108

THE ROLE OF PLACENTA PATHOLOGY IN RECURRENT PREGNANCY LOSSES

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Purpose: As part of the examination into recurrent pregnancy losses, a blood test, villous pathologic examination, the examination of the uterus, of the villous chromosome and of the mother's and father's chromosomes were performed. All testing is important for treatment, but the villi causing the recurrent pregnancy losses are often found to show abnormality. I examined the villi in single miscarriage as well as the examination villi in recurrent pregnancy losses, the villi in both cases determines what kind of problems there are mostly exist and this information may be able to help in future clinical treatment.
Method: I performed pathologic studies of the villi in 10 recurrent pregnancy losses and 30 recent miscarriages.
Results:

1. The placenta villi which showed chromosomal aberration was large, many had variant forms, was strong and showed trophoblast islands, so-called dysmature villi. As for the pathologic examination of the miscarriage villi and the recurrent pregnancy losses, dysmature villi were high rate.
2. There were cases of extensive deposition of fibrin to decidua, and severe fibrin deposition around villous trophoblast.
3. There were also cases of repeat intrauterine infection.

Discussion: The gene abnormality in the villi as well as chromosome aberration, led to anomaly, dysmature villi and recurrent pregnancy losses. Coagulation disorder that was not proved hematologically was found in the extensive fibrin deposition around the decidua and the villi. In these cases, anticoagulation therapy helps produce satisfactory outcome. Repeat intrauterine infection has increased dramatically recently, and the epidemiological investigation will be necessary in future, in order to find the cause of infection.

P2.109

THE LONG NON-CODING RNA ASSOCIATED WITH THE HELLP-SYNDROME IS EXPRESSED IN THE EXTRAVILLOUS TROPHOBLAST AND FUNCTIONS IN THE CELL CYCLE

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Objective: The pregnancy-related HELLP- syndrome leading to Hemolysis, Elevated Liver enzymes and Low Platelet count is linked to the 12q23 chromosomal region, a gene desert flanked by *PMCH* and *IGF1*, both transcribed from the Crick (-) strand. Initial work in this gene desert showed Watson (+) strand-specific transcription with the 5'end overlapping exon 1 of *PMCH*, and without open reading frames to form a protein, supporting the existence of a long non-coding RNA within the HELLP linkage region.
Methods: To analyze the 3'end of the transcript, RACE (Rapid Amplification of cDNA Ends) experiments were carried out on total RNA isolated from an extravillous trophoblast cell line. The exact location of the ncRNA transcript within the placenta was determined by Fluorescent *in situ* Hybridization (FISH). siRNA-mediated knockdown of the transcript was performed followed by confirmation using semi-quantitative and quantitative RT-PCR. Samples with confirmed knockdown were subsequently used in whole genome RNA-sequencing.
Results: The 3'end was found to be located within the 3'UTR of *IGF1*, yielding a non-coding RNA with a length of 205kb. FISH experiments showed nuclear and perinuclear localization within the extravillous trophoblast cell line. Placental sections showed predominant localization of the ncRNA transcript within the extravillous trophoblast columns.
Whole genome RNA-sequencing on the siRNA-mediated knockdown samples has yielded an extensive set of data which, when analyzed by Ingenuity Pathway Analysis (IPA), yielded top ranked networks and functions related to the cell cycle.
Conclusions: The identified long non-coding RNA associated with the HELLP syndrome is primarily localized in extravillous trophoblasts. This, along with IPA data obtained from whole genome RNA sequencing, will be helpful to further investigate the exact function of the long non-coding RNA and its dysfunction in the HELLP syndrome.

P2.110

DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING OF THE PLACENTA IN PREGNANCIES COMPLICATED BY SMALL FOR GESTATIONAL AGE OR MORBIDLY ADHERENT PLACENTA

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Objectives: To evaluate diffusion-weighted magnetic resonance imaging (MRI) of the human placenta in normal pregnancies and those complicated by morbidly adherent placenta (MAP) or small for gestational age (SGA).
Methods: In this prospective observational study, we evaluate 1,5 T fetal MRI's. The local ethics committee approved the study. All patients were recruited from our foetal medicine unit. The apparent diffusion coefficient (ADC) of the whole placenta was calculated on diffusion-weighted MR images.
First were elaborated "norm" values in analysing control cases where MRI was realized in normal growing fetuses presenting an organic malformation like neural-tube defects or others. The placental function was supposed normal in these cases.
Then the placentas of MAP cases, fulfilling the ultrasound criteria's for placenta accreta, were analysed.
Finally, placentas of SGA foetuses, were biometries at ultrasound were below the P10, were studied.
MRI-Data were correlated to the ultrasound and the post-natal findings.
Results: In total, 48 pregnancies were included. The mean gestational age at MRI was 30 weeks of amenorrhea (range 18-36) and the mean ADC for the whole group was 1.9×10^{-3} mm^2/sec (range 1.0-2.9).
The mean ADC of controls (n=17) and MAP (n=5) were with 2.1 and 2.0×10^{-3} mm^2/sec in the same range. The SGA group (n=26) could be divided in two subgroups (13 with and 13 without placental insufficiency, based on ultrasound, neonatal and pathological information). The mean ADC was significantly different in these two groups (2.1 versus 1.5×10^{-3} mm^2/sec, $p<0.0001$).
Conclusions: SGA related to placental dysfunction is associated in MRI with restricted diffusion and reduced ADC compared to control or MAP placentas. Decreased placental ADC might be a sign of altered function in cases of intrauterine growth restriction (IUGR) caused by placental insufficiency.

P2.111

A CASE OF VASA PREVIA (TYPE 2)

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Vasa previa is an uncommon condition in which fetal vessels traverse the lower uterine segment in advance of the fetal presenting part. There are the two types of vasa previa; type 1 results from velamentous cord insertion and type 2 from vessels running between lobes of a bi-lobed or succenturiate lobed placenta. Since the vessels are not protected by either the umbilical cord or the placenta, they are prone to be compressed during labor and may tear as a result of the rupture of the membrane. Because rupture of vasa previa results in fetal exsanguination, hemorrhagic shock and death, the prognosis of fetus is poor when vasa previa is not diagnosed prenatally. We report a case of 32-year-old woman who is diagnosed of vasa previa (type 2) prenatally. She was referred to our hospital at 30 weeks of pregnancy because she had low-lying placenta and slight vaginal bleeding was observed. Transabdominal and trasvaginal ultrasound examination showed a horseshoe-shaped placenta nearly enclosed the inner cervical os, marginal placental venous sinus and vessels crossing over the inner cervical os. She was diagnosed as type 2 vasa previa and admitted to our hospital. Because uterine contraction increased, in spite of tocolysis, elective cesarean section was performed at 34 weeks of pregnancy. A vertical incision on the uterine body was used in order to avoid cutting the placenta, vasa previa and fetal vessels. The post operative examination revealed the horseshoe-shaped placenta and large vessels running on the extraplacental membrane between the both ends of the placenta crossing over the internal cervical os. The postpartum course of her and the infant was uneventful.

P2.112

CAN ULTRASONOGRAPHIC MEASUREMENT OF THE MAJOR AXIS OF THE PLACENTA AT 20-21 WEEKS OF ' GESTATION PREDICT FETAL GROWTH RESTRICTION ?

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Objective: Fetal growth depends on the placental size. An abnormally decreased placental weight has been linked to increased perinatal complications, including fetal growth restriction (FGR). However, there is no standardized method for measuring a placenta prenatally using ultrasonography. We herein propose a novel method using two-dimensional ultrasonography to predict fetal growth restriction from the diameter of the placenta at 20-21 weeks of gestation.
Methods: We performed a prospective study to measure the placental diameter in 471 pregnancies at 20-21 weeks of gestation.
Results: The relationship between the placental diameter and the birth weight was analyzed using a linear regression analysis. There was a direct correlation (R^2=0.416, P<0.001) between the placental diameter and the birth weight of the infants. The average placental diameter of LFD cases (n=29) was 10.4±0.6cm, while the average placental diameter of non-LFD cases (n=442) was 12.4±1.0cm. It is therefore possible to predict the FGR based on the placental diameter at 20-21 weeks of gestation. Using a ROC curve, the cutoff value for the placental diameter was set at 11.2cm. The sensitivity and specificity of the test were 97% and 88%, respectively. The positive predictive value and negative predictive value of the test were 35% and 99%, respectively. The risk of the endpoint was significantly higher in the LFD cases than in the non-LFD cases (incidence risk ratio: 134.8, 95% confidence interval: 18.6 to 976.7).
Conclusion: The measurement of the placental diameter as a screening method for the detection of FGR appears to be useful.

P2.113

A CASE OF LARGE PLACENTAL CHORIOANGIOMA USING THE TEI INDEX IN FETAL RIGHT VENTRICLE TO EVALUATE CARDIAC FUNCTION – COMPARISON WITH SIX RECIPIENT FETUSES WITH TWIN TO TWIN TRANSFUSION SYNDROME –

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Objectives: Placental chorioangioma is the most common benign tumor of placenta, found in approximately 1% of all pregnancies. Large chorioangioma is often associated with fetal complications including prematurity, heart failure and intrauterine death. Evaluation of the ventricular function with fetal heart failure is important to determine the perinatal management. The purpose of this study was to evaluate right ventricular function of fetus using the Tei index measured by Doppler echocardiography.
Methods: A control group consisted of 60 fetuses in normal pregnancies. The study group consisted of one fetus with large placental chorioangioma at 28 weeks, and six recipient fetuses with twin to twin transfusion syndrome. The Tei index in the right ventricle and Aorta Vmax were measured prenatally with the Aloka SSD 5000 ultrasonographic system.
Results: The Tei index for the control fetuses was 0.5 or less and had no correlation to gestational weeks. Large placental chorioangioma was measured 10 cm in diameter. Aorta Vmax were high values, but the Tei indices were within normal range. The new born infant was unremarkable without heart failure. For six recipient fetuses, amnioreduction was done in case numbers 1, 4, and 5, and fetoscopic laser photocoagulation (FLP) was performed in case number 6. In case numbers 2, 4, and 6, fetuses had high Tei indices. In case numbers 2, the neonate was treated for heart failure . In case number 4, the Tei indices was elevated over 1.0, Aorta Vmax fell below 20cm/sec, and the fetus died in utero at 24 weeks. In case number 6, after FLP the Tei indices were within normal limits, and the infant had an unremarkable postnatal course.
Conclusion: In this study, fetuses with right heart failure had high Tei indices. These results suggest that the Tei index may prove useful in assessing fetal right ventricular function.

P2.114

THE MATERNAL-TO-FETAL KINETIC ANALYSIS OF FRACTALKINE/CX3CL1

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Fractalkine is a CX3C chemokine that has chemoattractant activity for T cells, monocytes and natural killer (NK) cells. We have previously demonstrated that fractalkine protein was detected as a 95kDa band in both the amniotic fluid and the amnion during the second and third trimesters. Immunohistochemistry using an anti fractalkine polyclonal antibody revealed positive staining of epithelial cells in amnion and trophoblasts in both the second and third trimesters. RT–PCR detected fractalkine mRNA transcripts in the amnion. Neonatal urine also contained detectable amounts of fractalkine. CX3CR1 positive cells had migrated into the amniotic fluid and the amnion. Preterm labor and chorioamnionitis are strongly associated with chemokine network. In the present study, we demonstrate the association between maternal/neonatal fractalkine levels and preterm labor/chiriosomnionitis.
Materials and Methods: Samples of maternal and umbilical blood, placenta and membranes were collected. We assayed fractalkine in maternal sera before delivery and umbilical sera by ELISA. And we analyzed the expressions of fractalkine and its receptor in placenta and membranes by Western Blotting. We excluded the cases complicated with intrauterine fetal death, multiple pregnancies, fetal anomalies and abortion.
Results: We evaluated 151 deliveries. Fractalkine concentration in maternal sera had no significant differences between term and preterm deliveries. Fractalkine concentration in maternal sera had elevated in cases with histological chorioamnionitis at term. Fractalkine concentrations in umbilical sera were significantly higher those of maternal sera. The expression of fractalkine receptor in placenta was positively correlated with that in the membranes, and the expressions had no significant differences in the period of deliveries and chorioamnionitis. The expression of fractalkine in placenta was positively correlated with membranes, and the expressions of placenta in term deliveries had elevated compared to preterm deliveries.
Conclusion: Fractalkine was continually present in maternal sera. Fractalkine in umbilical sera might have important roles in immunological regulation.

P2.115

DRAMATIC ALTERATIONS IN MICROTUBULE NUCLEATION IN FUSED BEWO CELLS

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Objective: The formation of syncytia from mononuclear cells leads to a number of changes in cellular structure. We focus on alterations to the microtubule cytoskeleton in fused BeWo cells.
Methods: BeWo cells were used as surrogates for cytotrophoblasts and were induced to fuse by treatment with forskolin (FK). BeWo cells were treated with FK for 48 hours to induce cell fusion; this was followed by treatment with nocodazole to induce microtubule depolymerization and wash out of the drug to induce subsequent microtubule re-growth. Antibodies to α-tubulin, γ-tubulin, and pericentrin were used in immunofluorescence experiments to monitor microtubule organization.
Results: In both control (non-fused) and FK-treated (fused) cells microtubule depolymerization was observed following 30 minute exposure to ice-cold nocodazole. In both groups of cells, re-growth of microtubules was found soon after wash out of nocodazole and return to 37C. However, microtubule re-growth was different in the two types of cells. In controls, re-growth was centered at the centrosome and radiating outward like spokes of a wheel. In fused cells, the re-growth did not originate from single sites but appeared to be nucleated from broad areas that had increased labeling for pericentrin.
Conclusions: We show a dramatic alteration in the amount of and distribution of pericentrin in fused BeWo cells compared to mononuclear cells. Moreover, these expanded areas of pericentrin serve as sites for microtubule nucleation.

P2.116

IGFBP-3 ENHANCES IGF-II CELL SIGNALLING THROUGH ACTIVATION OF SPHINGOSINE KINASE AND S1P PRODUCTION IN HUMAN EXTRAVILLOUS TROPHOBLAST CELLS

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Introduction: IGF system plays an important role in placental development and fetal growth. It is well accepted that IGFBP-3 forms a complex with IGF-II thus preventing free IGF-II from accessing its receptor and activating cell signaling. However, in other cell types, IGFBP-3 has also been shown to positively regulate IGF cell signaling by up-regulating sphingosine kinase expression. Whether this may also occur in trophoblast cells is unknown.
Material and Results: Our study shows that human extravillous trophoblast cells (HTR8/SVneo cells) secrete IGFBPs, including IGFBP-3, and IGF-II. Pre-treatment of HTR8/SVneo cells with low concentrations of IGFBP3 enhances IGF-II-induced cell signaling as indicated by increases in phosphorylation of AKT and ERK. This also translated into increased trophoblast migration. In contrast, when cells are treated with higher dosages of IGFBP-3 and IGF-II, IGF-II cell signaling is inhibited. Co-treatment of IGFBP-3 and sphingosine induces ERK phosphorylation, an effect mediated by sphingosine phosphate (S1P, the product of sphingosine kinase). This effect is attenuated by DMS (sphingosine kinase inhibitor). Furthermore, low dose IGFBP-3 treatment for 1 hour increases sphingosine kinase protein content and its phosphorylation in the membrane fraction of trophoblast cells without affecting its total protein level, indicating IGFBP3 induces sphingosine kinase activation. Finally, we also observed that S1P itself enhances IGF-II-mediated AKT and ERK phosphorylation as well as IGF-II-induced IGF1R phosphorylation. \
Conclusion: At low concentrations, IGFBP3 enhances IGF-II-mediated signalling and cell migration by activating sphingosine kinase and S1P production in human extravillous trophoblast cells. This suggests the tight regulation of IGFBP-3 expression is important in the regulation of IGF-II –related functions, including trophoblast migration.

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FURIN GENE (FUR) REGULATION IN DIFFERENTIATING HUMAN TROPHOBLAST CELLS: INVOLVEMENT OF CREB IN REGULATING FUR P1 PROMOTER

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Furin promotes trophoblast cell fusion and both mRNAs and proteins of furin are upregulated during syncytialization of human trophoblast cells. Three promoters (P1, P1A and P1B) are reported to mediate transcriptional activation of furin. However, the exact role of these promoters and the transcriptional regulation of furin gene during syncytializaiton are largely unknown.

Objectives: The objective of this study is to uncover the mechanism of transcriptional regulation of furin in human trophoblast syncytialization.
Methods and Results: Furin mRNA level was increased during fusion of both BeWo carcinoma cells and primary term cytotrophoblast cells, and blocking PKA signalling pathway dramatically decreased the expression of furin mRNA during trophoblast cell fusion. Several potential CRE-sites were identified in human furin promoters region, and luciferase reporter studies led to the identification of CREB, a bZIP-type protein, stimulating the transcription of furin in BeWo cells. By performing EMSA assay, we found that CREB binds on the exact binding sites of furin P1 promoter. Finally, we showed that CREB mediated furin activation was critical during trophoblast cell fusion process.
Conclusion: CREB-dependent stimulation of the P1 promoter of furin is of great significance in furin transcriptional activation during human trophoblast syncytialization.