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Abstracts for the Forthcoming European Placenta Group Meeting (11th IFPA Meeting) in Glasgow

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In considering the diversity of Eutherian mammalian placental structure, it is helpful to keep in mind that both phylogenetically and ontogenetically a functional yolk sac placenta precedes development of the choioallantoic placenta. Usually the choioallantoic placenta progressively displaces the area of contact of the yolk sac placenta with the endometrium. It is also closely applied to the endometrium, increasing respiratory efficiency but tending to decrease histotrophic nutrition. Carcarnine primates have minimal histotrophic uptake, using hemotropic mechanisms throughout most of gestation. Rodents, by using partial or complete inversion of the yolk sac, have extensive regions available to histotrophy in later pregnancy. Most mammals, however, have developed specialized regions of the choioal- lantoic placenta for ingestion of uterine secretions, cell debris, and erythrocytes. These regions, such as areolae and hemophagous areas, are consistently lined by columnar cellular trophoblast across an extraordinarily wide group of species including not only those with an epitheliocorial placenta but also species with endotheliocorial and hemochorial placentas. Restricting phagocytosis to regions of columnar cells provides cells appropriately polarized for ingestion, breakdown and transport, and limits the ingestion of both beneficial and potentially toxic materials to expendable individual cells.

Placental hemodynamics during development in mice

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Deficient placental perfusion underlies common complications of human pregnancy and is associated with elevated Doppler resistance indices in the uterine and umbilical circulations. Genetically-altered mice are providing new models to explore the etiology of abnormalities in placental hemodynamics. We have developed a method to quantify placental hemodynamics in mice and thereby increase the benefit of these new models. We are using ultrasound biomicroscopy (30–40 MHz) to non-invasively image and record Doppler blood velocity waveforms in the uterine artery and intraplacental arterial canals of the mother, and the yolk sac and umbilical circulations of the embryo in normal pregnancy and in mouse models of intrauterine growth restriction (IUGR). Peak (PSV) and end-diastolic velocities (EDV) in the uterine artery increase and uterine arterial resistance index (RI = (PSV-EDV)/PSV) decreases progressively from the day the heart starts to beat (E8.5) to term (E18.5) whereas PSV in the vitelline artery to the yolk sac increases until E13.5, and then remains stable. In the umbilical artery, EDV increases from zero to become detectable in nearly all embryos at E18.5. Umbilical waveforms are similar to those observed in first trimester human pregnancy. As in human IUGR, we found elevated resistance indices in the umbilical and uterine arteries of two mouse models of IUGR; eNOS knockouts, and transgenics with perinatal undernutrition. Knockout of the placental-specific transcript (P0) of the insulin-like growth factor 2 (igf2) gene in the mouse conceptus results in placentas which are reduced in size (at E19 placental weight is reduced by the same amount but fetuses are normal size at this gestation). There is a clear association between the activity of several nutrient transporters in the human placenta and fetal growth. We have used mouse and human models of altered fetal growth to understand cause and effect relationships and to investigate the genetic and hormonal signals underlying them. Knockout of the placental-specific transcript (P0) of the insulin-like growth factor 2 (igf2) gene in the mouse conceptus results in placentas which are reduced in size compared to wild type (wt) by about 29% at E16 (term = E20) but the fetuses are normal size at this gestation. At E19 placental weight is reduced by the same amount but fetuses are now also growth restricted. Maternofetal transport of glucose and methylaminosobutyric acid (MeAIB, amino acid analogue, System A transporter substrate) is increased per g placenta at E16 in the P0 knockout mice and the Slc 38al, a2 and a4 isoforms of the System A transporter gene (Slc 38al, a2 and a4) are expressed in first trimester and term: SNAT4 protein expression at term is twice that in first trimester. These data show that placental transport can be upregulated in relation to fetal demand through transcriptional regulation of specific transporter genes. In the mouse, compromised placental amino acid transport can cause fetal growth restriction. Funded by The Wellcome Trust and BBSRC.
circulation of the mouse placenta. This enabled us to measure unidirectional maternofetal placental weight at 17.5d). Fetal blood $[Ca^{2+}]$ was measured using an ion sensitive electrode. Determination of the level of expression to increase in association with labour. Current studies are focusing on determining which of the TLR receptors are active in both term and preterm labour. We are currently exploring the expression of TLRs 1- 7 and that there is a tendency for the expression to increase in association with labour. Current studies are focusing on determining which of the TLR receptors are active in both term and preterm labour.

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Activation of hypoxialmic-pituitary-adenal (HPA) function occurs in the fetus in association with the onset of parturition at full term and in many instances of preterm birth. Fetal HPA activation occurs in human pregnancy in response to stressors and contributes to the onset of preterm labour. We have suggested that placentrial CRH in human pregnancy may be analogous to placental PGE2 in the sheep, and that it may have a prolonged effect with both NF-κB activity increases in human myometrium with the onset of labour. In the amnion, NF-κB plays an important role in regulating the expression of inflammatory cytokines and chemokines such as IL-1b, or other factors may activate NF-κB.Whilst it is possible that IL-1b or other factors may activate NF-κB, it appears that proinflammatory cytokines provoke co-ordinated upregulation of several genes in the PG biosynthetic pathways, and coincident increases in stimulatory PG receptor levels in intrauterine tissues. Recently, we found that chorion contains 1b-hydroxysteroid dehydrogenase-1 activity, and converts inactive cortisone locally to cortisol. This observation suggested a potential further local source of cortisol that drives a series of feed-forward cascades leading to birth. We now believe that in women, the maternal progesterone levels do not decline in normal gestation as a means of maintaining relaxation in the lower uterine segment, but that local progesterone withdrawal occurs in the fundal region of the uterus in response to co-repressors such as PSF that block progesterone transactivation. Cytokines promote PSF output, suggesting a further link between fetal HPA activation, placental function and local control mechanisms in the birth process. Supported by the Canadian Institutes for Health Research (CIHR)

MECHANISMS OF PRETERM LABOUR

John RG Challis
Vice-President Research and Associate Provost
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There is a strong association between early preterm labou and chorionamnionitis. In the majority of cases it is probable that infection is ascending from the vagina and that chorionamnionitis begins in the lower pole of the uterus before spreading to the upper segment and then ultimately to the fetus itself. The onset of labour, both at term and preterm, requires changes in the biochemistry of the uterus to convert the cervix from a ripe soft structure which will dilate and induce fundally dominant contractions. Changes in the lower segment of the uterus include upregulation of prostaglandin synthesis via COX-2 and of inflammatory cytokines and chemokines such as IL-1b, IL-6 and IL-8. These changes, especially in the fetal membranes occur in the lower segment before they occur in the upper segment. Increased contractility in the myometrium is associated with an increase in the expression of contraction associated proteins including gap junctions and oxytocin receptor. Over the past five years we have demonstrated that the transcription factor Nuclear Factor kappa-B plays an important role in regulating the expression of proteins associated with the onset of labour. NF-xB is central to the expression of COX-2 and IL-8 in the amnion and also appears to down regulate prostaglandin dehydrogenase expression. NF-xB also plays a role in regulation of OTR. The activity of NF-xB is increased in amnion at the time of both term and preterm labour. Furthermore, stretch of the amnion also activates NF-xB which may be one mechanism explaining the regionalisation of prostaglandin synthesis within the fetal membranes.

Studies by others, in animal models suggest that NF-xB is also activated in the myometrium at the time of the onset of labour. We have shown that myometrial cells respond in the classic manner to stimuli such as IL-1β, TNFα or LPS leading to a rapid activation and then deactivation of NF-xB. However, in response especially to IL-1b, there appears to be a prolonged effect with both NF-xB activity and COX-2 expression persisting beyond 24 hours following stimulation. We have found it more difficult, however, to prove that NF-xB activity increases in human myometrium with the onset of labour. In the amnion activation of NF-xB appears to be permanent and persists in cell culture for days after labour and delivery. It is probable that this represents a state of biochemical commitment to labour. Whilst it is possible that IL-1β or other factors may activate NF-xB in myometrium during labour, this does not appear to be persistent as we have found in the amnion. Bacterial infection is almost certainly linked to activation of NF-xB and therefore to the onset of preterm labour through Toll-like receptors. We are currently exploring the expression and activation of TLRs in the fetal membranes in both term and preterm labour. Current data suggests that there is expression of TLRs 1-7 and that there is a tendency for the level of expression to increase in association with labour. Current studies are focusing on determining which of the TLR receptors are active in both term and preterm labour.
The putative benefits of sample fractionation with MALDI-ToF mass spectrometry (MS) and is manifested in strategies. into the underlying biology, and contribute to the development of novel treatment identification based upon changes in the pattern of (for the most part) unidentifiable single assay. While direct mass spectrometry protein profiling offers the opportunity for diagnosis based upon changes in the pattern of (for the most part) unidentified proteins using pattern recognition software. Both approaches are currently being applied to the identification of biomarkers to identify women at risk of complications of pregnancy. Succeeding investigations promise new diagnostic tools for pregnancy outcome, insights into the underlying biology, and contribute to the development of novel treatment strategies.

Understanding Embryonic Stem Cells
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Embryonic stem (ES) cells were originally derived by plating preimplantation mouse embryos on a feeder layer of mitotically inactivated fibroblasts in medium containing serum. ES cells have been invaluable as an in vitro model system for early embryonic development and as a means to introduce genetic modifications into mice. The important component produced by the feeder cells was subsequently found to be leukemia inhibitory factor (LIF), which maintains self-renewal by activating the signal transducer and activator of transcription, STAT3. We have recently developed a serum free culture system that has allowed identification of another factor that acts in concert with LIF to maintain ES cell self-renewal. It has been shown previously that the addition of BMP4 to ES cells in serum free medium prevents differentiation into neurons. We have exploited this observation and have consequently demonstrated that BMP4 operates in this context by activation of IκB genes via the SMAD pathway. The addition of LIF and BMP4 to serum free medium afforded improved assay sensitivity and specificity achieved by combining multiple (up to 100) known biomarkers of disease into a single assay. While direct mass spectrometry protein profiling offers the opportunity for diagnosis based upon changes in the pattern of (for the most part) unidentified proteins using pattern recognition software. Both approaches are currently being applied to the identification of biomarkers to identify women at risk of complications of pregnancy. Succeeding investigations promise new diagnostic tools for pregnancy outcome, insights into the underlying biology, and contribute to the development of novel treatment strategies.

Cytotrophoblast Stem Cell Lines Derived from Human Embryonic Stem Cells
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Trophoblast stem cell lines are important tools to investigate early placentation. They have been derived from mouse embryos but not from human embryos so far. We aimed to generate cytotrophoblast stem (CTBS) cell lines from human embryonic stem cells (HESCs). In defined culture, embryoid bodies were generated from HESCs and selected for trophoblast enrichment by rounds of cellular aggregation and disaggregation. Human chorionic gonadotrophin (hCG) was used as a marker of trophoblast differentiation. Three CTBS lines were derived which could be maintained in the absence of feeder cells, extracellular matrix or residual HESCs, and displayed a variety of trophoblast lineage markers. Spheroid CTBS aggregates were generated and their interaction with luteal-phase endometrial stroma assessed by real-time image analysis. CTBS cells displayed typical cyto- and syncytiotrophoblast characteristics and exhibited further differentiation to the invasive endovascular phenotype involved in uterine blood vessel remodelling. Spheroid CTBS cells mimicked closely the early invasive stages of implantation when incubated with human endometrial stroma in vitro. These human CTBS cell lines are a significant model for investigating human placentation and may also have considerable potential in cell therapies related to vasculogenesis.
AN EMBRYONIC STEM CELL MODEL FOR HUMAN DIFFERENTIATION AND DEVELOPMENT. T. Golos, Department of Obstetrics and Gynecology and the Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison, WI, USA

Specification of the trophectoderm lineage, trophoblast differentiation, and placental morphogenesis are critically important not only for pregnancy initiation, but also for the achievement of an optimal maternal-fetal dialogue and environment for fetal growth, yet the precise mechanisms controlling human placental development remain elusive due to the inability to investigate differentiation at the level of the embryo and the early implantation site during human pregnancy. Our overall hypothesis is that the initiation of intrinsic pathways and response to extrinsic influences/cues coordinates lineage determination, trophoblast differentiation and formation of the chorionic villi. We have explored whether human embryonic stem cells (hESC) represent a opportunity to develop an embryonic surrogate model which will allow investigation into this critical stage in human development. While spontaneous differentiation of hESC into trophoblasts (as detected by hCG secretion) is consistent but of low efficiency, we have found that differentiation of trophoblasts in hESC-derived embryoid bodies (EBs) initiates elevated hCG secretion. Embryoid bodies were transferred to 3-dimensional Matrigel droplets as a first approximation of the implantation environment and in this model there is a profound increase in sustained secretion of hCG, progesterone and estriol, as well as the development of cellular outgrowths from the EBs into the surrounding Matrigel. Intriguingly, the pattern and dynamics of hCG secretion is quite different in culture of EBs on "2-dimensional" thin coating of Matrigel, with a truncated elevation of hCG secretion which is not sustained in prolonged culture. We are continuing to explore the balance of differentiation/proliferation in the 2-dimensional vs. 3-dimensional models, with the expectation that in vitro systems can be devised which will allow not only trophoblast differentiation, but the recapitulation of placental morphogenesis. Supported by NIH Grants HD34215, RR14040, HD35843, and RR000167.

HUMAN DECIDUAL NK CELLS: UNIQUE PHENOTYPE AND FUNCTIONAL PROPERTIES
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Natural Killer (NK) cells are lymphocytes of the innate immune system that recognize and induce lysis of a variety of target cells, including virally-infected cells and tumour cells. In addition, NK cells produce a variety of cytokines which influence adaptive immunity. NK cells are present in peripheral blood (PB) and secondary lymphoid organs. NK cells are massively recruited at the site of implantation, constituting the major immune cell type in the decidua in early pregnancy. In humans, decidual NK (dNK) cells are mostly CD56dimγδT which differ from the major CD56bright PB-NK subpopulation. Using a panel of monoclonal antibodies directed against various activating (NKG2D, NCR, NKG2C, CD160, CD16) and inhibitory (KIR, NKG2A, ILT2) NK cell receptors we found additional phenotype differences between CD56dim and CD56bright PB-NK and dNK. The functions of dNK in human are still unknown but a number observations suggest that, in normal pregnancy, they do not lyse the HLA-C, -E, -G positive invading extravillous cytotrophoblast they are in contact with. In contrast, after activation by these HLA or non HLA ligands, their vast range of cytokine production may affect placental vascularization and maintenance of successful pregnancy. A recent report showing that a particular combination of maternal KIR and foetal HLA-C genes favours preclampsia emphasised the crucial role dNK cells exert at the maternal-foetal interface.

MECHANISMS REGULATING IMMUNE CELL CONTRIBUTIONS TO SPIRAL ARTERY MODIFICATION
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Objectives: Massive endometrial enrichment in uterine Natural Killer (uNK) cells occurs early in pregnancy. In rodents, uNK cells are key in establishing a permissive environment for spiral artery modification by steps leading to interferon-γ production. Human uNK cells are also angiogenic. We wished to address regulation of uNK cell precursor homing, steps in uNK cell activation and to determine if uterine lymphocyte-promoted angiogenesis occurs in species having pregnancy without endometrial decidualization.

Methods: Homing potential of human blood CD56+ cells was assayed by adhesion to frozen sections of decidualized mouse uterus. Murine uNK cell activation was assessed by realtime PCR analysis of laser-capture, microdissected, uNK cell subsets and morphometric analyses of implant sites from mice genetically null in NK cell receptor signaling pathways. RNA from adipocytokine, peri-attachment stage pig implant was analysed as endometrial biopsies, lymphocytes and trophoblasts.

Results: Surge in luteinizing hormone or rising estrogen promotes CD56+ cell interaction with uterine endothelium. In mouse uNK cells, the transcription factor Eomes is more important than T-bet for induction of interferon-γ. Internal cell signals depend on FcγR > DAP12 but not on DAP10. Porcine uterine lymphocytes transcribe angiogenic molecules.

Conclusions: Uterine lymphocytes are key contributors to endometrial angiogenesis early in gestation. Supported by NSERC, OMAFRA, Ontario Pork, CIHR, Ontario Womens Health Scholar and Canada Research Chairs Programs.

FROM MORPHOLOGY TO STEREOLOGY: MORPHOLOGICAL BASIS OF PLACENTAL INSUFFICIENCY. Graham J Burton, Department of Anatomy, University of Cambridge, Cambridge, UK

In the past, placental insufficiency was largely defined in clinical terms, and the concept was reinforced by widespread belief that the organ normally functions to the maximum of its capacity. It might be expected therefore that any deficiency in function would be reflected in changes in placental structure. Our present understanding of placental architecture is based heavily on seminal papers produced by Peter Kauffman and his colleagues in the late 1970s. They developed the classification system of stem, intermediate and terminal villi, defining these in terms of their size, morphology, vascularity and function. Stereology allows values for three-dimensional parameters to be estimated from two-dimensional sections using geometrical probes. The total surface area of intermediate and terminal villi can be substituted into the Fick equation, along with mean membrane thickness, to estimate the morphometric diffusing capacity of the placenta. This index provides an estimate of the physiological capacity of the organ, and when related to fetal weight provides a measure of efficiency. In the human, stereological studies of clinically well-defined cases of intrauterine growth restriction have shown a proportional reduction in villous surface area, most likely secondary to pathology in the utero-placental arteries. Although of a different origin, similar miniaturisation of the placenta is seen in mice in which the placental-specific transcript for insulin-like growth factor 2 has been deleted and there is late-onset fetal growth restriction. Stereology can therefore provide a valuable link between clinical, genetic and physiological studies by quantifying changes in structural parameters of direct physiological importance.
FROM MORPHOLOGY TO MOLECULE: THE MOLECULAR BASIS OF PLACENTAL DEVELOPMENT.

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The trophoblast lineage arises at the blastocyst stage first as a simple epithelium (the trophotroctoderm) that then goes on to differentiate into a variety of cell subtypes after implantation. Only ~60 trophoblast cells are present at the mouse blastocyst stage and, therefore, considerable proliferation occurs after implantation. Because the ~50 mural trophctodermal cells are post-mitoic and committed to forming primary trophoblast giant cells (TGCs), the polar trophotoderm has been thought to give rise to the rest of the trophoblast lineage. In the post-implantation placenta, trophoblast stem (TS) cell lines can be derived from the extraembryonic ectoderm/chorion and either remain as stem cells or differentiate into more TGCs, or alternative differentiated cell types including spongiotrophoblast, glycogen trophoblast cells, or syncytiotrophoblast cells of the labyrinth. Despite considerable molecular insights into factors required for differentiation, the lineage origins and functions of these cell subtypes have remained elusive. Indeed, to date only indirect lineage tracing studies had been done. During TGC differentiation, cultured cells first express genes typical of the ectoplacental cone/spongiotrophoblast layer, before eventually expressing TGC-specific genes. The assumption has been that during differentiation of trophoblast stem cells to TGCs, they first pass through an intermediate stage. Indeed, isolated ectoplacental cone cells rapidly differentiate into TGCs in culture. In the last few years we have done several studies that indicate that the picture is more complicated and more interesting.

FROM MORPHOLOGY TO CLINICS: IMAGING PLACENTAL INSUFFICIENCY

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Objectives: To review the developmental biology and molecular pathology of severe “placental insufficiency”, to apply this information to the identification of at risk women, and thereby create a therapeutic “window of opportunity” for disease prevention.

Methods: Since 1999 we have prospectively evaluated the role of integrating maternal serum biochemistry, placental gray-scale morphology, and uterine artery Doppler (triple placentaion test - TPT), for the identification of high-risk women destined to develop severe placental insufficiency resulting in preterm death or delivery.

Results: Amongst 60 women with combined elevations in HCG and AFP, the combination of abnormal placental morphology and uterine artery Doppler identified all women at risk of severe disease with a 75% PPV. In a cohort of 325 clinically high-risk women, TPT screened 75% of women with a 50% PPV for developing AREDs in the umbilical arteries. Most have ischemic-thrombotic placental disease, thus a heparin intervention trial is planned across Ontario for women with abnormal biochemistry who have one or more ultrasound features of placental dysfunction.

Conclusions: Placental insufficiency is the “ovarian cancer” of pregnant women. The ratio of research for preventing placental versus myocardial infarction is a depressing 1/140 (www.pubmed.com, accessed 09-04-05). We can only address this serious disadvantage through the development of clinical screening networks that encourage women at risk of serious disease to participate in clinical trials.

Funding: CIHR and Rose Torno Chair (to JK).
L21
NUTRITIONAL MODULATION OF ADOLESCENT PREGNANCY OUTCOME
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The risks of miscarriage, prematurity and low birth weight are particularly acute in adolescent girls who are still growing at the time of conception. The role of maternal nutrition in mediating pregnancy outcome in this vulnerable group has been examined in sheep models. When singleton bearing adolescent dams are overnourished to promote rapid maternal growth throughout pregnancy, growth of both the placenta and fetus is impaired, and birth occurs prematurely relative to control adolescents of equivalent age (model 1). Studies at mid-gestation, prior to alterations in placental mass, suggest that reduced proliferation of the fetal trophectoderm, impaired angiogenesis, and attenuated uteroplacental blood flows are early defects in placental development. By late pregnancy, relative placental mass is reduced by 45%. The asymmetrically growth restricted fetuses are hypoxic, hypoglycemic and have reduced insulin and IGF-1 concentrations. However, fetal utilization of glucose and oxygen remain normal on a fetal weight basis. This suggests altered sensitivities to metabolic signals and may have implications for subsequent metabolic health. At the other end of the nutritional spectrum, many girls who become pregnant have inadequate or marginal nutritional status during pregnancy. This situation is replicated in model 2, whereby dams are prevented from growing during pregnancy by relatively underfeeding. Limiting maternal intake in this way gradually depletes maternal body reserves leading to a lower transplacental glucose gradient and a modest slowing of fetal growth in late pregnancy. These changes appear to be independent of alterations in placental growth per se. Thus, while the underlying mechanisms differ, maternal intake at both ends of the nutritional spectrum is a powerful determinant of fetal growth in pregnant adolescents.

L22
FETAL PROGRAMMING: CAUSES AND CONSEQUENCES
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Objectives: There are now many studies to show that maternal nutrition alters pregnancy outcome, both short and long-term. This presentation will provide data on a clinically relevant model – that of maternal Fe deficiency – and will discuss which might be the periods when the fetus is most sensitive to maternal nutritional imbalance.

Methods: Female weanling rats were fed for two weeks on normal diet followed by four weeks on an iron-deficient diet (15% control). They were mated and pups collected at different times. In some experiments, the embryos were cultured from D10.5 for up to 48h in different media. In others, the fetuses or offspring were killed and tissues collected. RNA, protein and enzyme levels were measured appropriately. All experiments were approved by the Ethics Committee and carried out under Home Office Licence.

Results: Maternal Fe deficiency causes an increase in blood pressure in the offspring. Additionally, there is an increase in obesity, without a change in total fat content. When embryos are cultured for different periods in Fe-deficient or control serum, we demonstrate that the period D10.5 to D12.5 is critical for the effect on cardiovascular development, while growth retardation of deficient embryos is not reversed by culturing in normal serum. We have examined gene expression but to date have not identified relevant changes that will explain the results. It is unlikely to be a simple consequence of Fe deficiency, however.

Conclusions: The data demonstrate that there are critical windows in development, when the embryo or fetus is susceptible to maternal nutrient deficiency and that the mechanism(s) that operate may be different. Given the relevance of maternal anaemia to the human population, our data would support the prophylactic use of Fe supplementation as early in pregnancy as possible.

L23
THE EARLY EMBRYONIC ENVIRONMENT: IMPLICATIONS FOR EMBRYO POTENTIAL AND FETAL/POSTNATAL HEALTH
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Recent evidence from several laboratories and in different species indicates that the pre-implantation embryo is sensitive to environmental conditions which may impact not only on immediate aspects of morphogenesis but also in setting long-term growth and physiological characteristics, both pre- and post-natally. From our own studies, using rodent models comprising either in vitro culture or in vivo maternal dietary protein undernutrition, such environmental conditions can alter blastocyst proliferation and gene expression profiles. Later consequences of periconceptional undernutrition include reduced fetal growth, post natal ‘catch-up’ growth, and increased systolic blood pressure in adult offspring, even if normal control diet is supplied for post-implantation and post-natal life. A similar phenotype is evident following preimplantation culture and embryo transfer. Low protein diet during preimplantation development significantly alters the composition of uterine fluid with respect to amino acids at the time of blastocyst morphogenesis which may initiate changes in embryo metabolism and signalling activity. Epigenetic effects are implicated since the pattern of imprinted gene expression with respect to H19 and IGF2 are altered in blastocyst and fetal liver in response to preimplantation dietary challenge. In addition, fetal liver exhibits alteration in expression of genes controlling metabolic activity. Our current studies are extending analysis of postnatal phenotype following periconceptional undernutrition to include behaviour and endothelial function. Collectively, the effects of embryo environment on long-term phenotype represent a complex interaction of multiple mechanisms spanning the developmental period into adult life.
TROPHOBLAST-INDUCED REDISTRIBUTION OF ICAM-1 IN UTERINE ENDOTHELIAL CELLS UNDER FLOW

Methods: Cocultures were examined using immunocytochemistry and image analysis.

Results: Endothelial ICAM-1 was redistributed towards the downstream edge when the cells were cocultured with trophoblasts under a steady shear stress of 15 dyn/cm² for 24 h. ICAM-1 asymmetry was less pronounced at 7.5 dyn/cm² and was not observed when cocultures were maintained under static conditions or when endothelial cells were cultured alone under steady shear stress conditions. Redistribution of ICAM-1 to the downstream edge was also observed, albeit to a lesser extent, when endothelial cells were incubated with trophoblast-conditioned culture medium and subjected to flow. Confocal microscopy showed that some trophoblasts had penetrated and migrated under the endothelial monolayer. The incidence of sub-endothelial trophoblasts was greater under flow than under static conditions.

Conclusions: These data suggest that endothelial ICAM-1 expression is regulated by direct contact with trophoblasts and as yet unknown secreted factors. ICAM-1 asymmetry has been associated with endothelial cell migration and ICAM-1 plays a major role in leukocytic and metastatic transmigration across endothelium. We therefore speculate that trophoblasts and fluid shear induce uterine endothelial mobility, which reduces the extent of endothelial cell-cell adhesion and facilitates penetration by trophoblasts.

SCA-1 AS A MARKER OF TROPHOBLAST STEM CELLS IN THE MOUSE PLACENTA

Objective: To use scanning (SEM) and transmission (TEM) electron microscopy to investigate development of the definitive mouse placenta, describing the interhemal membrane (IM) in the labyrinth zone, and spongiotrophoblasts (Sp) and glycogens cells (GC) of the junctional zone (Jz).

Methods: Placentas from B16 mice from E12.5 to E19.5 were immersion-fixed and pro-processed for TEM. Further placentas at E16.5 were perfusion-fixed from the maternal side, cryofractured and processed for SEM.

Results: SEM revealed the superficial cytotrophoblast (CT) layer to be highly fenestrated, allowing direct interaction between the syncytiotrophoblast and maternal blood. Secretion from the syncytiotrophoblast fenestrations was observed as gestation advances the CT form bridges that subdivide the maternal blood spaces, and the organisation of the LIM becomes more ordered. The interface between the two layers of syncytiotrophoblast is very regular, although the apical and basal membranes become increasingly vacuolated and microvillous. The fetal capillary endothelium is also fenestrated. In the Jz, the Sp t vary in size and shape, and their cytosol becomes more complex and variable in degree of osmiophila. From E12.5 populations of small cells become surrounded by extracellular matrix and begin to accumulate glycogen. As glycogen occupies more space subcellular structures are lost.

Conclusions: This study demonstrates new features of the interhemal membrane of the mouse placenta, and provides the first description of early morphological differentiation of glycogen cells.

EGF MODULATES TROPHOBLAST MIGRATION THROUGH REGULATION OF CONNEXIN 40.

Methods: JAR cells were cocultured with trophoblasts under a steady shear stress of 15 dyn/cm² for 24 h. ICAM-1 expression was barely detectable after 2 days of differentiation. In contrast, Sca-2 protein was increased in intensity until day 6 of differentiation. In contrast, Sca-2 expression was restricted to the labyrinth layer. In TS cells, both Sca-1 and Sca-2 mRNAs were highly expressed in undifferentiated, proliferating cultures. However, under differentiating conditions, whereas Sca-1 expression was barely detectable after 2 days, Sca-2 increased in intensity until day 6 of differentiation. In contrast, Sca-2 protein was detectable by FACs analysis in approximately one third of undifferentiated TS cells and until 2 days of differentiation after which time it was undetectable. Sca-1 protein was detectable in approximately 95% of undifferentiated TS cells and decreased markedly as the cells differentiated, though was still present in approximately 10% of cells after 6 days of differentiation. We further investigated the Sca-1 population of TS cells and found a subpopulation (≤ 5%) in both undifferentiated and differentiated cultures that were Sca-1+/Hoechst−, Hoechst+ (SP) cells are present in many tissues and often indicative of stem cell populations. Our results identify a potential trophoblast stem cell marker for the mouse placenta.
DIFFERENTIAL INSULIN RECEPTOR EXPRESSION CAUSES DIFFERENTIAL INSULIN SIGNALLING IN THE TWO INTERFACES OF THE HUMAN PLACENTA.
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Objectives: The human placenta expresses high amounts of insulin receptors (IR) with developmental changes of location from trophoblast in first trimester to the endothelium at term. Two IR isoforms exist (IRB, IRA) that elicit diverse cellular responses. We hypothesized that first trimester trophoblasts (FT) and term endothelial cells (EC) express different IR isoforms resulting in diverse signalling. Thus, IR isoform expression and insulin stimulation of IR, PKB, ERK1/2 and fos mRNA expression was examined.

Methods: Serum starved FT and EC isolated from human placentas as well as NIH 3T3 cells over-expressing IRB (3T3 B) or IRA (3T3 A) were stimulated with 1 (mRNA isolation) or 10 nM insulin (protein extraction) for 0-60 min. IR isoforms and fos mRNA expression (RT-PCR) as well as phosphorylation of IR, PKB and ERK1/2 (western blot) was determined.

Results: FT expressed IRB while EC mainly expressed IRA. The ratio of IR and PKB activation was similar in FT and IRA expressing cells. Insulin induced a strong increase of ERK1/2 phosphorylation and fos mRNA expression in IRB expressing cells (FT: 5x, 5x; 3T3 B: 15x, 12x) but not in both IRA cells (EC; 3T3 A). Inhibition of the ERK1/2, but not of the PKB pathway blocked fos mRNA induction.

Conclusion: Trophoblasts and endothelial cells express different IR isoforms resulting in activation of diverse signalling pathways. Comparison with model-cells expressing IRB or IRA suggests the prominent activation of the ERK1/2 pathway in FT to be characteristic for the IRB isoform. Expression of distinct IR isoforms on the placental surfaces (trophoblast, endothelium) allows maternal and fetal insulin to regulate different processes in the human placenta. (European Commission PERILIP grant QLRT-2001-00138; ONB Jubilee Fund 10896)

REGULATION OF PLACENTAL EFFICIENCY FOR NUTRIENT TRANSPORT BY IMPRINTED GENES
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Objectives: Imprinted genes play key roles in resource acquisition during fetal life. Paternally expressed genes increase maternal nutrient transfer through the placenta, whereas maternally expressed genes reduce it. We are studying how imprinting regulates placental resource provision to the fetus.

Methods: We use imprint knock-out mouse models that show disproportionate growth of the placenta and study growth kinetics, placental morphology, in vivo placental capacity assays, and expression levels of nutrient transporter genes.

Results: A knockout lacking paternally expressed placental-specific Igf2 leads to reduced growth of the placenta and compromised passive diffusion at E16, but normal fetal growth. The fetal to placental weight ratio is thus increased. Increased placenta efficiency is due to upregulation of key placental nutrient transport systems, such as system A amino acids and glucose, and this occurs through increased transcription of specific transporter genes (imprinted Slc38a4 gene and Slc2a5). Decreasing fetal demand by removal of fetal Igf2 abolished upregulation of both transport systems. Knock-outs of the maternally expressed Ipl and H19 genes result in placental overgrowth and show decreased fetal to placental weight ratios. Decreased placental efficiency correlates with down-regulation of System A transporter Decreased placental efficiency correlates with down-regulation of System A transporter genes Slc38a2 and Slc38a1.

Conclusions: Our results reveal a system that senses fetal growth demand and signals it back to the placenta which responds by regulation of nutrient transport genes. Crossover between imprinted genes may be a component of the genetic regulation of nutrient supply and demand during mammalian development.
O09

PLACENTAL GLUCOSE-6-PHOSPHATASE: A REASON FOR DIFFERENCES IN GLUCOSE METABOLISM IN NORMAL PREGNANCY AND SMALL FOR GESTATIONAL AGE.

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Objectives: Term placenta may be capable of glucose production due to the presence of glucose-6-phosphatase (G6Pase). We hypothesised that there would be placental glucose secretion (PGS) in normal term pregnancy (controls) but this secretion would be decreased in Small for gestational age (SGA) pregnancies which could be due to differences in G6Pase activity in these placentas.

Methods: A primed (5.25mg/kg) infusion (4.7mg/kg/hr) of 6,6,2H2-glucose was infused before elective caesarean section in 16 normal pregnancies and 11 SGA pregnancies. Maternal arterialised blood (MAB) representing the uterine artery, umbilical vein and umbilical artery blood samples were taken for isotopic enrichment from MAB to UV (3.47 ± 0.21 to 3.14 ± 0.2, p = 0.039). In the SGA group there was an insignificant dilution in the enrichment from MAB to UV (3.47 ± 0.21 to 3.14 ± 0.2, p = 0.276). G6Pase activity was higher in the placenta of controls compared to SGA subjects with the substrates glucose-6-phosphate (11.19 ± 3.3 nmoles/min versus 9.12 ± 2.8 nmoles/min (p = 0.037), Beta-glycerol phosphate (3.98 ± 1.06 nmoles/min versus 3.038 ± 1.062, p = 0.008) and Glucose adenosine-6-phosphate (3.91 ± 1.2 versus 3.01 ± 1.0, p = 0.014).

Conclusions: Placental glucose secretion occurs in normal pregnancy and may not occur in SGA. These results may be explained by a decrease in G6Pase activity in the placenta of SGA subjects.

O10

OVINE ENDOGENOUS BETARETROVIRUSES (enJSRVs) REGULATE CONCEPTUS GROWTH AND DEVELOPMENT.

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The ovine genome contains multiple copies of endogenous betaretroviruses (enJSRVs) that are related to an exogenous oncogenic virus, Jaagsiekte sheep retrovirus, for which hyaluronidase 2 (Hyal2) is the cellular receptor. This study tested the hypothesis that the enJSRVs envelope (Env) is involved in regulation of placental morphogenesis. We investigated expression of enJSRVs env and hyal2 mRNAs in the ovine uterus and conceptus. enJSRVs env expression was detected in the conceptus at Day 12, while hyal2 was evident from Day 16. enJSRVs env mRNA was specifically expressed in endometrial luminal and glandular epithelium, trophoblast giant binucleate cells (BNC), and the multinucleated syncytiotrophoblast of the placenta. Hyal2 mRNA was only detected in the BNC and syncytiotrophoblast of the placenta. Partial sequencing of transcribed enJSRVs from uteroplacental tissues revealed expression of enJSRV loci containing an open reading frame in env similar to enJSF16 and enJS56AI proviruses. To determine function of enJSRVs Env in vivo, morpholino antisense oligonucleotides (MAO) were designed to inhibit translation of enJSRVs Env. Ewes were mated at estrus (Day 0) and the uterine horn ipsilateral to the corpus luteum was double ligated near the uterine body and injected with either control or enJSRVs Env MAO on Day 8. On Day 16, control MAO concepti were elongated and filamentous, while the concepti recovered from enJSRVs Env MAO treated ewes were fragile, growth retarded and lacking BNC. These results further support the hypothesis that enJSRVs Env regulate conceptus growth and placental morphogenesis.

O11

NEEDLE-WIRE TARGETTED CORRELATION OF PLACENTAL ULTRASOUND FINDINGS WITH PATHOLOGICAL LESIONS AND OBSTETRICAL OUTCOME

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Objective: Placental texture should be homogenous with placental villi evenly and symmetrically distributed; recent studies have correlated abnormalities in texture with pathological lesions and adverse perinatal outcome. In the present study, echogenic, cystic lesions (ECL) within the placenta identified on antenatal ultrasound as having ECLs were correlated with pathological lesions and adverse perinatal outcome. Perinatal outcome data was recorded.

Methods: Patients identified on antenatal ultrasound as having ECLs were followed prospectively through pregnancy. Post delivery needle localization wires were placed within these lesions under ultrasound guidance; placentas were submitted for independent pathological evaluation. Perinatal outcome data was recorded.

Results: Seven ECLs were identified and correlated with the following pathological lesions: 4 intervillous thrombosis (1 thrombus was contiguous with perivillous fibrin deposition), 1 chorioangioma, 1 villous infarction and 1 dilated intervillous space consistent with a “lake”. Other significant lesions identified within the placenta included: perivillous fibrin deposition, decidual vasculopathy, and destructive chorionic villi. Each placenta was complicated by pregnancy induced hypertension, premature pre-term ruptured membranes and/or poor fetal well-being. Conclusions: ECLs encompass a spectrum of pathology but are most commonly due to intervillous thrombosis. Further correlative studies using needle-wire localization are ongoing to define the clinical significance of these lesions in high and low risk pregnancies.

Funding: Departments of OBGYN and Pathology/Laboratory Medicine

O12

VITAMINS C AND E SUPPRESS ACTIVATION OF P38 AND NF-κB PATHWAYS IN PLACENTAL EXPLANTS.

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Objective: To investigate placental signalling pathways activated by acute oxidative stress.

Methods: Placental samples were collected with consent from uncomplicated elective caesarean deliveries, into medium equilibrated with 5% O2,5% CO2. Tissue was incubated under 10% O2, continuously; subjected to hypoxia (0.5% O2) for 1 hr and reoxygenation (H/R) at 10% O2 for 6 hr or 15 hr or incubated under 10% O2, with or without 1 mM H2O2. The p38 inhibitor PD169316 was tested at 10 µM, and ascorbic acid and Trolox at 2 mM and 1 mM respectively. Samples were snap-frozen for Western blotting or fixed for immunohistochemistry (IHC) Superantigens were collected for ELISA analysis.

Results: Samples treated with H/R or H2O2 showed higher oxidative stress through increased levels of Hsp27, Hsp90, HIF-1α and COX-2, localised primarily to the trophoblast. H/R and H2O2 induced phosphorylation of p38, SAPK and IkB, an increase in caspase-3 and caspase-9 and PARP cleavage, which were localised by IHC to syncytiotrophoblast. In addition, H/R and H2O2 induced secretion of TNFα. Both the p38 inhibitor and vitamins powerfully suppressed phosphorylation of IkB, stimulation of HIF-1α, Hsp27 and COX-2, reduced secretion of TNFα, and apoptosis.

Conclusion: Oxidative stress activates the p38, SAPK and NF-κB pathways in placental explants, leading to apoptosis and secretion of inflammatory cytokines. Vitamins C and E suppress these effects. Supported by The Wellcome Trust.
NITRIC OXIDE AND CARBON MONOXIDE INHIBIT HYPOXIA/REOXYGENATION INDUCED APOPTOSIS IN SYNCTIOTROPHOBLASTS

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Hypoxia-reoxygenation (H-R) insult to the placenta has been shown to initiate the apoptotic cascade in the syncytiotrophoblast. It is postulated that in pre-eclampsia shedding of apoptotic syncytiotrophoblasts into the maternal circulation, following chronic H-R insult, can initiate a maternal inflammatory response with subsequent endothelial dysfunction. Nitric oxide (NO) and carbon monoxide (CO) both appear to display anti-apoptotic properties. Therefore, we sought to determine whether NO and CO are capable of inhibiting H-R induced apoptosis in syncytiotrophoblasts. Placental villous explants were exposed to repeated hypoxia-reoxygenation insults that lasted three hours each, over a twelve-hour period. Throughout the experiments the explants were bathed in normal explant medium, explant medium treated with 1 µM glyceryl trinitrate (GTN) or explant medium treated with 75 µM CO. The villous explants were then analyzed for syncytiotrophoblast apoptosis by TUNEL assay with subsequent morphological studies using electron microscopy. A reduction in TUNEL-positive staining was observed in explants treated with both NO and CO; the effect of NO appeared to be more pronounced. Both nitric oxide and carbon monoxide are capable of reducing syncytiotrophoblast apoptosis following repeated H-R insult. This would suggest that NO and/or CO have therapeutic potential in the treatment of pre-eclampsia.

DIFFERENTIAL DISTRIBUTION OF CD4+CD25BRIGHT T-CELLS IN DECIDUA AND MATERNAL PERIPHERAL BLOOD DURING HUMAN PREGNANCY

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Objectives: During pregnancy several maternal and fetal mechanisms are established to prevent a destructive immune response against the allogenic fetus. Despite these mechanisms, fetus specific T-cells persist throughout gestation and little is known about the regulation of these T-cells. Recently a role of decidua CD4+CD25+ T-cells was suggested for maintenance of allogenic pregnancy in mice.

Methods: Lymphocyte isolates from human decidua (d.) basalis, d. parietalis and peripheral blood from uncomplicated early- and term pregnancy were analysed for expression of CD3, CD4, CD25 and additional surface markers using flow cytometry.

Results: We show a differential distribution of CD4+CD25+ T-cells in decidua and maternal peripheral blood during pregnancy. Decidua derived CD4+CD25+ T-cells have a significantly higher expression of CD25, CD152 and HLA-DR compared to CD4+CD25+ T-cells derived from peripheral blood. In addition, a significant increase in percentage of CD4+ CD25+ T-cells is shown in d. parietalis but not in d. basalis and peripheral blood during pregnancy.

Conclusion: Differences in phenotypic characteristics between decidua and peripheral blood derived CD4+CD25+ T-cells, may reflect a specific immunomodulatory function of these cells during pregnancy.
Blood Vessels and Endothelium

P1.01
ULTRASTRUCTURAL FEATURES OF ISOLATED PRESSURISED HUMAN PLACENTAL ARTERIES
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Objectives: The ability of a blood vessel to develop tone is dependent upon a number of morphological parameters of the smooth muscle cells (SMC), including density, relationship with the endothelium and subcellular distribution of myofilaments and intracellular organelles. Wall ultrastructure of isolated human placental chorionic plate arteries (PA), pressurised to mimic their in vivo geometry, was examined using electron microscopy (EM), and compared with maternal arteries (MA).

Methods: PA (n=12) and MA (omental, n=10 and myometrial, n=6) were isolated from biopsies, taken with LREC approval from women with uncomplicated pregnancies, pressurised, tested for contractile viability then fixed for EM, with some postfixed in osmium ferricyanide for sarcoplasmic reticulum (SR) identification.

Results: In contrast to PA, MA have no internal elastic lamina but considerable extracellular matrix separates circularly orientated SMC. The SMC contain tightly packed arrays of myofilaments running parallel to the plasma membrane, enveloping cellular organelles. Synthetic SMC, with few myofilaments and much rough SR, are observed in PA only. SR in MA is located centrally, often encircling mitochondria, and also near the plasma membrane associated with caveolae. Positive SR staining was rarely observed in SMC of the PA.

Conclusions: This study highlights ultrastructural differences in PA from those of MA that may underlie specialised mechanisms of regulating vascular tone in the placenta.

P1.02
ANGIOGENIN IS ASSOCIATED WITH BLOOD VESSEL FORMATION DURING EARLY HUMAN PLACENTAL DEVELOPMENT.
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Objectives: Human angiogenin is a potent inducer of neovascularisation in experimental models, in vivo. The secreted 14-kDa protein is largely expressed in human term placenta (Pavlov et al. Angiogenesis 2003; 6: 317). We studied angiogenin expression along placental development to question its involvement in blood vessel formation.

Methods: Angiogenin was localised in situ by indirect immunofluorescence in sections of human first and second trimester placentas. Its cellular distribution was established by double immunolabelling with cell markers expressed by epithelial cells (cytokeratin), endothelial cells and their precursors (vWF, PECAM-1, CD34, VEGF-R2, VE-cadherin, Tie-2, Epo-R), smooth muscle cells (alpha-smooth muscle actin), hematopoietic cells (CD45) and marker for proliferative cells (Ki-67). Angiogenin expression was confirmed by RT-PCR on placental tissues and primary culture of trophoblastic cells, and by detecting the protein in conditioned media.

Results: Angiogenin was expressed in villous and extravillous trophoblasts, and blood vessels. In addition, in early placental villi, angiogenin immunoreactivity was detected in some single cells co-labelled for early endothelial markers in close vicinity of the trophoblastic layer, and in nascent fetal blood vessels located deeper in the villous stroma.

Conclusions: Given angiogenin biological activities in vitro, these data suggest that angiogenin might be involved in placental villous vasculogenesis.

P1.03
ENDOTHELIAL CELLS PHAGOCYTE DEAD TROPHOBLASTS.
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Objectives: A number of lines of evidence suggest that preeclampsia is caused, in part, by a factor or factors from the placenta which induce endothelial cell activation. Trophoblasts are, like other epithelia, shed from the placenta as they become aged or damaged. The shed trophoblasts are then deported from the placental site in the maternal blood. Trophoblast shedding occurs in normal pregnancy but may be increased in pre-eclampsia. Most deported trophoblasts do not appear to remain in the maternal circulation but are trapped in the pulmonary or other capillary beds. The ultimate fate of deported trophoblasts is currently unknown but to prevent clogging of the pulmonary circulation they must be cleared from the capillary beds. We undertook this study to examine whether endothelial cells might be involved in clearing deported trophoblasts and the possible effects of the deported trophoblasts on the endothelial cells.

Methods: We labelled Jeg-3 and Jar choriocarcinoma cell with red fluorescent cell tracker stain, then induced them to undergo either apoptotic or necrotic death, and added the dead cells to monolayers of HMEC-1 endothelial cells.

Results: Using confocal microscopy we found the endothelial cells phagocytosed both apoptotic and necrotic trophoblasts (Jar and Jeg-3) and that the phagocytosis of the trophoblasts was blocked by cytochalasin B. Importantly, phagocytosis of necrotic but not apoptotic trophoblasts induced ICAM-1 expression by the HMEC-1 cells.

Conclusions: This work suggests that endothelial cells could potentially phagocytose deported trophoblasts and that the mechanism of trophoblast death (apoptotic or necrotic) could have major effects on the maternal vascular response to shed trophoblasts.

P1.04
EFFECTS OF THE ANTI-ANGIOGENIC FACTOR TNP-470 ON GROWTH OF THE MOUSE FETUS AND PLACENTA.
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Objectives: The placenta adapts to meet fetal demands and vascular growth and development are critical features of this adaptation. Interference with these processes may compromise placental and fetal growth. Here, we describe the effects of TNP-470 on placental composition and induction of a form of IUGR in mice.

Methods: At 2-day intervals between days E10.5 and E16.5, dams were injected subcutaneously with TNP-470 and killed at E18.5. Random samples of placenta were analysed by 1H-thymidine autoradiography and stereology.

Results: TNP-470 administration did not affect litter sizes but was associated with decreased placental and fetal sizes. Placentas at E13.5 and E18.5 showed no changes in the volume densities of compartments (labyrinth, blood vessels, spongiosarclathoblast, giant trophoblast cells, decidual layer, yolk sac, chorion, Reichert's membrane) or combined lengths of fetal or maternal vessels. Mean cross-sectional areas of fetal and maternal vessels of TNP-470-treated mice were reduced at E13.5 (by 40%) but not at E18.5. Endothelial S-phase indices were reduced (by 40%) at E13.5 and E18.5. No other structural differences were detected between TNP-470-treated and control mice at E18.5.

Conclusions: TNP-470 interferes with placental vascular development, including endothelial proliferation and vessel calibre, and offers a reproducible murine model of IUGR.
HOMEOBOX GENE EXPRESSION IN PLACENTAL MICROVASCULAR ENDOTHELIAL CELLS

HOMEBOX GENE EXPRESSION IN PLACENTAL MICROVASCULAR ENDOTHELIAL CELLS

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Objectives: Angiogenesis is fundamental to placental development, which is evident from the microvascular defects seen in major pathologies. Our limited knowledge of human placental angiogenesis comes primarily from cell culture studies employing macrovascular HUVEC. HUVEC may not be a suitable model for placental microvascular endothelial cells (PLEC) [Lang et al (2003) Eur J Cell Biol. 82(4) 163-73]. Homeobox genes are transcription factors that regulate cardiovascular endothelial cell development but little is known about their role in placental vasculature. The aim of this study was to measure homeobox gene expression in isolated PLEC.

Methods: PLEC were isolated by perfusion of the fetal placental vasculature with proteolytic enzymes.

Results: Yields of 5x10^6 to 6x10^6 cells per cotyledon, with 93-98% purity and 66.3-100% viability, were obtained. Immunostaining with the endothelial cell markers vWF and CD34 confirmed their identity. Using RT-PCR, we measured the expression of homeobox genes DLX3, DLX4, MSX2, GAX, HLX1 in PLEC. These homeobox genes had previously been identified in placental tissue sections and extracts. We also identified six homeobox genes not previously described in the placenta, including HEX. Finally, we showed HLX1 was expressed at low levels in PLEC compared to HUVEC.

Conclusion: A variety of homeobox genes are expressed in PLEC, including HEX and GAX, which are important in cardiovascular development. We also provided further evidence of the heterogeneity of gene expression between macrovascular HUVEC and microvascular PLEC.

PLACENTAL SECRETION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) INTO THE FETAL AND MATERNAL CIRCUMATIONS

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Objectives: VEGF is a potent vasodilator of the fetal placental vasculature. It is expressed in placental villi, amnion, chorion and maternal decidua, and is present in the fetal and maternal circulations. The study aim was to test for placental secretion of this hormone.

Methods: Human placental lobules (31.5 ± 3.1 g, mean ± SE) from 6 term healthy pregnancies were dually perfused in vitro, and fetal and maternal venous perfusates were sampled periodically. Following centrifugation, supernatants were assayed for combined levels of total VEGF_{α}, and VEGF_{β}, isoforms using RIA. Venous and arterial umbilical cord sera from 8 healthy term pregnancies were assayed similarly.

Results: Fetal and maternal venous perfusate levels of VEGF decreased to plateaus after 1 hour, suggesting steady-state secretion (0.40 ± 0.30 and 1.92 ± 0.05 ng / ml, respectively at 3 hours, mean ± SE). The mean [VEGF]_{venous} was greater than the mean [VEGF]_{umbilical} (9.10 ± 0.32 and 8.60 ± 0.21 ng / ml, respectively, mean ± SE) but this difference was not significant at n=8.

Conclusions: These data confirm the placenta as an important paracrine and endocrine organ with respect to VEGF secretion. We speculate that regulation of these polarised (maternal > fetal) secretions is important in the control of fetoplacental blood flow and maternal blood pressure.

ROLE OF EPHB4/EPHRIN-B2 IN TROPHOBLAST AND ENDOTHELIAL CELL INTERACTIONS OF HUMAN PLACENTA.

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The placenta fulfills life-sustaining functions during the intra-uterine development of the mammalian embryo ensuring the exchange of nutrients and gases between the developing embryo/fetus and the mother. Successful implantation and placentation involves invasion, vasculogenesis and angiogenesis orchestrated by the placental trophoblast cells. In particular the proper interaction between the trophoblast and the maternal endothelium represents a critical step in the establishment of the utero-placental circulation. The Eph receptors and their ephrin ligands are involved in several biological processes during embryonic development such as cell migration, cell communication and cell interaction. Specifically, EphB4 and its ligand ephrin-B2 are crucial in the formation of capillary network. We have previously shown that this receptor-ligand pair shows a spatially and developmentally controlled expression in the human placenta. Moreover, expression was found to be regulated by hypoxia indicating an important role of the molecules during placentation.

To study the role of EphB4 and ephrin-B2 in trophoblast-endothelial interactions, we have investigated the expression profile and protein localization in the HIPEC-65 extravillous trophoblast cell line and in primary human endothelial cells isolated from the umbilical cord (HuVEC cells) under normal and hypoxic conditions. Moreover, coculture experiments involving the addition of synthetic ephrin-B2 proteins are undertaken to unravel the role of EphB4 and ephrin-B2 in trophoblast-endothelial attraction, proliferation, migration and interaction.

PLATELET-ACTIVATING FACTOR ACETYLHYDROLASES ARE REGULATED IN BOVINE PLACENTOMES AT PARTURITION

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Objectives: Platelet-activating factor (PAF) is a potent lipid messenger playing an important role during ovulation, implantation and parturition. Inactivation of PAF is carried out by specific acetylhydrolases (PAF-AH) removing the sn-2 acetyl group. The aim of our study was to elucidate the role of PAF for bovine placentomal angiogenesis and trophoblast biology via detection of the signal terminator.

Methods: PAF-AH mRNA was detected in bovine placentomes from day 30 to day 270 and immediately prepartum by RT-PCR (isoforms PAF-AH Ib β, PAF-AH Ib γ, PAF-AH II, plasma-PAF-AH) and localized in situ hybridization for isoform Ib, which is the only PAF-specific isoform. Immunohistochemistry was used to demonstrate PAF-AH protein in tissue sections.

Results: Specific mRNAs of PAF-AH-isoforms Ib β, Ib γ, II and the plasma-isoform were present in the bovine placenta throughout gestation. During gestation, mRNA of PAF-AH Ib and PAF-AH protein were co-localized mainly in immature trophoblast giant cells (TGC) and to lesser extent in maternal epithelial cells and fetal and maternal vascular endothelium. In contrast, in placentomes taken immediately prepartum PAF-AH was expressed predominantly by maternal stroma cells, while most TGC were negative.

Conclusion: The specific localization of PAF-AH in immature TGC throughout gestation suggests a role for PAF in TGC programming and paracrine stimulation of angiogenesis. The parturition-related switch to the maternal stroma implies the involvement of PAF in parturition and re-programming of prepartum tissue.

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MATERNAL DIABETES REDUCES THE FRACTIONAL AREA OF VESSELS IN PLACENTAL VILLI.

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Objectives: Pregnancies with Type 1 diabetes mellitus (DM) show increased angiogenesis of feto-placental vessels. Here we test whether the diabetic environment alters the fractional area of vessel tissues in sectioned villi in different groups with DM.

Methods: Paraffin embedded villous tissues from the following groups were analysed: controls, diet-treated gestational DM (GDM), insulin-treated GDM, type-1 DM without and with further complications. Sections were stained for CD34 (QBend-10) and the fractional areas occupied by villi and vessels were determined using randomly-selected microscopic fields of villous tissue.

Results: In control placentas the fractional area of vessels occupied more than 50% of the cross sectional area of terminal villi and was significantly higher compared to all DM groups. In insulin-treated GDM, the fractional area of vessels was lowest (about 20%). While in GDM the number of vessel cross sections per villus was similar to controls (about 4 per villus), type 1 DM cases tended to have more vessel cross sections per villus (about 5.7 per villus).

Conclusion: Despite an increased proliferation of placental vessels combined with a higher number of vessel cross sections per villus, the fractional area occupied by these vessels is profoundly reduced in diabetes. This suggests an elongation of the vessels without widening of their lumen in DM. If not compensated for by an increased placental mass, this may lead to a decreased oxygen supply of the fetus.

P1.10

QUANTIFICATION OF MURINE FETOPLACENTAL VASCULATURE USING MICRO COMPUTED TOMOGRAPHY.

MY Rennier, KJ Whiteley, SL Adamson and JG Sled. *Mouse Imaging Centre, Hospital for Sick Children, Toronto, Canada and †Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

Objectives: The recent availability of mutant strains of mice with placental insufficiencies has emphasized the need for methods to quantify the normal and abnormal vascular structure in the murine placenta. This study evaluates micro-CT as a new method to quantify the three dimensional structure of the murine placental vasculature and examines changes during development.

Methods: The arterial feto-placental vasculature was perfused with Microfil, a radio opaque silicone rubber contrast agent, at two gestational ages (day (D) 15.5 and 18.5 (term)). The specimens were scanned in a micro-CT scanner to produce data sets with a voxel size of 13µm. Reconstruction produced three dimensional images and enabled generation of geometric models of the lumen surface, excluding capillaries. Surface area and diameter measurements were made in 7 D18.5 and 6 D15.5 specimens.

Results: Average surface areas were 102 ± 20 mm² at D15.5 and 200 ± 30 mm² at D18.5 (p<0.0001). Average umbilical artery diameter was 0.54 ± 0.02mm at D15.5 and 0.64 ± 0.02mm at D18.5 (p<0.0001). Diameters (mm) in subsequent vessel generations (1st, 2nd, and 3rd) for D15.5 vs. 18.5 were 0.34 ± 0.05 vs. 0.48 ± 0.07, 0.25 ± 0.04 vs. 0.34 ± 0.06, and 0.18 ± 0.02 vs. 0.24 ± 0.04 respectfully, each differing with p<0.005.

Conclusions: We have demonstrated the first use of micro-CT for quantitative evaluation of the murine feto-placental vasculature. Results suggest this method will be useful for future evaluation of murine models with placental insufficiencies and show it is sufficiently sensitive to detect growth of the placental vasculature in late gestation.

P1.11

VASCULAR ENDOTHELIAL GROWTH FACTOR UPREGULATES THE EXPRESSION OF ENDOTHELIN-1 BY MATRIX METALLOPROTEINASE-2.

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Objectives: We investigated the mechanism of VEGF-induced, endothelin-dependent inhibition of vasorelaxation using Human Umbilical Vein Endothelial Cells (HUVECs) and serum levels of VEGF, Matrix Metalloproteinase-2 (MMP-2) and Endothelin-1 (ET-1) in preeclampsia patients.

Methods: HUVECs were incubated for 6–48 hours in medium of various concentrations (0.1–100 ng/mL) of VEGF. They were then incubated with phosphoramidon which is known as endothelin converting enzyme-1 (ECE-1) inhibitor or tissue inhibitors of metalloproteinase(TIMP-2). The cell-free supernatant is known as endothelin converting enzyme-1 (ECE-1) inhibitor or tissue inhibitors of metalloproteinase (TIMP-2) with 10 ng/mL of VEGF. The cell-free supernatant was then assayed for ET-1 using ELISA. To measure levels of VEGF, MMP-2 and ET-1 was measured by ELISA. To measure levels of VEGF, MMP-2 and ET-1 by ELISA, blood samples were obtained from pregnant women with (n=20) or without (n=20) preeclampsia before labor. The results were analyzed using Mann-Whitney U test and linear regression analysis.

Results: ET-1 levels increased in the media of VEGF treated HUVECs in dose-dependent manner. ET-1 levels in the media were decreased not by phosphoramidon but by TIMP-2. The preeclampsia group had higher levels of VEGF, MMP-2, ET-1 (VEGF level, 2.39±0.75 vs 0.28±0.14, p<0.001; MMP-2 level, 31.99±6.05 vs 11.8±11.75 ng/ml, p<0.001; ET-1 level, 43.54±13.92 vs 14.83±5.77 pg/ml, p<0.0001) than the control group. There were positive correlations among them(VEGF and MMP-2, r=0.91, p<0.0001; MMP-2 and ET-1, r=0.780, p<0.0001; VEGF and ET-1, r=0.671, p<0.0001).

Conclusion: These findings suggest that VEGF-induced, endothelin-dependent inhibition of vasorelaxation might be mediated by increased ET-1 via MMP-2 upregulation rather than ECE-1.
P2.01

GLYCOXYLATION AT THE FETOMATERNAL INTERFACE OF THE HAEMOMONOCORHAL PLACENTA IN TENREC, HYENA AND HUMAN.

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Objectives: Haemochorial placentation has evolved independently on different occasions. It is known that the maternal-facing trophoblast surface is heavily glycosylated and here we examine whether glycosylation is conserved between evolutionary diverse species that share a haemomonochorial form of placenta; two (hyena and human) have a syncytial and the other (tenrec) a cellular type of trophoblast.

Methods: A 65 day placenta (term 110 days) from hyena (Crocuta crocuta), near-term placenta from two tenrecs (Echinops telfairi) and a placenta from a normal human delivery were processed and embedded in epoxy resin. Semithin sections were stained with 24 biotyi- nylated lectins using an avidin peroxidase revealing system.

Results: There were significant differences in glycosylation of the trophoblast in the three species, with absence of group A substance bound by Dolichos biflorus lectin, and GalNAc1→3Galβ1 (Vicia villosa lectin) in hyena and human, but with heavy binding in tenrec. There was also no terminal GalNAc1→3Galβ1 (Helex pomatia lectin) or GalNAc1→6Galβ1 (Wisteria floribunda lectin) on hyena or human apical trophoblast though present in tenrec. Complex N-glycan was sparse in hyena trophoblast as was apical α2,6-linked sialic acid (Sambucus nigra lectin) in human, and there were significant apical-betaal differences between syncytial plasma membrane glycans in both hyena and human, especially with respect to N-acetyl glucosamine oligomers.

Conclusions: Convergent evolution of haemochorial placentation is not necessarily accompa- nied by similar patterns of glycosylation, and species continue to exhibit distinctive glyco- types at the fetomaternal interface.

P2.02

PLACENTATION IN THE AMAZONIAN MANATEE, TRICHECHUS INUNGUIS

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Objectives: To determine the nature of the interhemal membrane in a representative of Sirenia (manatees and dugong) and relate the findings to placation in the elephant and other members of the superordinal clade Afrotheria.

Methods: A captive breeding program for the Amazonian manatee (Trichechus inunguis) is ongoing at the National Research Institute for Amazon (INPA). Placentae and membranes were secured immediately upon delivery. The membranes were examined and photographed. Pieces of tissue were fixed in phosphate-buffered paraformaldehyde for histology and paraformaldehyde-glutaraldehyde for transmission electron microscopy (TEM). Tissues were processed by standard methods for histology and TEM.

Results: We could confirm that the manatee has a large, four-lobe allantoic sac. The cord is rather short and sends four sets of branches to the placenta. These vessels subdivide the folds of membrane that divide the allantoic sac. The placenta is zonary in shape. The interhemal membrane is of the endotheliochorial type. An irregular interstitial lamina underlies the endothelium of the maternal vessels whereas there is a normal basal lamina around the fetal vessels. There is a single layer of cytrophoblast. This is in contrast to the interpretation of Wislocki, who interpreted the interhemal membrane as hemochorial and the trophoblast layer as syncytial.

Conclusions: Placation in the manatees resembles that of elephants to which they are related on morphological grounds as well as by molecular systematics. The four-lobe allantoic sac is a feature shared by all six orders that constitute the clade Afrotheria.

P2.03

TRANSFER OF THE HUBRECHT LABORATORY COLLECTION FROM UTRECHT TO THE MUSEUM FUR NATURKUNDE, BERLIN

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The Hubrecht Lab Collection is an outstanding collection of comparative embryology that is based on the material of the collection by A.A.W. Hubrecht who started to collect animals in the late 19th century. Besides collecting local species, Hubrecht’s deep interest in comparative embryology resulted in a vast number of vertebrate embryos from animals in the late 19th century. Besides collecting local species, Hubrecht's deep inter- est in comparative embryology resulted in a vast number of vertebrate embryos from around the world. His work led to the foundation of the Hubrecht Laboratory in 1916 which today still bears his name. The Hubrecht collection served as the nucleus for the embryological collection of the Hubrecht Laboratory, which was enlarged by donations from developmental biologists such as Hill, Bolk, Mangold, Speman and others. In total, the collection is by far the largest of its kind and houses 30,000 to 80,000 histological slides, 2,000 jars of specimens stored in alcohol and numerous specimens embedded in paraffin representing an estimated 600 species in 175 families and 10 classes. Due to a shift in research techniques in the Hubrecht Laboratory, this collection was transferred to the Museum für Naturkunde in Berlin as a permanent loan in order to keep this invaluable collection complete and open to researchers. In order to publicise the new location of the collection, the transfer of the collection to its new home in Berlin is illustrated in this poster presentation.

P2.04

TROPHOBLAST INVASION IN MICE AND RATS ACCORDING TO MATHIAS DUVAL (1844-1907)

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Objectives: Recent immunohistochemical studies have clarified patterns and pathways of interstitial and endovascular trophoblast invasion in mice and rats. We re-examined a much quoted but rarely read comprehensive study by Duval on rodent placation in order to compare his views on trophoblast invasion to present insights, with the addi- tional aim to uncover still unanswered questions.

Methods: A close reading of “Le placenta des Rongeurs” (Paris: Felix Alcan; 1892) with reference to our own histological collection of pregnant rat and mouse uteri.

Results and Conclusions: Duval recognized invasion of trophosphagial cells into the decidua, correctly identified endovascular trophoblast invasion of spiral arteries in the rat, but erroneously extrapolated and over-generalized this vascular pathway to explain placental outgrowth into the decidua in the mouse. This made him to interpret the trophosphagial glycogen cells as islands of decidua incorporated within the placenta, an idea which was recently refuted by cytokerin immunohistochemistry. In both spe- cies interstitial and endovascular trophoblast are now known to represent two different trophoblastic subpopulations. While the glycogen cell origin of interstitially invading trophoblast is now well established, the precursors of endovascular and/or perivascular trophoblast in rats and mice are not yet clearly identified.

IFPA 2005 Meeting
**P2.05**

**CO-LOCALIZATION OF \( \beta_1 \) INTEGRIN AND CYTOSKELETON-ASSOCIATED PROTEINS IN PRIMARY BOVINE CARUNCULAR CELLS**

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**Objectives:** Binding of integrins to the extracellular matrix may induce signal cascades which are involved in embryo implantation, and migration of bovine trophoblast giant cells. Functional activation of integrin receptors is accompanied by the accumulation of cytoketol-associated proteins and specific phosphorylation products. The aim of our study was to show whether or not this ligand binding is functional in cultures of primary bovine caruncular cells.

**Methods:** Caruncular cells were isolated by collagenase I incubation, and when grown to confluence after the second passage, subjected to immunofluorescence for cytoketol filaments \( \alpha \)-smooth muscle actin (\( \alpha \)), cytokeratin (CK), desmin (D) and vimentin (V) as well as for the integrin subunit \( \beta_1 \), for the associated proteins talin (T) and \( \alpha \)-actinin (AC), and for phosphotyrosin (PT). The presence of the respective proteins in caruncular tissues was confirmed by Western blot. **Results:** Epitheloid cells were positive for CK, and showed a weak immunoreaction for V, while the few fibroblastoid cells expressed A, D and V. AC was either located in the vicinity of the Golgi apparatus or along the lateral cell-cell borders, where it was co-localized with \( \beta_1 \) integrin, which was also observed as small plaques towards the culture flask ground. T and PT were co-localized to \( \beta_1 \) integrin plaques towards the flask ground, however at the border of single cell colonies both were arranged as dense waves.

**Conclusion:** The specific co-localization of AC, T and PT to the \( \beta_1 \) integrin subunit suggests that in cultured bovine caruncular epithelial cells the binding of integrins is functional, thus, these cells may be used for integrin binding studies.

**P2.06**

**POSSIBLE ROLE OF BOVINE TROPHOBLAST GIANT CELLS IN THE VERTICAL TRANSMISSION OF NEOSPORA CANINUM IN CATTLE.**

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**Objectives:** Neospora (N.) caninum is an apicomplexan parasite that has brought several concerns to brazilian cattle raisers due to its relationship to fetal loss in *Bos taurus* and *Bos indicus* breeding. However, the precise mechanism of the parasite’s vertical transmission and induced abortions are not completely understood. The bovine trophoblast giant cells (TGC) play a major role in the maternal-fetal interactions. These cells migrate during the entire pregnancy period, from the chorionic connections to uterine epithelium. This study aimed to investigate the possible role of TGC as plasmodic cells and consequently their participation in the bovine vertical transmission cycle of *N. caninum*.

**Methods:** The TGC were isolated by discontinuous Percoll gradient at 1043 and 1060 g/ml in DMEM medium supplemented with 10% bovine fetal serum, and characterized with Hoechst 33342 staining specific to the nucleus. Isolated TGC were cultured in the same medium described above, and infected with 107 tachyzoites of *N. caninum* NC-1 strain.

**Results:** Slides with smears of culture derived normal and infected TGC were stained by Giemsa technique. Multiplication of parasites took place in 2-3 day cycles. Healthy cows’ placenta and normal and infected cultured TGC were immunostained with monoclonal antibodies MAC-387, against TGC, and anti-iNOS demonstrating their phagocyte capacity against the parasite. Thus, TGC were characterized as cells with macrophagic activity (lineage is too strong), which may host *N. caninum* in vitro.

**Conclusion:** Therefore, we may conclude that TGC could potentially participate in the vertical transmission (from mother to fetus) of the bovine neosporosis.

**P2.07**

**EXPRESSION OF THE CYTOKINE MIF (MACROPHAGE MIGRATION INHIBITORY FACTOR) IN THE PLACENTA OF THE SMOOTH HOUND SHARK.**

Mustelus canis. 1. P. Paulesu, 2. E. Bigliardi, 3. S. Jantra, 1. R. Romagnoli and 3. W. C. Hamlett. 1Department of Physiology; 2Department of Evolutionary Biology, University of Siena, Siena, Italy and 3Department of Anatomy & Cell Biology, Indiana University School of Medicine, Notre Dame, IN, USA.

**Objectives:** The role of cytokines in maternal-fetal immunotolerance has been largely investigated in mammals. We recently showed that cytokines, namely the Interleukin-1 family, are also expressed at the maternal-fetal interface of non-mammalian vertebrates including squamate reptiles and elasmobranch fishes. In this study we focused our attention on MIF (macrophage migration inhibitory factor), a pro-inflammatory cytokine recently shown to be involved in human and murine reproductive events including ovulation, embryo receptivity and gestation. Specifically, expression of MIF was investigated in the placenta of *M. canis*, a placental viviparous shark of the family triakidae.

**Methods:** Formalin-fixed, paraffin embedded tissues were examined by immunohistochemistry and anti-human MIF polyclonal antibodies. **Results:** a positive staining for MIF was shown both, in the placenta and uterus. **Conclusions:** The presence of MIF at the maternal-fetal interface of elasmobranch fishes, as well as in mammals, suggests that MIF is a universal mediator of maternal immunotolerance in viviparous placental vertebrates.

**P2.08**

**GROSS MORPHOLOGY AND ULTRASTRUCTURE OF THE SUBPLACENTA IN OCTODON DEGUS AND PETROUSM TYPICUS – TWO HYSTRICOGNATH RODENTS WITH UNUSUAL PLACENTAL STRUCTURE.**

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**Objectives:** Hystricognath rodents show a disjunct distribution in South America and Africa, yet are widely accepted as a monophyletic taxon. One of the defining characters is the occurrence of a subplacenta, a structurally distinctive area that is regarded as functionally linked to placental lobulation. However, recent studies revealed hystrocnagths without marked lobulation, drawing into question the homology of the subplacenta character. **Methods:** We investigated ontogenetic stages from early pregnancy to near term from the South American degu Octodon degus (d 25 – d 84) and the African dassie rat Petromus typicus (d 35 – d 85) with respect to conventional light and electron microscopy. **Results:** Although pronounced placental lobulation is absent, the subplacenta of both species share important similarities to other hystrocnagths: The organ develops during early and mid-term stages and degenerates towards term. At first, a close association to maternal arteries is acquired, but it is lost during mid-term and followed by access to the fetal blood supply system. Internally, the subplacenta possess folded layers of cellular trophoblast (cytotrophoblast) that surrounds internally situated syncytial trophoblast and connective tissue, becoming the main source of cellular trophoblast during ontogeny. **Conclusions:** The subplacenta of Octodon and Petromus is regarded as homologous to other hystrocnagths, confirming the systematic unity of the group. Currently, a new hypothesis according to the functional relevance of this region is tested together with other authors (see Zaki et al., this volume).
A COMPARATIVE VIEW AT THE PLACENTATION OF SUNCUS MURINUS AND CROCIDURA RUSSULA

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Objectives: Suncus murinus and Crocidura russula, representatives of the two most diverse genera of the Crocidurinae, were used for a comparative study of the morphology and morphogenesis of Crocidurine placentation, which is still insufficiently known for Crocidura.

Methods: The placentae of three gestational stages (14, 22, 26 dpc) of Suncus and seven stages (12, 15, 17, 18, 21, 25/26, 29/30 dpc) of Crocidura have been histologically studied by light microscopy. Additionally, two placentae (14, 22 dpc) of Suncus were prepared for ultrastructural investigation using transmission electron microscopy.

Results: Both species have a discoidal, definitive chorioallantoic placenta of the labyrinthine type, that is situated at the antimesometrial side of a bicornuate uterus. They show a large, incompletely inverted, permanent yolk sac with a splanchnopleure, which functions as a haemopoietic organ. A sheath-like structure with still unclear origin surrounds the attachment site of the allantoic stalk. Suncus and Crocidura both have a broad haemophagous region, responsible for the iron transfer between mother and fetus. The ultrastructural studies of the Suncus placentae reveal that the interhemal membrane is mostly of endothelio-chorial nature.

Conclusions: Suncus murinus and Crocidura russula show many similarities in their mode of placentation, which reflects the close phylogenetic relationship of these two genera.

CORRELATION OF TROPHOBLAST GIANT CELL INVASION AND CONNEXIN 43 EXPRESSION IN THE BOVINE PLACENTOME

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Objectives: Connexins (cx) are structure proteins of gap junctions differing according to their location and function. In human and rodent reproduction, deficiencies in their expression lead to inadequate embryo implantation and trophoblast invasion and also characterize stages of malignancy in endometrial carcinogenesis. Therefore, cell-cell communication via gap junctional cx may be essential for the restricted trophoblast invasion in the synepitheliochorial placenta of the bovine. In this study the possible role of cx43 during placentomal development was investigated.

Methods: Frozen sections, from day 90-210 of pregnancy, were analysed using indirect immunofluorescence and confirmed by Western blot analysis.

Results: Cx43 was localised in the caruncular stroma, fetal mesenchyme, mononuclear trophoblast and trophoblast giant cells (TGC). The mononuclear trophoblast showed an apical-lateral cell membrane associated cx43 expression. In TGC, cx43 signals differed depending on the localisation within the placentome. In the centre of the placentome cx43 was associated to the cell membranes whereas at the base of the fetal villi TGC additionally showed cytoplasmatic cx43 specific fluorescence. In contrast, TGC which were about to fuse with uterine epithelial cells and hybrid cells were negative.

Conclusions: Apical cx43 localisation supports the hypothesis that cx43 connexons may be involved in the regulation of cell proliferation without forming gap junctions. The correlation of TGC invasion with the loss of cx43 suggests that cx43 plays an important role for the differentiation and migration of TGC. Funded by the German Research Foundation (DFG).
INTERACTIONS OF MACAQUE BLASTOCYSTS WITH EPITHELIAL CELLS IN VITRO.

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Objectives: Early in vitro studies of blastocyst formation in several primate species have demonstrated the feasibility of such studies. An initial study of in vitro fertilized oocytes cultured with buffalo rat liver cells suggested that nonuterine epithelial cells might be used to assess blastocyst adherence and penetration in vitro.

Methods: Macaque blastocysts were incubated with different epithelial cell lines or with matrigel. The interaction was studied using light and transmission electron microscopy.

Results: In general, zona-free blastocysts attached two days after placing on the substrates. MDCK cells provided optimal conditions for blastocyst development. The best preparations showed some development of epiblast and an amniotic cavity as well as cytotrophoblast and syncytial trophoblast. Distribution of syncytial trophoblast at the margin of the site and cytotrophoblast centrally was similar to that seen at the trophoblastic plate stage in this species. However there was less syncytiotrophoblast than is normally found at this stage, and total time from fertilization to the trophoblastic plate stage was delayed.

Conclusions: While in vitro studies with blastocysts cannot completely mimic the intrauterine environment, they can illustrate some of the potential interactions and provide a situation in which parameters may be manipulated.

PATTERN OF EXPRESSION OF TUMOR NECROSIS FACTOR AND TUMOR NECROSIS FACTOR RECEPTOR 1 IN RAT UTERINE TISSUES DURING PREGNANCY

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The uterine tissue remodeling that occurs throughout pregnancy is associated with the apoptotic process, though the mechanisms involved are still poorly understood. Tumor necrosis factor (TNF) is a cytokine with multiple effects on the cells. It can act as a pro-apoptotic or as a survival factor. It exerts its functions by binding to its receptors 1 and 2. In order to investigate the involvement of TNF/TNFRI signaling pathway the expression of these two factors in the uterine tissue during rat pregnancy was carried out by immunohistochemistry. Apoptotic cells were detected using anti-active caspase-3 antibody. Similar immunoreactivity of TNF and TNFRI was observed in the antimesometrial decidua (AMD), mesometrial decidua (MD) and granulated metrial gland (GMG) cells in the metrial gland. The peak of expression was found on day 8 in AMD, on day 14 in the MD and on day 16 in GMG cells. This was correlated with the expression of active caspase-3. These results suggest that regression of decidual tissue may involve the death receptor pathway mediated through TNFRI. On the other hand, the smooth muscle cells of the blood vessels and the circular muscle layer revealed a different pattern of expression. The circular muscle layer was TNFR1-positive, whilst active caspase-3 negative, suggesting that TNF/TNFRI signaling pathway may perform not a proapoptic but a survival role in this tissue. The smooth muscle cells of blood vessels expressed TNF and active caspase-3 till day 12 of pregnancy, though TNFR1 was not detected, suggesting that apoptosis is triggered by other factors, such as the Bc12 family members associated to the mitochondrial pathway.

MECHANISMS REGULATING THE EXPRESSION OF INDOLEAMINE 2,3-DIOXYGENASE DURING DECIDUALIZATION OF HUMAN ENDOMETRIUM

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Objective: To clarify the physiological importance of indoleamine 2,3-dioxygenase (IDO) in human pregnancy, we have studied how the expression of this enzyme changes during decidualization of human endometrium at both the cell and tissue level.

Methods and Results: The level of IDO mRNA expression (determined by RT-PCR) was higher in decidual than in endometrial tissue. Uterine decidual tissue in ectopic pregnancy similarly showed increased mRNA expression. Immunohistochemistry demonstrated that IDO protein immunoreactivity was found in glandular epithelium and in stromal cells. The intensity of this immunoreactivity was increased in decidualized tissue. In a cell culture model, the level of IDO mRNA was suppressed specifically by progesterone-induced decidualization of isolated endometrial stromal cells. IDO protein abundance (determined by Western blot) was also decreased by progesterone-induced decidualization. However interferon-γ, a potent stimulator of IDO gene expression, increased the level of IDO mRNA and protein in both non-decidualized and in decidualized cells. IDO activity was also decreased by progesterone-induced decidualization but enhanced following interferon-γ treatment. Expression of other interferon-γ inducible genes (STAT1 and tryptophan-1-d RNA synthetase) showed the same pattern as that of IDO in tissue samples, but was not changed by decidualization in the cell culture model.

Conclusion: These data suggest that despite suppression by progesterone, IDO expression in endometrial stromal cells may increase during decidualization due to stimulation by interferon-γ secreted by infiltrating leukocytes.
LIF REGULATED GENES IN THE LUMINAL EPITHELIUM AT EMBRYO IMPLANTATION

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Objective: Leukemia Inhibitory Factor (LIF) is essential for converting a non-receptive uterus to a fully receptive uterus allowing the blastocyst to implant. Previous studies have shown that LIF, produced in the glandular epithelium, binds to LIF receptors that are expressed on the Luminal Epithelium (LE), so activating the JAK-STAT signaling pathway. We have initiated a series of studies to identify which genes are regulated by LIF in the LE at the time of implantation, as they may be important to regulating the change in receptivity and implantation. This study aimed to identify LIF regulated genes in contrast to the effects of estrogen which, in the mouse, also increases at implantation.

Methods: Using array technology, we compared the gene expression profiles of LE from wildtype delayed implantation (DI) mice injected with LIF and LIF deficient DI mice injected with estrogen. Each sampling point was set at 0, 1, 3, 6 hours after injection. All experimental procedure was followed by the technical manual from the supplier.

Results and Conclusion: We identified some 320 genes that show statistically significant differences among each time point in wildtype DI mice. Annotation of these genes reveals that many of the genes regulate angiogenesis, cell adhesion/extracellular matrix formation, and removal of metabolic/degradation products. A few regulate transcription and signaling. Most genes that show significant differences in wildtype DI mice did not correspond to those in LIF deficient DI mice. Genes up-regulated just 1 hour after LIF administration were compared to LIF deficient DI mice. Some genes showed no significant difference at each time point in LIF deficient DI mice. Two of the genes were ESTs. They are cloned and their localization is examined at gene and protein levels. We are also using morpholinos, introduced into the uterine LE, to determine whether these two ESTs may also mediate interactions between the blastocyst and LE at implantation.

MATRIX METALLOPROTEINASE (MMP)-26 EXPRESSION IS LOCALIZED IN DECIDUA

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Objective: The human matrix metalloproteinase (MMP)-26, also called matrilysin-2 or endometase, has been isolated as a matrilysin (MMP-7) homolog. MMP-26 mRNA is primarily expressed in epithelial cancers, such as lung, breast and prostate carcinomas, in their corresponding cell lines, and in a very limited number of normal adult tissues, such as the uterus, kidney and placenta. We focused on placenta and examined the localization of MMP-26 expression.

Methods: Total RNAs were isolated from 9 normal chorionic villi and 12 deciduals. Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed to evaluate the MMP-26 mRNA expression level. To identify MMP-26 protein localization, we performed immunohistochemical analysis. We examined the effect of estrogen and its receptor (ER) on MMP-26 expression in choriocarcinoma and endometrial carcinoma cell lines by real-time RT-PCR, western blot and luciferase assays.

Results: Real-time RT-PCR analysis revealed that MMP-26 mRNA expression was significantly higher in the normal human deciduals, compared with that in the chorionic villi. MMP-26 expression was localized only in the endometria. Estrogen and ER not only transactivated the MMP-26 promoter activity but also enhanced endogenous MMP-26 expression.

Conclusions: We found that MMP-26 expression was detected only in deciduals and ER overexpression induced MMP-26 expression. Our results provide evidence that regulation of MMP-26 promoter activity by estrogen may represent a mechanism for placental function.
Fetal and Placental Growth

P4.01
EXOGENOUS INSULIN LIKE GROWTH FACTORS IN EARLY PREGNANCY ACT VIA DIFFERENT MECHANISMS TO PROMOTE FETAL GROWTH
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Objectives: Gene ablation studies in mice have shown that insulin like growth factor (IGF)-II deficiency results in impaired fetal and placental growth, whereas IGF-I deficiency reduces fetal growth only. This study aimed to determine if IGFs can act in an endocrine manner to promote growth of the placenta and hence of the fetus, in guinea pigs.

Methods: Pregnant dams received 1mg/kg/day IGF-I, IGF-II or vehicle subcutaneously from day 20 to 38 of pregnancy (term 70 days) and were killed on day 62. Fetal and placental weights and maternal body composition were measured. Using Masson’s trichrome stain and double label immunohistochemistry, placental structure was assessed.

Results: IGF-II increased the number of viable fetuses by 25% (p<0.03), while total litter size was unaffected by either IGF. IGF-I and IGF-II increased fetal weight by 17% and 11%, respectively (p<0.04). IGF treatment did not alter placental weight but IGF-II increased the cross-sectional area and volume of placental labyrinth (exchange region) both by ~30% and the total exchange surface area by 39% (p<0.05). Within the labyrinth, IGF-II increased the volume of trophoblast and maternal blood space by 29% and 46%, respectively (p<0.02). IGF treatment did not affect maternal weight gain, however IGF-I reduced maternal adipose tissue weights by 30% (p<0.05).

Conclusions: Maternally derived IGF-II in early pregnancy promotes placental structural and functional development and fetal growth and viability, while IGF-I appears to act via the mother to enhance fetal growth. Increasing maternal IGF-II in early pregnancy may be a potential therapeutic approach to prevent placental insufficiency.

P4.02
MATERNAL ASTHMA, GlUCOCORTICOID TREATMENT AND PLACENTAL COMPOSITION.
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Objectives: Maternal pregnancy during leads to increased perinatal mortality and reduced birth weight which may be related to asthma severity, fetal hypoxia or drug treatment. Here, we test for differences in placental morphometry.

Methods: With informed consent, we studied non-asthmatic and asthmatic pregnancies grouped by treatment (mild cases received no glucocorticoids, moderate/severe cases received high doses of glucocorticoids). Random tissue samples of placenta were prepared for light microscopic stereology. Villi and capillaries were analysed using measures of height growth, remodelling, vascularization and branchiogenesis. Comparisons were drawn by 2-way ANOVA.

Results: No significant effects involving maternal age, weight gain or haematocrit, gestational age, birthweight, fetal haematoctrit or placental weight were detected. Within placentas, measures of angiogenesis, vascularization and capillary remodelling did not vary between groups or genders. There were significant group x gender interaction effects involving trophoblast volume (greater in asthmatics with female fetuses, smaller in those with males) and villus branching (greater in glucocorticoid-treated subjects with females, reduced in those with males).

Conclusions: Changes differ from those seen in pregnancies complicated by fetal hypoxia and do not support the notion that glucocorticoid treatment affects placental morphology regardless of fetal gender.

P4.03
INTRAUTERINE GROWTH RESTRICTION:RELATIONSHIP OF LACTATE CONCENTRATION TO PLACENTAL WEIGHT AND SEVERITY
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Objectives: to explore the relationship between fetal lactate concentration in IUGR fetuses of different clinical severity and to correlate it to fetal oxygenation and placental weight. Clinical severity was based upon fetal heart rate and umbilical pulsatility index (PI): Group 1: normal FHR and PI; Group 2: normal FHR and abnormal PI; Group 3: abnormal FHR and PI.

Methods: 70 IUGR were compared to 70 AGA pregnancies studied at the time of elective cesarean section. Maternal and umbilical blood samples were obtained. Fatty acids were extracted by the Folch method and relative concentrations (%) were determined by HRGC.

RESULTS: IUGR were delivered earlier (33.5±3.4wks, p<0.001) and had a lower birth weight (1536.9±569.1g, p<0.001) than AGA (38.5±11.1 wks, 3274.5±392.0) pregnancies. Placental weights were significantly decreased in IUGR (AGA 508.8±112.2g; IUGR 242.6±84.5g, p<0.001). Maternal plasma % of linoleic and (AGA 22.6±4.1; IUGR 19.3±2.9, p<0.01) and alpha-linolenic acid (AGA 0.41±0.32; IUGR 0.23±0.16, p<0.05) were decreased in IUGR, while arachidonic acid was slightly increased (AGA 5.1±1.0; IUGR 5.6±1.1, p<0.08): the conversion ratio arachidonic/linoleic acid resulted significantly increased in IUGR mothers (AGA 0.23±0.07; IUGR 0.29±0.07, p<0.05). In both venous and arterial umbilical samples arachidonic acid (vein: AGA 12.1±1.9; IUGR 10.8±2.2, p<0.05; artery: AGA 12.2±2.4; IUGR 10.2±3.5, p<0.05) and the conversion ratio arachidonic/linoleic acid were significantly higher in AGA than IUGR (vein: AGA 1.4±0.4; IUGR 1.4±0.3, p<0.01; artery: AGA 1.5±0.4; IUGR 1.2±0.4, p<0.08).

CONCLUSIONS: The different fatty acids profile present in IUGR pregnancies could be related to an inadequate transplacental transport as well as to a lack of the enzymes involved in the essential fatty acid biosynthesis. (The study was part of Perlip, a project supported by the EU). We thank the technical assistance of M. Morante.

P4.04
FATTY ACIDS IN NORMAL (AGA) AND INTRAUTERINE GROWTH RESTRICTED (IUGR) PREGNANCIES.
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OBJECTIVE: to compare fatty acid levels in mothers and fetuses of normal pregnancies (AGA) and pregnancies complicated by intrauterine growth restriction (IUGR).

METHODS: 31 AGA and 24 IUGR pregnancies were studied at the time of elective cesarean section. Maternal and umbilical blood samples were obtained. Fatty acids were extracted by the Folch method and relative concentrations (%) were determined by HRGC.

RESULTS: IUGR were delivered earlier (33.5±3.4wks, p<0.001) and had a lower birth weight (1536.9±569.1g, p<0.001) than AGA (38.5±11.1 wks, 3274.5±392.0) pregnancies. Placental weights were significantly decreased in IUGR (AGA 508.8±112.2g; IUGR 242.6±84.5g, p<0.001). Maternal plasma % of linoleic and (AGA 22.6±4.1; IUGR 19.3±2.9, p<0.01) and alpha-linolenic acid (AGA 0.41±0.32; IUGR 0.23±0.16, p<0.05) were decreased in IUGR, while arachidonic acid was slightly increased (AGA 5.1±1.0; IUGR 5.6±1.1, p<0.08): the conversion ratio arachidonic/linoleic acid resulted significantly increased in IUGR mothers (AGA 0.23±0.07; IUGR 0.29±0.07, p<0.05). In both venous and arterial umbilical samples arachidonic acid (vein: AGA 12.1±1.9; IUGR 10.8±2.2, p<0.05; artery: AGA 12.2±2.4; IUGR 10.2±3.5, p<0.05) and the conversion ratio arachidonic/linoleic acid were significantly higher in AGA than IUGR (vein: AGA 1.4±0.4; IUGR 1.4±0.3, p<0.01; artery: AGA 1.5±0.4; IUGR 1.2±0.4, p<0.08).

CONCLUSIONS: The different fatty acids profile present in IUGR pregnancies could be related to an inadequate transplacental transport as well as to a lack of the enzymes involved in the essential fatty acids elaboration. (The study was part of Perlip, a project supported by the EU). We thank the technical assistance of M. Morante.
IDENTIFYING MODIFIERS OF PLACENTAL DEVELOPMENT IN EGFR DEFICIENT MICE.

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The Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase known to activate multiple pathways regulating cell proliferation, differentiation, migration or invasion. Mice homozygous for a null allele of Egfr, Egfr<sup>tmlMag</sup>, display strain-dependent placental abnormalities resulting in embryonic lethality on some genetic backgrounds. Previous attempts to map modifiers of placental development in EGFR deficient mice have revealed that the phenotype is polygenic. Taking into account this genetic heterogeneity, we have developed a strategy for mapping genomic regions associated with adequate placenta in the absence of EGFR.

Methods: F1 hybrids were generated by crossing ALS/LtJ, which supports survival of EGFR deficient embryos, to 129S1/SvImJ and FVB/NJ, strains exhibiting embryonic lethality. The ALS/129 and ALS/FVB hybrids were backcrossed for four generations to 129 and FVB, respectively. ALS loci required for placental development in an EGFR-deficient context were isolated by selecting animals that produced Egfr<sup>tm<sub>Mag</sub></sup> homozygous pups to establish each subsequent backcross.

Results: Six independent lines of Egfr<sup>tm<sub>Mag</sub></sup> mice have been generated in which ALS placental modifiers were selected for and isolated on a 97% 129 background. One line of Egfr<sup>tm<sub>Mag</sub></sup> mice with ALS modifiers has been obtained on a 94% FVB background.

Conclusions: Dominant loci from the ALS strain are better able to support placentation in combination with the 129 genome compared to FVB. Groups of ALS modifiers have been captured in the 129 and FVB lines and genome scans performed on Egfr<sup>tm<sub>Mag</sub></sup> homozygous pups will reveal retained regions of the ALS genome. Candidate genes in ALS intervals include molecules involved in cell signaling and placentaion.

EFFECT OF MATERNAL ASTHMA ON BIRTHWEIGHT IN A BRITISH INNER-CITY POPULATION.

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Background: A study of selected, pregnant, asthmatic Australian women found that female offspring of untreated mothers had reduced growth and alterations in placental function. Objective: To evaluate the effect of asthma management declared during pregnancy on birthweight at an inner city hospital in England.

Methods: Between June 2001 to December 2003, 10,983 unselected women at antenatal clinics were questioned about asthma. Women with singleton uncomplicated pregnancies ending at term were selected (n ~ 718) and retrospectively matched with non-asthmatic controls (n ~ 718).

Results: Among boys born to women using inhaled steroids and bronchodilators (n ~ 170), 43% had birthweight < 10<sup>th</sup> centile; compared with 27% of controls (P = 0.011; OR (95% CI): 2.51 (1.52-4.14)). Taking account of smoking, ethnicity, gestational age and parity the mean birthweight reduction (95% CI) was 106g (2.71-210); P < 0.05. For girls the proportions were 28% and 27%. In women using bronchodilators only (n ~ 178) or declaring no treatment (n ~ 370) birthweights were not significantly reduced in boys or girls.

Conclusions: Our finding that boys born to asthmatic women, rather than girls, were at higher risk of low birthweight merits further investigation. Exploring the impact of the differences in patient management and study design between the two studies could suggest novel influences on placental function.

PERINATAL OUTCOME IN ELBW ACCORDING TO MODE OF DELIVERY.

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Objectives: Birthweight and gestational age at delivery determine morbidity and mortality in preterm babies particularly in extremely low birthweight (ELBW ≤ 1000 gr) babies. The objective of this study was to evaluate whether mode of delivery might influence neonatal outcome.

Methods: Mode of delivery (vaginal or cesarean — VD, CS), neonatal mortality and morbidity of 55 ELBW fetuses born between 1996 and 2002 were analyzed. Results are mean ± sd. Data were analyzed by χ<sup>2</sup> and Student t test for unpaired samples.

Results: Mean gestational age at delivery was 28 ± 2.2 weeks (range 23-32) whereas mean birthweight was 705 ± 177 grams. 49 were livebirths with 34 babies < 10<sup>th</sup> percentile (62%), 40 (81.6%) were delivered by CS whereas 9 cases (18.4%) delivered vaginally for irreversible preterm labour. Indication for CS was IU/GR (75%), abrubtio placentae (7.5%), preeclampsia (12.5%) or pPROM (5%). Birthweight, Apgar score at 1’ and 5’, and intubation at 5‘ were not different according to mode of delivery however gestational age at delivery was significantly lower in the VD group compared with the CS group (24.5 ± 1 vs 28 ± 2; p < 0.001). More neonates delivered by CS underwent mechanical ventilation (100%) compared to those delivered by VD (66.7%) (p < 0.001) although corticosteroids were administered only in the CS group. Neonatal survival rate was not different (VD 39/3 vs CS 24/40).

Conclusions: In our population vaginal delivery proved to be a reasonable option for ELBW babies: mechanical ventilation was less frequent in babies born by VD even though corticosteroids were administered only in CS patients. Neonatal survival rate was similar.
INTRAUTERINE GROWTH RESTRICTION (IUGR): DOES WEIGHT AT BIRTH MATTER?

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Objective: IUGR determines an increased risk of perinatal outcome and of long term effects in adult life. The aim of the study was to evaluate whether birthweight might influence neonatal outcome in severe IUGR.

Methods: We studied 56 IUGR pregnancies (AC in utero <10th centile) with abnormal PI of the umbilical artery classified, according to FHR, as Group 2 (FHR normal: 22 cases) and Group 3 (FHR abnormal: 34 cases). At delivery (28-33 wks) 9 of Group 2 (IUGR 2 AGA) and 16 of Group 3 (IUGR 3 AGA) had a birthweight >10th centile whereas 13 of Group 2 (IUGR 2 SGA) and 18 of Group 3 (IUGR 3 SGA), <10th centile. Obstetrical complications, data at delivery, neonatal mortality and morbidity were compared to 83 AGA fetuses of similar GA.

Results: Maternal age, weight, height, BMI were similar among groups. PIH was more frequent in IUGR than AGA and, among IUGR, in SGA. GA at birth was similar: only IUGR 3 SGA were delivered earlier compared to IUGR 3 AGA (30 ±1.7 vs 31 ± 1.8: p<0.001). Birthweight, neonatal CC and length were significantly lower in IUGR than in AGA; IUGR 3 SGA presented significantly lower weight and length than IUGR 3 AGA. IUGR 3 was associated with lower Appgar score. Perinatal mortality did not differ among groups, late neonatal mortality was higher in IUGR than in AGA. Major neonatal complications (RDS, IVH, ROP, Sepsis, NEC, DIC) were increased in IUGR 2 AGA (p<0.01), IUGR 3 AGA (p=0.05) and IUGR 3 SGA (p<0.001) than AGA. Minor neonatal complications (anemia, hypoglycemia) were more frequent in IUGR than AGA.

Conclusions: Prematurity represents an important factor affecting neonatal outcome. However, IUGR is a crucial criterion for the prediction of neonatal outcome, independent of the actual weight centile at birth.

PERINATAL OUTCOME

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Objective: Recent studies have suggested that umbilical cord characteristics are related to perinatal outcome. In the present study, associations between umbilical cord diameter through gestation and perinatal outcome were evaluated.

Methods: 140 umbilical cords and placentas were collected prospectively from women between 20 and 42 weeks gestation delivering at Mount Sinai Hospital (Toronto, Canada). Cord length, weight, diameter (UCD) and placental weight were measured; the ratio of placenta weight and UCD was calculated (cUCD). Perinatal outcomes were recorded.

Results: Over four week intervals through gestation, cUCD progressively increased from 175 g/cm at 20 weeks to 481 g/cm at 39 weeks. Within each gestational age category there was no correlation between UCD and placental weight. At term, both mean UCD and cUCD less than the tenth percentile were correlated with birth weight less than the tenth percentile. Coiling index and insertion site were not related to UCD or cUCD. There were no significant associations between UCD or cUCD and mode of delivery, intrapartum non-reassuring fetal status, fetal anomalies or Apgar scores at any gestational age.

Conclusion: Thin umbilical cord diameter is associated with intrauterine growth restriction at term. Cord diameter may be an independent risk factor or part of the spectrum of utero-placental insufficiency leading to growth restriction. Funded by the Departments of Pathology/Laboratory Medicine and OB/GYN; Mount Sinai Hospital.
P4.13

THE BRUCE STORY CONTINUED - THE CELLULAR BASIS FOR PLACENTAL INSUFFICIENCY IN BRUCE-DEFICIENT MICE

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BRUCE is a highly conserved 528 kDa peripheral membrane protein of the trans-Golgi network. Owing to the presence of an N-terminal single baculovirus inhibitor repeat (BIR), BRUCE might function as an inhibitor of apoptosis protein(IAP). In addition, due to the presence of a C-terminal ubiquitin-conjugating (UBC) domain, BRUCE can covalently attach ubiquitin to substrates. Recently we reported that the inactivation of the BRUCE gene resulted in growth retardation and perinatal lethality of homozygous mutant mouse embryos. This effect could be linked to an impaired placental development characterized by a significant reduction of the spongiotrophoblast layer and impaired maturation of the labyrinth. No evidence for an elevated apoptosis rate was detectable in embryonic and extra-embryonic tissues and in knockout fibroblasts. Instead embryonic and placenta-derived fibroblasts show morphological and transcriptional alterations as well as changes in the proliferating activity. Similar changes can be observed in situ in trophoblast cells of the placental labyrinth and spongiotrophoblast layer. Despite the drastically reduced number of cells in S-phase, enlarged trophoblast cells with atypical expression pattern accumulate in the placental tissue of mutant embryos, predominantly within the spongiotrophoblast layer. Our data indicate that BRUCE deficiency causes irreversible cell cycle arrest and rapid entry into the stage of cellular senescence in a p53-dependent manner. Since this process is not exclusively restricted to trophoblast cells, the same effect in other BRUCE-expressing cell types may additionally contribute to intrauterine growth restriction and perinatal death of the mutant embryos.

P4.14

PROLIFERATION DEFECTS IN EPIDERMAL GROWTH FACTOR RECEPTOR NULL PLACENTAE CAN BE RESCUED IN THE ABSENCE OF CYCLIN-DEPENDENT KINASE INHIBITORS.

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Decreased Epidermal Growth Factor Receptor (EGFR) signaling in the placenta has been associated with intrauterine growth retardation (IUGR) in humans, and mice homozygous for a null allele of Egfr, EgfrtmlMag, display a strain-dependent placental phenotype that results in embryonic lethality on many genetic backgrounds. Most strains examined to date exhibit a reduced spongiotrophoblast layer but strains dying before mid-gestation are distinguished by a disorganized labyrinth layer. Previous studies using BrdU have shown that e30.5 Egfr null placentae contain fewer proliferating trophoblasts than wildtype placentae.

Methods: To characterize interactions between EGFR and cell cycle machinery in murine trophoblasts mice heterozygous for EgfrtmlMag and null alleles for genes regulating the cell cycle were intercrossed.

Results: Egfr nullizygous embryos were rescued 30% of the time in a Cdkn1a (p21)- but not Trp53- or pRb-deficient background. Histological analysis of the p21, Egfr nullizygous placentae showed a less severe labyrinth defect compared to Egfr nullizygous placentae with wildtype p21.

Conclusions: To better understand the P53-independent interaction between EGFR and P21 we have set up intercrosses with mice heterozygous for null alleles of Smad3, Cdkn1b (p27) and Cdkn2c (p18). Data from EgfrtmlMag crosses with SMAD3-deficient mice may identify a role for TGF-β signaling. Intercrosses with P27- and P18-deficient mice will reveal whether the relationship between EGFR and cyclin-dependent kinase inhibitors (CKIs) is specific for the CIP family of CKIs or is a more general interaction with CIP (P21, P27, P57) and INK4 (P16, P17, P18, P19) CKIs.
**Gene Expression**

**P5.01**

**COLONY STIMULATING FACTOR-1 REGULATES HOMEBOX GENE HLX1 EXPRESSION IN THE HUMAN PLACENTA**

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**Objectives:** Trophoblast cells perform essential functions in placental development. Decreased trophoblast proliferation is a hallmark of pregnancy disorders such as fetal growth restriction (FGR). Colony Stimulating Factor-1 (CSF-1) binding to its cell surface receptor, initiates a signal transduction pathway that leads to activation of transcription factor expression in the nucleus and culminates in increased trophoblast proliferation. Homeobox gene HLX1 is a transcription factor implicated in controlling trophoblast proliferation. CSF-1 and HLX1 levels are decreased in FGR. We examined whether CSF-1 acts through HLX1 to control trophoblast proliferation.

**Methods:** The cytotrophoblast cell line SGHPL-4 was used. CSF-1 stimulation of HLX1 mRNA expression was shown by RT-PCR. Downregulation of HLX1 expression was carried out by short interfering RNA (siRNA). Trophoblast proliferation was measured by 3H-thymidine uptake. To determine whether CSF-1 acts through HLX1, HLX1 was downregulated with siRNA, in the presence of CSF-1.

**Results:** CSF-1 stimulation increased trophoblast proliferation (p<0.001, n=6, t-test) as expected. CSF-1 stimulation also resulted in increased HLX1 mRNA expression (p<0.001, n=3, t-test). Downregulation of HLX1 by siRNA resulted in decreased trophoblast proliferation (p<0.001, n=4, ANOVA). Following siRNA-mediated HLX1 downregulation, proliferation was reduced even when stimulated by CSF-1 (p<0.001, n=4, ANOVA).

**Conclusions:** HLX1 is regulated by the cytokine CSF-1 in cultured trophoblast cells. CSF-1 acts through HLX1 to control trophoblast proliferation.

**P5.02**

**PLACENTAL GENE EXPRESSION IS ALTERED BY REPEATED ANTENATAL BETAMETHASONE TREATMENT IN SHEEP**

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Maternal treatment with antenatal glucocorticoids improves neonatal outcome following preterm birth. In sheep, both maternal and fetal intramuscular betamethasone injections improve preterm lung function. However, only maternal treatment causes fetal growth restriction suggesting impaired placental function. We aimed to quantify placental Igf2, Igf2r and Glut-1 mRNA expression in sheep following 3 repeated maternal (M3) or fetal (F3) intramuscular injections of betamethasone (3-beta).

**Methods:** Twenty six ewes were injected with 150mg medroxy-progesterone acetate on day 98 of pregnancy (term is 150 days) and randomised to maternal (M) or fetal saline (FS) (104, 111, 118 days) or 3-beta (104, 111, 118 days) treatment groups. At 146 days, ewes were killed and fetuses weighed. All placentomes were weighed and snap frozen. To determine if betamethasone treatments caused sustained changes in placental gene expression, RNA was extracted from B and C type placentomes from MS, M3, FS and F3 groups. Gene expression was quantified by real time RT-PCR.

**Results:** We have previously reported that only M3 reduced fetal weight (22%, p=0.038) but total placental weight was unaffected. Placental Igf2 and Igf2r mRNA were reduced by 35% and 26%, respectively in M3 compared to MS (p<0.05). Both genes were expressed more abundantly in placentas from fetal compared to maternal treatment groups (P<0.05). Placental Glut-1 expression was not altered by treatment.

**Conclusion:** These results suggest that repeated maternal betamethasone treatment causes sustained reductions in placental expression of genes involved in tissue remodelling (igf2 and igf2r) and placental transport and growth (igf2).

**P5.03**

**THREE PATIENT-SPECIFIC METHYLATION PROFILES IN THE H19 DMR IN HUMAN PLACENTA.**

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1aH Jammes, 2IG Gut and 1D Vaiman.

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**Objectives:** Methylation differences are found in several regions of the human genome during normal development. Abnormalities in DNA methylation patterns might play a role in the pathogenesis of human diseases. In particular, aberrant methylation at DMRs, homologous to mouse Igf2/H19, is present in several tumours and is known to be associated with disease. Thus, the study of specific DMR methylation levels in the normal adult tissue is of importance. We intended to examine differences in methylation levels of the H19 DMR in human placenta. For this purpose, we focused on one of the best characterized human DMRs, which has been linked to a number of pathological conditions such as diabetes and growth retardation. The H19 DMR is a DNA segment of unknown genes. BeWo cells were exposed to nutritional (amino acid restriction) or endocrine (cortisol) stress. PolyA+ RNA was isolated from treated and control cells, reverse transcribed and hybridised to the expression array in a dye-swap experiment. Differentially expressed genes were identified by sequencing of unknown genes. BeWo cells were exposed to nutritional (amino acid restriction) or endocrine (cortisol) stress. PolyA+ RNA was isolated from treated and control cells, reverse transcribed and hybridised to the expression array in a dye-swap experiment. Differentially expressed genes were identified by sequencing of the corresponding PCR products and comparison with gene databases using a BLASTn search.

**Results:** The following results were obtained. Cortisol exposure reduced expression of genes that regulate growth factor signalling cascades and increased expression of classical stress response genes. In contrast, amino acid restriction decreased expression of these stress genes but increased expression of alternative stress pathways and genes involved in positive regulation of members of the insulin-like growth factor family. Nutritional stress not only triggers the regulation of transport mechanisms but also initiates a stress response, different to that of endocrine stress and changes the expression of genes involved in placental growth and development. Supported by SEERAD, ICA and Framework V QLK-1999-003377.

**P5.04**

**GENE REGULATION BY NUTRITIONAL AND ENDOCRINE STRESS: INVESTIGATION USING A HUMAN PLACENTA-SPECIFIC EXPRESSION ARRAY**

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Many placental transport mechanisms are regulated by nutritional and endocrine stress, for example, amino acid and iron transport systems. The signalling events that underlie this regulation are unknown. To investigate these we have used a human placenta-specific expression array. Not only can we identify regulated known genes but this array also allows us to express regulated proteins to aid identification of unknown genes. BeWo cells were exposed to nutritional (amino acid restriction) or endocrine (cortisol) stress. PolyA+ RNA was isolated from treated and control cells, reverse transcribed and hybridised to the expression array in a dye-swap experiment. Differentially expressed genes were identified by sequencing of the corresponding PCR products and comparison with gene databases using a BLASTn search.

Cortisol exposure reduced expression of genes that regulate growth factor signalling cascades and increased expression of classical stress response genes. In contrast, amino acid restriction decreased expression of these stress genes but increased expression of alternative stress pathways and genes involved in positive regulation of members of the insulin-like growth factor family. Nutritional stress not only triggers the regulation of transport mechanisms but also initiates a stress response, different to that of endocrine stress and changes the expression of genes involved in placental growth and development. Supported by SEERAD, ICA and Framework V QLK-1999-003377.
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Objectives: Since the common ancestor, mouse and human pregnancy-specific glycoproteins (PSG) have expanded independently into multigene families expressed virtually exclusively in placental trophoblasts, suggesting convergent evolution. However, within each species, it is unknown whether multiple paralogs have been selected for diversification of function, or for increased dosage of monofunctional PSG. We analysed the evolution of the mouse PSG sequences, and compared them to rat, human and baboon PSGs to attempt to understand the evolution of this complex gene family.

Methods & Results: Phylogenetic tree analyses indicate that the human N-domain and the mouse N1-domains exhibit a higher degree of conservation than that observed in a comparison of the mouse N1 and N2-domains, or mouse N1 and N3-domains. Compared to human PSG N-domain exons, mouse PSG N-domain exons have undergone less sequence homogenisation. High non-synonymous substitution rates in the CFG face of the mouse N1-domain, within a context of overall conservation, suggests divergence of function of mouse PSGs. The rat PSG family appears to have undergone less expansion than the mouse, exhibiting lower divergence rates and increased sequence homogenisation in the CFG face of the N1-domain. In contrast to most of the human PSG N-domains, the mouse PSG N1-domains do not contain an RGD motif. However, the conservation of RGD-like motifs in the N1-domains of mouse PSGs, which is not mirrored in the N2 and N3-domains, suggests possible functional significance of RGD-like motifs in the mouse PSGs.

Conclusions: Relative conservation between the human N-domain and the mouse N1-domain suggests that, despite independent gene family expansions, mouse and human PSGs retain conserved functions.
ENDOMETRIAL NK CELLS ARE DECREASED BY PREDNISOLONE AND ARE ASSOCIATED WITH αβ, INTEGRIN EXPRESSION
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Objectives: Recurrent miscarriage (RM) is a distressing condition that results from foiled implantation. Uterine natural killer cells (uNK) and integrins are thought to be important in pregnancy implantation. We aimed to investigate the relationship between endometrial integrin expression and uNK cells and the effect of prednisolone on these.

Methods: 110 women with RM of no known aetiology had their endometrium sampled in the mid-luteal phase. Immunohistochemistry with CD56 was used to identify uNK cells and αβ, nα, nβ, nγβ, for integrins detection. 29 women agreed to take oral prednisolone 20mg/day from one day of the menstrual cycle for 21 days when second biopsy was obtained and analysed. Integrins were analysed using the HSCORE system. 5000 cells per patient grin in women with RM.

Results: αβ, nα, nγβ integrins were not correlated with uNK cell number, but αβ, was strongly correlated with NK cell number (r=0.0004). Prednisolone reduced both uNK cells, (p=0.0004) and αβ, integrin expression (P=0.011), but not nα, and nγβ, expression.

Conclusions: Endometrial stromal αβ, but not nα, and nγβ, integrin expression was associated with uNK cell number. Prednisolone reduced uNK cell number and αβ, integrin in women with RM.

ENDOMETRIAL CD56+ CELLS AND IMPLANTATION FAILURE AFTER IVF
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Objective: Previous reports have suggested that CD56+ cells play a role in infertility and pregnancy loss. In this study we determine the number of endometrial CD56+ cells in women with implantation failure after IVF treatment.

Methods: Measurement of LH from the mid-follicular phase was used to identify the LH surge in 10 women with ≥ 2 implantation failures after IVF and 10 normal fertile women (both groups mean age 34 years, range 29-39). Endometrial biopsy was taken on day LH+7, fixed in formalin and embedded in paraffin wax. Immunohistochemistry was performed on 5μm dewaxed rehydrated sections. The antigen was unmasked by micro-waving in 10nmol citrate buffer. Slides were blocked for 1 hour and incubated overnight at +4°C with mouse anti-human CD56 at 1:100 dilution. An ABC kit and DAB substrate was used to visualise binding of antibody to antigen. The number of CD56 positive cells plus total stromal cells was counted in 10 ×400 magnification microscope fields for each biopsy.

Results: The endometrium of women with implantation failure after IVF had a significantly (P = 0.004 Mann-Whitney) higher number of CD56+ (21.95%, range 2.15%-13.87%) than the endometrium of the normal control women (6.23%, range 2.15%-13.87%). Conclusion: Implantation failure appears to be associated with raised levels of endometrial CD56+ cells.

IUGR IS ASSOCIATED WITH REDUCED NUMBER OF NK CELLS IN DECIDUA
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Objectives: Intrauterine growth restriction (IUGR) and pre-eclampsia (PE) may be due to impaired trophoblast invasion and maladaptation of uterine arteries. Maternal immune cells, especially natural killer (NK) cells, probably contribute to regulation of trophoblast invasion. In this project, we have studied leukocyte subpopulations in decidua basalis from women with PE and/or IUGR.

Methods: Decidua basalis tissues from pregnancies complicated with IUGR (birth weight ≤ 2.5 percentile) (n = 5), IUGR combined with PE (n = 18), isolated PE (n = 8) and healthy controls (n = 31) were collected by vacuum aspiration of the placental bed. Leukocytes were stained with monoclonal antibodies and counted in serial sections. The relative number of NK cells (CD56+, CD16+), T cells (CD3+), activated lymphocytes (CD25+, CD69+) was calculated (with total leukocyte count (CD45+ cells) as denominator).

Results: The proportion of NK cells in decidua basalis in IUGR was significantly lower (p = 0.02) than in healthy controls. Concomitantly, the CD25/CD45 ratio was increased in the IUGR group (p = 0.04).

Conclusions: We conclude that IUGR is associated with altered immune responses at the feto-maternal interface, as suggested by the reduced NK ratio and increased lymphocyte activation found in this study.

THE IMMUNOMODULATORY EFFECT OF MESENCHYAL STEM CELLS DERIVED FROM THE FOETO-MATERNAL INTERFACE
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Objectives: Protective mechanisms are likely to be present at the foeto-maternal interface during pregnancy, as foeto-specific alloreactive T cells in the decidua do not harm the foetus.

Methods: We isolated maternal and foetal mesenchymal stem cells (MSC) from vaginally acquired amnion fluid and second trimester placenta (amnion) obtained from women undergoing termination of pregnancy for social reasons. Culture-expanded cells were identified as MSC based on phenotype and their multilineage potential.

Results: Co-culture of MSC in a primary mixed lymphocyte culture (MLC) of unrelated HLA-mismatched responder-stimulator combinations resulted in a dose dependent inhibition of proliferation and of TH1 cytokine production. Moreover, MSC inhibited the proliferation of previously primed alloreactive T cells and of an antigen-specific T cell clone. Strikingly, foetal MSC showed a more potent inhibitory effect on both the primary and secondary responses compared to maternal MSC. Conclusion: MSC derived from the foeto-maternal interface have the capacity to inhibit naive and antigen-experienced (memory) T cells. Further investigation into the functional role of these cells may be useful for understanding the immunology of pregnancy and may lead to tolerogenic protocols in transplantation.
P6.05
UTERINE NK CELLS PERSIST AFTER THE 1ST TRIMESTER BUT FEWER EXPRESS PERFORIN AND GRANZYM B
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Background: Uterine NK cells (uNKs; CD56+ CD16- ) are prominent in the 1st trimester of pregnancy but thereafter are thought to decline in number. Our recent immunohistochemical studies of placental bed biopsies have not demonstrated a significant reduction in CD56+ cell numbers in decidua basalis between 1st (7-12 weeks) and early 2nd trimester (13-19 weeks). Previous studies documenting the reduction in uNKs have often relied on identification of cytoplasmic granules.

Hypothesis: The apparent reduction of uNKs after the 1st trimester is due to a reduced proportion containing cytoplasmic granules.

Methods: Placental bed biopsies were obtained from women undergoing elective surgical termination of pregnancy at 8-10, 12-14 and 16-20 weeks gestation (n=4 each group) at Royal Victoria Infirmary, Newcastle. Biopsies were formalin fixed, embedded in paraffin wax and serial 3 µm sections were stained with phloxine tartrazine or immunostained for CD56, perforin and granzyme B. The number of cells positive for CD56, perforin and granzyme B and with phloxinophilic cytoplasmic granules in decidua basalis was compared.

Results: CD56+ cells did not alter between the 3 gestational age groups. The number of cells with phloxinophilic granules was reduced at 16-20 weeks (30.4±5.2) compared with both 8-10 (52.5±1.0) and 12-14 (52.0±5.3) weeks. The number of CD56+ cells containing perforin and granzyme B decreased significantly with increasing gestational age (8-10, granzyme 56.0±5.2; perforin 54.3±2.9; 12-14, granzyme 43.5±2.9; perforin 46.0±2.5; 16-20, granzyme 28.8±1.4; perforin 27.7±1.8).

Conclusion: Although CD56+ cells persist into the second trimester of pregnancy, the proportion expressing perforin and granzyme B is reduced.

P6.06
INDOLEAMINE-DIOXYGENASE IS INVOLVED IN THE FETO-MATERNAL IMMUNOLOGICAL DIALOGUE
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Objectives: Indoleamine-2,3-Dioxygenase (IDO) cooperates with the enzyme tryptophan-dehydrogenase in the degradation of the essential amino acid tryptophan. Tryptophan deficiency leads to the inhibition of T-cell proliferation and differentiation. In the mouse it was shown that blocking IDO leads to the rejection of allogeneic fetuses by the maternal immune system.

Methods: To find out the possible significance for IDO in regulation of trophoblast invasion and maternal tolerance we studied IDO-expression in human endometrial tissue and specimens from 1st and 2nd trimester pregnancy by real-time PCR, immunohistochemistry and Western-blotting. In addition, mRNA expression was analyzed in isolated cell populations from endometrial and decidual tissues as well as 1st and 2nd trimester villous trophoblast by semiquantitative RT-PCR.

Results: In decidua of the 1st trimester IDO is expressed predominantly in the epithelial cells and leukocytes as shown by semiquantitative RT-PCR in isolated cell populations. This could be confirmed by immunohistochemistry using a monoclonal antibody which displays one clear band of the expected size by Western blotting. Expression of IDO is reduced from the 1st to 2nd trimester as revealed by real-time PCR. In contrast, in villous trophoblast, IDO expression increases from 1st to 2nd trimester. During the menstrual cycle IDO is expressed predominantly within the surface and glandular epithelium. Expression of mRNA is low during the proliferative phase and starts to increase at the time of a possible blastocyst implantation around cycle day 18-22. Expression remains high until the end of the luteal phase. Protein expression seems to be prolonged to the first days of the next cycle within the basal glands.

Conclusions: Expression of IDO in epithelial cells of the receptive endometrium and the 1st trimester of pregnancy on the one hand may be involved in regulation of trophoblast proliferation and invasion. On the other hand, IDO may inhibit proliferation of activated T-cells recognizing fetal antigens and therefore help to establish the maternal tolerance. IDO expression in villous trophoblast may tolerize maternal T-cells entering the placenta with the maternal blood supply.

P6.07
PROLACTIN (PRL) UP-REGULATES IDO (INDOLEAMINE-2,3-DIOXYGENASE) EXPRESSION IN CD14+ CELLS
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Purpose; Recently, it was shown that IDO plays essential roles for successful pregnancy. The physiological concentration of PRL increases dramatically during pregnancy and its receptor shares its structure and signal transduction pathway with type-1 cytokine receptors that induce IDO expression. Thus, we examined the possible cooperation between PRL and type-1 cytokine on IDO expression.

Method; Peripheral blood samples were obtained from 12 non-pregnant women who gave informed consent before sampling. Mononuclear cells were prepared by gradient centrifugation and cultured for 24-48 hours in the presence or absence of stimuli. (IFN-γ: 5 or 100 IU/ml, PRL: 10, 50 or 500 ng/ml). IDO protein level was evaluated by flow cytometry and IDO gene trancriptions were analyzed by semi-Quantitative RT-PCR in combination with NIH Image.

Results; 1) Both PRL and IFN-γ increased IDO expression by monocytes in a dose-response manner. 2) Both PRL and IFN-γ increased IDO expression by monocytes in a dose-response manner. 3) The up-regulation of IDO expression by PRL was observed only when PRL was added before IFN-γ stimulation.

Conclusions; These findings suggest PRL may act as a primer in the induction of IDO thereby playaing a facilitating role in maintenance of pregnancy. Our data favor the view that physiological concentrations of IFN-γ are important for pregnancy.

P6.08
PHAGOCYTOSIS OF SHED TROPHOBLASTS INCREASES THE EXPRESSION OF THE IMMUNOSUPPRESSIVE ENZYME, INDOLAMINE 2,3-DIOXYGENASE, BY MACROPHAGES.
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Objectives: The fetus and placenta are immunoloigically foreign to the mother yet, large quantities of apoptotic trophoblasts are shed daily from the placenta into the maternal blood at the end of their life cycle. A mechanism must exist to clear the trophoblasts from the maternal circulation but this mechanism is not known. It is likely that maternal macrophages are important in clearing shed trophoblasts but phagocytosis of foreign cells by macrophages usually leads to an immune response. However, evidence from other systems suggests that the uptake of apoptotic cells by macrophages induces an immunosuppressive response by the macrophages even if the phagocytosed apoptotic cells were immunologically foreign. We undertook this study to investigate the effect of shed trophoblasts on macrophages.

Methods: We induced U937 cells to an adhesive, macrophage-like phenotype by treatment with PMA. We labelled villous explants with green fluorescent cell tracker stain then harvested trophoblasts shed from explant cultures and exposed them to the U937 cells. Phagocytosis was examined by confocal microscopy and the expression of indoleamine 2,3-dioxygenase (IDO) by the U937 cells was examined using a cell-based ELISA.

Results; After exposure of the shed trophoblasts to U937 cells green fluorescent trophoblast-derived particles were present within the U937 cells. The expression of IDO by the U937 cells more than doubled (p<0.01) following phagocytosis of the shed trophoblasts.

Conclusions; Phagocytosis of shed (apoptotic) trophoblasts leads to an immunosuppressive type of response by macrophages. Thus, trophoblast shedding may be important for the development of maternal immune tolerance towards placental/fetal antigens.
P6.09
HLA-G IS ON THE SURFACE OF NORMAL EXTRAVILLOUS TROPHOBLAST IN A DIMERIC FORM
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Objectives: We investigated whether multimeric forms of HLA-G exist on normal trophoblast. The functions of HLA-G are unknown. It is expressed only by invading extravillous trophoblast (EVT) cells, which are in close contact with maternal decidua leukocytes. LIR-1 and LIR-2, expressed by macrophages and dendritic cells, are well documented HLA-G receptors, but the NK cell receptor, KIR2DL4, is less certain to bind HLA-G. Recently, HLA-G was found to exist as disulphide-linked homodimers in HLA-G transfectants. We show these dimers are present on normal trophoblast.

Methods: Primary trophoblast, cell lines and HLA-G transfected cells were surface biotinylated, lysed and covalently associated class I complexes immunoprecipitated using HLA-G gene structure, siRNA to knock down single isoforms seem to be not realizable.

Results: Immunoprecipitation with HLA-G specific antibodies, separated on non-reducing gels, produced two forms of HLA-G: the expected 40kDa band and an 80kDa complex. Only the 40kDa band appears in reducing conditions, suggesting the 80kDa complex is a covalently associated dimeric form of HLA-G. HLA-G immunoprecipitation from trophoblast as well as transfected cells gave this 80kDa complex, although the choriocarcinoma cell line JEG-3 displayed only established monomeric HLA-G. Both the 40 and 80 kDa complexes of HLA-G include β2m specific antibodies. The complexes were resolved by reducing or non-reducing SDS-PAGE, western blotted and probed with streptavidin-HRP.

Conclusions: We have shown a novel form of HLA-G on the surface of normal trophoblast. The possibility that dimeric HLA-G may bind new receptors, or induce functional signalling of described receptors on maternal leukocytes, either by increased avidity or inducing cross-linking is being explored.

P6.11
EXPRESSION OF CXCR1 AND CXCR3 IN HUMAN ENDOMETRIUM: EFFECTS OF THEIR LIGANDS ON ENDOMETRIAL CELL FUNCTION.
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Objectives: Chemokines and their receptors are proposed to play a role in human endometrial function. The expression of CXCR1 and CXCR3 in human endometrium in vivo and in cultured stromal and epithelial cells was investigated. We also investigated the effect of CXCL10 (IP10) and CXCL5 (ENA78) on actin filament rearrangement and MMP production by these cells.

Methods: Expression of CXCR1 and CXCR3 was determined by immunofluorescence in 12 biopsy samples obtained throughout the menstrual cycle and in cultured stromal and epithelial cells. Stromal and epithelial cells were incubated with CXCL10 and CXCL5 (1-50 ng/ml) for 15-40 mins and actin filaments visualised using FITC-phalloidium. Cells were also incubated with chemokines for 48 hrs and the secretion of MMPs determined by zymography.

Results: CXCR1 and CXCR3 were expressed by stromal and epithelial cells as well as cells associated with the blood vessels in whole tissue. Intense staining for both receptors was also seen in clusters cells within the stroma, possibly leukocytes. Epithelial cell expression of CXCR1 was less than in stromal cells, while for CXCR3 expression in epithelial cells was greater than in stromal cells. Both CXCR3 and CXCR1 were expressed in cultured stromal and epithelial cells, with increased expression of CXCR1 in stromal cells. Addition of CXCL10 and CXCL5 had no effect on actin rearrangement, but CXCL10 caused a dose-dependent increase in MMP activity.

Conclusions: The chemokine receptors CXCR1 and CXCR3 are expressed by endometrial stromal and epithelial cells. Ligands of these receptors may play a role in stromal and epithelial cell function.

P6.10
HLA-G1 PROTECTS JEG-3 CHORIOCARCINOMA CELLS AGAINST NK CELL CYTOTOXICITY
TG Poehlmann, H Winzer, T Wengenmayer, S Busch, UR Markert
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Objectives: Trophoblast cells and choriocarcinoma cells escape from NK cell killing by interaction of HLA-G with killing inhibitory receptors. The role of HLA-G isoforms is not yet exactly revealed.

Methods: RNA interference (RNAi) was applied to Jeg-3 cells to knock down total HLA-G and HLA-G1 and soluble HLA-G1 (HLA-G5). Small interfering RNA (siRNA) and scrambled oligonucleotide controls were self-designed. Due to homologies in the HLA-G gene structure, siRNA to knock down further single isoforms seem to be not realizable. HLA-G knock down was analyzed by Western blot and flow cytometry. NK cells were isolated from peripheral blood of healthy donors. For cytotoxicity assays, Jeg-3 cells were stained with CFSE and coincubated with NK cells. Death rate was measured by flow cytometry.

Results: HLA-G1 knock down was successfully performed. Increase of cytotoxicity after HLA-G1 knock down was similar to that after total HLA-G knock down. Among the different HLA-G isoforms, HLA-G1 plays a key role for protection of trophoblastic cells from NK cell cytotoxicity.

P6.12
JEG-3 CHORIOCARCINOMA CELLS ARE PROTECTED BY HLA-G1 AGAINST NK CELL CYTOTOXICITY
TG Poehlmann, H Winzer, T Wengenmayer, S Busch, UR Markert
Placenta-Labor, Department of Obstetrics, Friedrich-Schiller-Universität Jena, Germany

Problem: Trophoblast cells and choriocarcinoma cells escape from NK cell killing by interaction of HLA-G with killing inhibitory receptors. The role of HLA-G isoforms is not yet exactly revealed.

Methods: RNA interference (RNAi) was applied to Jeg-3 cells to knock down total HLA-G and HLA-G1 and soluble HLA-G1 (HLA-G5). Small interfering RNA (siRNA) and scrambled oligonucleotide controls were self-designed. Due to homologies in the HLA-G gene structure, siRNA to knock down further single isoforms seem to be not realizable. HLA-G knock down was analyzed by Western blot and flow cytometry. NK cells were isolated from peripheral blood of healthy donors. For cytotoxicity assays, Jeg-3 cells were stained with CFSE and coincubated with NK cells. Death rate was measured by flow cytometry.

Results: HLA-G1 knock down was successfully performed. Increase of cytotoxicity after HLA-G1 knock down was similar to that after total HLA-G knock down. Among the different HLA-G isoforms, HLA-G1 plays a key role for protection of trophoblastic cells from NK cell cytotoxicity.
P6.13
LOW IFN-γ DOES DURING THE POST-IMPLANTATION PHASE DO NOT INTERFERE WITH MICE REPRODUCTIVE PERFORMANCE, BUT INDUCE A DIFFERENTIAL GENE EXPRESSION ON TROPHOBLAST CELLS
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Objectives: There is evidence that IFN-γ can act as an abortion inductor factor, in spite of being an important immunoregulatory cytokine in biological defense’s processes and of co-existing at the maternal-fetal interface.

Methodological strategy: In this study, we ascertain in vivo, the concentrations of IFN-γ compatible with gestation, and in vitro, the IFN-γ direct effect on the trophoblast.

Results: When IFN-γ was administered to mice females (100, 1000 and 2000 U/ml) on the days 5.5, 6.5 and 7.5 of gestation, it did not change reproductive parameters, although disturbances in the IFN-γ and TNF-α serum profiles were detected. None of these treatments was considered incompatible with the gestation. In vitro, IFN-γ (100 U/ml) did not change the morphology, proliferation and rate of cellular death in the trophoblast cells, but it led to a differential expression of 42 genes, suggesting a functional role for this cytokine in the placenta. Part of the more expressed genes was related to immune response processes or cell activation mediated by IFN-γ.

Conclusions: Thus, it is possible that IFN-γ participates in the maternal-fetal interface’s physiology, activating trophoblast cell defense functions in a similar way to what was found for macrophages.

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P6.14
EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS ON THE OVINE PLACENTA.
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Objectives: The mammalian fetus inherits approximately half of its genetic material from its father and therefore could express major histocompatibility complex (MHC) antigens which are foreign to the maternal immune system. This means that the fetus could potentially be recognized as foreign and so be rejected in a similar way to a tissue graft. The aims of this study were to investigate the expression of the MHC class I molecules on the ovine placenta throughout gestation and characterize the expression of both polymorphic and non-polymorphic regions of the class I mRNA.

Methods: Immunohistochemistry and immunocytochemistry were carried out in ovine placenta and on isolated trophoblast binucleate cells (BNC) at various stages throughout gestation. Polymorphic and non-polymorphic regions of MHC class I cDNAs were amplified and sequenced from maternal, fetal and placentomal samples.

Results: This study indicates for the first time that there is MHC class I expression on both the maternal and fetal sides of the ovine placenta throughout gestation. Expression in the BNC population is controlled according to both the location of the cell and the stage of gestation. Paternally and maternally inherited MHC class I molecules are co-expressed on the ovine placenta at day 90 gestation and there is also expression of placentomal specific MHC class I molecules.

Conclusions: The BNC population up regulates MHC expression immediately prior to fusion with the feto-maternal hybrid syncytiotrophoblast at a time when one might expect down regulation of MHC molecules in order to protect the fetus from maternal immune attack. Further investigation is required to clarify whether the dominant pattern of MHC class I expression on the ovine placenta is polymorphic or of a non-classical type.

P6.15
CHANGES IN DECIDUAL LEUKOCYTES IN HUMAN PREGNANCY ASSOCIATED WITH INTRAUTERINE GROWTH RESTRICTION AND PREECLAMPSIA
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Objectives: Maternal leukocytes form a major component of human decidua, and throughout pregnancy a varying proportion of uterine NK cells and T cells is found. Preeclampsia and intrauterine growth restriction (IUGR) are significant complications of pregnancy associated with high perinatal morbidity and mortality. The role of leukocytes located at the feto-maternal interface in the pathogenesis of preeclampsia and IUGR is not defined. The aim of this study was to determine the role of decidual leukocytes in preeclampsia and IUGR by quantifying and comparing the distribution and percentage of leukocytes in decidua basalis and parietalis from placentas associated with preeclampsia and/or IUGR.

Methods: Paired decidua basalis and parietalis samples were obtained from three groups: pregnancy associated with IUGR alone (n=11), pregnancy associated with IUGR and pre-eclampsia (n=10), and normal term pregnancy (n=30). Percentages of leukocytes subpopulations within the CD45+ cell fraction were determined by flow cytometry.

Results: The percentage of TCRγδ T cells within the CD45+ cell population was significantly lower in decidua basalis and in decidua parietalis associated with IUGR alone and also with preeclampsia/IUGR when compared with that of normal pregnancy. Furthermore, a lower percentage of CD4+CD25+ cells was found in IUGR decidua basalis and parietalis. In the decidua basalis, there was a significant difference in the percentage of uterine NK and of cytotoxic NK cells among the three groups with the highest percentage in IUGR decidua.

Conclusions: A typical difference in immune-regulation in pregnancy complications is suggested by the finding of both a reduction in the percentage of TCRγδ cells and of CD4+CD25+ cells in the deciduas of (near) term IUGR and preeclampsia pregnancies.

P6.16
HLA-G IN DECIDUA FROM NORMAL AND PRE-ECLAMPTIC PREGNANCIES
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Objectives: Trophoblast expression of HLA-G has probably a role in implantation and placental development. The present study was performed to analyze the expression of different HLA-G mRNA isoforms (HLA-G1-7) and total HLA-G protein in decidua tissues of women with different HLA-G mRNA isoforms and total HLA-G protein in decidua tissues of women with different HLA-G mRNA isoforms. Expression of HLA-G mRNA was studied by RT-PCR. HLA-G gene expression was studied by Affymetrix analysis. Trophoblast expression of total HLA-G protein was detected by immunohistochemical techniques, combining antibodies against trophoblasts (mab CK7) and HLA-G (mab 4H84).

Results: Gene expression of the membranebound forms HLA-G1, -G3 and–G4 and the soluble forms HLA-G5, -G6 and -G7 were detected in decidua of both cases and controls. Detection of HLA-G2 awaits further experiments. No obvious differences in HLA-G isoform gene expression were detected between cases and controls, however, HLA-G5 mRNA levels seems to vary between individuals in the study. Cases tended to have a lower HLA-G gene expression in Affymetrix analysis. The proportion of HLA-G positive trophoblasts, as assessed by immunohistochemistry, did not differ between cases and controls. However, individuals that demonstrated the lowest HLA-G in trophoblast were all cases.

Conclusion: HLA-G mRNA isoforms are widely expressed in decidua tissues, but a potential involvement in placental disease must be studied at the protein level. The data indicate a tendency of reduced level of total HLA-G in decidua tissues of women with PE and/or IUGR.
THE EFFECT OF VITAMINS C AND E ON THE SHEDDING BY PLACENTAL VILLOUS TISSUE OF CELL FREE NUCLEIC ACIDS AND PLACENTAL MICROPARTICLES

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Objectives: Preeclampsia is a disorder of the second half of pregnancy which is associated with placental oxidative stress. Women with preeclampsia also have increased circulating plasma levels of cell free fetal DNA and RNA. Suggesting abnormal trophoblast turnover.

Methods: Villous tissue explants from normal placentas were cultured for 30h under normal or oxidative conditions in the absence or presence of the antioxidant vitamin C and Vitamin E. The release by the villosus tissue of cell free DNA and microparticles was quantified.

Results: Villous tissue cultured under oxidative conditions released increased levels of cell free DNS, which shows characteristic apoptotic features. Under normal culture conditions, vitamin C reduced the release of DNA and the shedding of placental alkaline phosphatase (PLAP)-associated microparticles. Under oxidative conditions, a combination of vitamin C and E was effective at decreasing cell free DNA release, however the shedding of microparticles was not changed.

Conclusions: Antioxidant vitamins potentely reduce the shedding of apoptotic cell free DNA by placental villous tissue, suggesting that these may play a role in regulating trophoblast survival under both normal condition and oxidative stress. Our data also suggest that the shedding of PLAP-and RNA-associated microparticles uses distinct mechanisms that those used to release the apoptotic DNA.

OXIDATIVE STRESS INCREASES PLACENTAL ACTWIN A SECRETION

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Objectives: Maternal serum and placental activin A levels are increased in women with preeclampsia. The mechanisms underlying this observation are not known. We set out to determine whether oxidative stress, a feature of preeclampsia, alters placental activin secretion.

Methods: Placental explants (~150mg/well) cultures were established in vitro from the terminal regions of term placental villi collected from 11 elective caesarean sections and cultured in 3.5mL serum free DMEM/F12 in 5%CO2 and 95% air for 48 hours before treatment. Explants were then cultured either in DMEM/F12 alone (controls) or with 2.3mM xanthine and 0.015U/mL xanthine oxidase (X/XO treatment) for a further 48 hours. Activin A and 8-isoprostane were measured by commercial assay in conditioned media at 24 and 48 hours and corrected for explant wet weight.

Results: In X/XO treated explant media, mean±SEM 8-isoprostane at 24 hours was 41.7±10.3pg/mL/mg significantly higher than controls (11.9±2.7pg/mL/mg, p<0.005), consistent with oxidative injury. In X/XO treated explant media activin A at 24 and 48 hours was 145±47 pg/mL/mg and 323±89.7pg/mL/mg, significantly higher than controls (59.6±9pg/mL/mg and 139±27.4pg/mL/mg, p>0.04).

Conclusions: Oxidative stress increases term placental secretion of activin A in vitro and may be the mechanism underlying increased circulating maternal activin A and placental activin A in vivo in preeclampsia.

DECI TRANSCRIPTION IS OXYGEN RESPONSIVE IN HUMAN PLACENTA

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Objectives: The hypoxia inducible transcription factor differentiated embryo chondrocyte expressed gene 1 (DEC1), highly expressed in the human placenta, is thought to mediate inhibitory effects of hypoxia on differentiation. As placental oxygenation increases during the course of pregnancy, we tested the hypothesis that expression of DEC1 mRNA is higher at first trimester than at term and sensitive to oxygen status.

Methods: Villous tissue from first trimester (6-9 and 10-13 weeks) and term placentas (38-40 weeks) collected under LREC guidelines was stored in RNAlater at −80°C. Oxygen effects on DEC1 expression were performed on first trimester villous explants cultured for 7 days in CMRL + FCS at either 1% oxygen or switched on day 5 from 1% to 8% oxygen. Total RNA was extracted, cDNA generated using random primers and quantitutive PCR performed using DEC1 specific primers and SYBR Green I quantification with melt curve analysis.

Results: DEC1 expression was significantly higher at 6-9 weeks vs. term and 10-13 weeks vs. term (Kruskal Wallis p<0.001; ρ=0.05, n=5). Switching first trimester villous explants (6-13 weeks, n=5) from 1% to 8% oxygen, lowered DEC1 expression in all experiments (mean 5 fold percentage drop from 1-8% oxygen). DEC1 expression did not alter in explants maintained at 1% oxygen for 7 days.

Conclusions: DEC1 mRNA expression decreases as placental oxygenation increases. We propose that DEC1 may provide a link between placental oxygenation and differentiation.

Smoking is associated with systemic oxidative stress which may effect antioxidant (AO) engine systems in the placenta. We have previously found that smoking in pregnancy upregulates the AO enzyme haeme oxygenase (HO) in the basal plate. HO degrades haeme into carbon monoxide (CO) (a vasodilator) and biliverdin (an AO) and has two isoforms: HO-1 (inducible) and HO-2 (constitutively active). The aim of this study was to determine the effect of cigarette smoke exposure on HO expression in the HTR-8 trophoblast cell line.

Methods: A cigarette smoke extract (CSE) was prepared by bubbling the smoke from 3 cigarettes through 15mL of RPM media. This 100% CSE was syringe filtered and then diluted to 0.1, 0.5, 1, 5, and 10% concentrations. HTR-8 cells were then cultured with the CSE (n=3 for each dilution) for 48 hrs. A control group was incubated with plain RPMI for 48 hrs (n=3). The cells were harvested, protein was extracted and run on SDS-PAGE gels, and western blot analysis was earned out for HO-1 and HO-2.

Results: The 5% and 10% dilutions were toxic to the cells. The 0.1% CSE solution did not significantly alter HO-1 expression. Treatment with the 0.5% and 1% CSE solutions resulted in a 2-fold and 3-fold increase in HO-1 expression respectively. None of the CSE treatments resulted in a significant alteration in HO-2 expression.

Conclusion: Treatment with CSE increases the expression of HO-1 in a dose-dependent manner in trophoblast cells, but at the concentrations used does not alter HO-2 expression. Increased HO-1 expression leads to an increase in the production of the AOs biliverdin and bilirubin and the vasodilator CO, all of which may contribute to the decreased incidence of pre-eclampsia in smokers.
ENDOTOXIN (LPS) AFFECTS SYNCTIOTROPHOBLAST AND ENDOTHELIUM IN DUALLY PERFUSED TERM PLACENTAS.
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Objectives: Intrauterine infections lead to increased perinatal mortality and morbidity. Using dual perfusion of the human placenta we studied the effects of endotoxin (LPS, lipopolysaccharides) on villous tissues.

Methods: Following C-section normal term placentas were dually perfused for 6h using medium with and without 0.1g, 0.5g, or 1.0g LPS/1 medium. After perfusion perfused and non-perfused tissues were paraffin embedded. Sections were stained for cytokeratin 18, CD31, E-selectin and ICAM-1.

Results: Circulation of endotoxin in the fetal blood system lead to an increase in the expression of E-selectin and ICAM-1 on fetal endothelium. Circulation of endotoxin on the maternal side of the villi, in the intervillous space lead to a similar upregulation of cytokeratin 18 expression within this layer.

Conclusions: Circulation of endotoxin in the placenta leads to the activation of fetoplacental endothelium or syncytiotrophoblast and shows a negative effect on the trophoblast cytoskeleton. Activation and alterations of specific placental tissues may disturb placental function and subsequently lead to growth restriction of the fetus.

CIRCUITRY OF OXYGEN-MODULATED GENE EXPRESSION IN EARLY HUMAN PLACENTA BY SYSTEMATIC SEQUENCING OF SUPPRESSIVE SUBTRACTION HYBRIDIZATION PRODUCTS.
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Villi from first trimester human placentas were exposed to oxygen concentrations of either 2%, or 20% during 3 hours to construct two reciprocally subtracted libraries using the Suppressive Subtractive Hybridization (SSH) methodology. After cloning, sequencing and mapping of genes, we identified 822 different sequences. We then developed a Logarithm of the Odds (LOD) test in order to identify a possible excess of genes in each group. We show that genes involved in angiogenesis are significantly over-represented in the “hypoxic” condition (2% O2), while apoptotic genes are significantly over-represented in the “normoxic” condition (20% O2). Further, we observed an excess of kinases relative to phosphatases and an excess of genes involved in proliferation over genes involved in cell growth in the “hypoxic” condition. To validate our results, we used quantitative RT-PCR to analyze the set of 8 genes involved in angiogenesis on 9 independent placentas. Finally, we studied the distribution of gene clusters on human chromosomes to check whether their chromosomal distribution was random or not. We observed on HSA11 a clear clustering of genes regulated similarly by O2 tension, and we also discovered indications that such clustering exists on chromosomes 6 and 12.

PRE-TRANSLATIONAL REGULATION OF ACTIVIN A BY OXYGEN TENSION
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Objective: Increasing evidence suggests that circulating activin A is increased in response to hypoxia. However, it is currently unknown at what level oxygen regulates activin A. Our objective was to examine the effect of oxygen on mRNA of activin A and activin A protein using in vivo and in vitro models.

Methods: Total RNA from placental tissues across gestation, high altitude (HA, 3100m) and preeclamptic placentae and villous explants (5-9 weeks of gestation) was subjected to quantitative RT-PCR using primers for activin/inhibin β subunit and activin A protein using in vivo and in vitro models.

Results: Activin A expression was higher during early gestation and decreased at term, with the highest expression observed between 7-10 weeks of gestation when O2 tension is low. A significantly higher expression of activin A was observed in HA placentas as compared to that from sea level (P<0.05). Moreover, increased activin A level was detected in preeclamptic placenta as compared to age-matched controls (P<0.05). In vitro studies demonstrated that activin A level in villous explants treated with 3% O2 was significantly higher than that treated with 20% O2 (P<0.05). Interestingly, explants treated with hypoxia/reoxygenation have significantly higher activin A compared to that exposed to 20% O2 (P<0.05).

Conclusions: Both low oxygen and hypoxia/reoxygenation play an important role in the regulation of activin expression. The regulation of activin A by oxygen tension occurs at a pre-translational level, which subsequently leads to increased protein synthesis and secretion (supported by CHIR).
THE INFLUENCE OF OXYGEN TENSION ON GENE REGULATION AND CELL BEHAVIOUR IN HUMAN LATE FIRST TRIMESTER CYTOTROPHOBLASTS IN PREGNANCIES WHOSE OUTCOME IS KNOWN.

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Objectives: Precisely regulated endovascular invasion of the uterus by cytotrophoblasts is critical to the success of human pregnancy. Although cytotrophoblasts from first trimester termination tissue have been used to examine mechanisms underlying placenta, the lack of ability to predict development of pre-eclampsia limits the use of this tissue to clarify abnormalities that may subsequently occur. We have developed methods to isolate from late first trimester chorionic villi from ongoing pregnancies cytotrophoblasts that retain function after freezing and thawing. This will allow exposition of cytotrophoblast functional abnormalities that precede pre-eclampsia at a critical period of gestation in pregnancies whose outcomes are known to be either normal or abnormal (such as pre-eclampsia).

Methods: Surplus tissue is collected from patients undergoing diagnostic chorionic villous sampling (CVS) at ~11-14 weeks gestation. Cells are cultured until confluent, frozen until the pregnancy outcome is known, then thawed and utilised in experiments. In preliminary experiments we have examined the gene expression (using reverse transcriptase-polymerase chain reaction) of cells from normal pregnancies subjected to 2% O2 (oxygen-deprived) and 3% O2 (oxygen-rich) over various time frames.

Results: Growth characteristics were unchanged in an oxygen-deprived environment. Expression of vascular endothelial growth factor (VEGF)-1, hypoxia-inducible factor pro-lyl hydroxylase (HIF)-1 and HPH-2 are markedly increased following oxygen deprivation.

Conclusions: We have developed a method of isolating CVS-derived cytotrophoblasts from ongoing pregnancies whose outcomes are known. Preliminary results indicate that the hypoxia pathway is active in these cells.

P7.09

P7.10

UP-REGULATION OF GADD34 IN TROPHOBLAST - A POTENTIAL PROTECTIVE FUNCTION AGAINST OXIDATIVE STRESS.

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Objectives: Eukaryotic cells respond to oxidative stress by phosphorylating the alpha subunit of translation initiation factor 2 (eIF2a) inhibiting protein synthesis, if not reversed this results in the activation of apoptotic pathways. GADD34 dephosphorylates eIF2a restoring protein synthesis. In the current study we determined whether there was a temporal association between GADD34 expression and the establishment of the maternal circulation with its documented rise in placental partial pressures of oxygen.

Methods: A developmental series of archived placental tissue from 6 to 40 weeks gestation was compared using immunocytochemistry with a primary antibody directed against GADD34. Two independent assessors blindly assessed staining intensity and localisation.

Results: GADD34 was constitutively expressed at very low levels by the cytotrophoblast as early as 6 weeks gestation. There was marked up regulation in GADD34 expression at 10 to 14 weeks gestation solely within cytotrophoblast with resolution back to control levels by 16 weeks. In placental sections from preterm LUSCS specimens GADD34 remains at low levels. The onset of labour both spontaneous and induced is associated with a mild increase in staining intensity and expression with the syncytiotrophoblast in addition to fetal endothelial cells.

Conclusion: The onset of the maternal circulation at 10-14 weeks gestation is associated with an increase in oxidative stress due to an increase in the partial pressure of oxygen within the placenta. There is specific up-regulation of GADD34 within trophoblast during this time period. This may represent an adaptive response by trophoblast to ensure continued protein synthesis and avoidance of cell death while it undergoes adjustment to higher oxygen levels. The labour dependent changes may similarly reflect an adaptive response to a minor degree of ischaemia.

PRE-ECLAMPSIA IS ASSOCIATED WITH AN UP-REGULATION OF GADD34 IN TROPHOBLAST - A POTENTIAL PROTECTIVE FUNCTION AGAINST OXIDATIVE STRESS.

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Objectives: Oxidative stress induces protein synthesis repression and activation of apoptotic pathways via phosphorylation of eIF2a. GADD34 dephosphorylates eIF2a facilitating continued protein synthesis and avoidance of cell death. Pre-eclampsia is associated with both oxidative stress and maintenance of protein synthesis by the trophoblast. We sought to determine whether GADD34 expression was up-regulated in pre-eclampsia as compared to intrauterine growth restriction and control populations.

Methods: Archive placental tissue from 23 control, 10 IUGR and 13 pre-eclamptic patients was used. GADD34 mRNA was quantified by RT-PCR and expressed relative to the trophoblast specific marker cytokeratin-7 given GADD43 protein distribution within placenta. Protein expression was compared semi-quantitatively using immunocytochemistry with a primary antibody directed against GADD34. Two independent assessors blindly assessed staining intensity and localisation. RT-PCR data was expressed as mean ± SEM and analysed by ANOVA.

Results: GADD34 mRNA was increased in pre-eclampsia (PE 26.7±2.6, IUGR 22.8±3.4, CON 18.5±1.3) over controls (p=0.02) but not IUGR (p=0.4). GADD34 was constitutively expressed by syncytiotrophoblast at low levels in the control population. IUGR was associated with a variable increase in staining intensity; in contrast pre-eclampsia had significantly stronger and consistent staining throughout the syncytiotrophoblast than observed in control or IUGR samples.

Conclusions: GADD34 mRNA and protein is up-regulated in trophoblast tissue from patients with pre-eclampsia as compared to control and IUGR populations. This may represent an adaptive response by trophoblast to oxidative stress ensuring maintenance of protein synthesis and avoidance of cell death.

P7.11

ROLE OF OXYGEN IN REGULATING INVASION OF FOUR TROPHOBLAST-LIKE CELL LINES

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Background: During early placental development O2 levels in the intervillous space are very low. We have previously shown that low O2 concentrations inhibit the invasiveness of extravillous trophoblast cells (EVT) derived from placental explants. Others have shown an opposite effect using a trophoblast-like cell line.

Aim: To determine role of low O2 in regulating invasiveness of four trophoblast-like cell lines.

Methods: ITR-8/6S/Vneo, SGHPL-4, JEG3 and JAR cell lines were cultured at 20% and 3% O2 for 24, 48 and 72 hours in Matrigel invasion assays. The number of cells on the underside of each filter was counted and the invasion index was determined by normalizing each experiment to its own 20% O2 control (n=4 each cell line).

Results: There was a significant increase in the invasiveness of ITR-8/8/S/Vneo (20% 1±0, 3% 2.8±0.4, P=0.001) and JEG3 (20% 1±0, 3% 2.1±0.6, P=0.04) cells after 24h at 3% O2 that was lost after 48h (ITR-8/8/S/Vneo 20% 1±0, 3% 1.9±1.0, JEG3 20% 1±0, 3% 1.7±0.6). After 72h culture at 3% O2 there was an inhibition in invasiveness of ITR-8/8/S/Vneo (20% 1±0, 3% 0.7±0.1, P=0.004) and JEG3 (20% 1±0, 3% 0.3±0.1, P=0.0004) cells. In contrast, there was a significant decrease in the invasiveness of SGHPL-4 (20% 1±0, 3% 0.5±0.3, P=0.02) cells after 24h at 3% O2, that was lost by 48h (ITR-8/8/S/Vneo 20% 1±0, 3% 2.1±1.5) and 72h (20% 1±0, 3% 2.8±1.5). The JAR cell line showed limited invasion at all time points which was not altered by culture in different O2 concentrations (24h 20% 1±0, 3% 1.2±0.4, 48h 20% 1±0, 3% 0.8±0.4; 72h 20% 1±0, 3% 3.3±1.5).

Conclusions: Low O2 concentrations have differential effects on invasiveness depending on cell type. Only SGHPL-4 cells show a similar regulation of invasive capacity in response to O2 as primary EVT.

P7.12

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THE INFLUENCE OF OXYGEN ON P53 AND MDM2 EXPRESSION IN CULTURED PLACENTAL VILLOUS EXPLANTS

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Objectives: The trophoblast cell cycle is influenced by oxygen. Oncoproteins p53 and Mdm2 may play a pivotal role. We investigated p53 and Mdm2 in placental villous explants cultured under various oxygen tensions.

Methods: Villous tissue was collected from 6 uncomplicated pregnancies. Fresh tissue was homogenized for Western blotting and fixed for immunohistochemistry. Explants were cultured for 4 days in 20%, 6% or 1% O2. Resultant tissues were processed for p53 and Mdm2 expression, Ki-67 immuno-recognition (proliferation) and TUNEL (apoptosis). Collected media were analysed for lactate dehydrogenase (LDH) and human chorionic gonadotrophin (hCG); markers of necrosis and cytotrophoblast (CT) differentiation, respectively.

Results: Over 4 days culture, villous explants generally shed their original syncytiotrophoblast (ST). p53 and Mdm2 in fresh tissue and 6%O2 were localised to the cytoplasm of this attached and liberated ST. At 20% oxygen, hCG levels in the media were significantly raised, indicating exaggerated CT differentiation (p<0.05). This was matched with a non-significant elevation in ST p53 and Mdm2. At 1%O2, hCG production was abolished and CT proliferation markedly enhanced. In this situation, p53 levels were also significantly raised with a concomitant rise in Mdm2 (p<0.05). Notably, p53 elevations at 1%O2 were localised to CT not ST. Overall, necrosis and apoptosis were greatest at 1%O2.

Conclusions: In general, p53 and Mdm2 were tightly balanced in trophoblast ST, with cultures at 6%O2 giving the closest match to fresh tissue. Hypoxia-induced CT proliferation was related to both p53 over-expression and exaggerated cell turnover. Our previously recorded elevations in p53 in pre-eclampsia could therefore reflect hypoxic events in villous tissue.

OXYGEN AND PLACENTAL ENERGETICS.

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Objectives. The availability of oxygen to the placenta determines which metabolic pathways predominate. Placental oxygen availability may be impaired due to low blood flow (first trimester) or by reduced atmospheric oxygen pressure (altitude). We hypothesized that placental metabolic response to low oxygen would be similar in early and term placentas.

Methods. Metabolic profiles were determined in 7-8 week and term placentas from sea level, and term placentas from 3100 m by magnetic resonance spectroscopy.

Results. First trimester placentas had greater polyol and inositol concentrations than sea level term placentas (29.94 ± 6.7 vs. 4.89 ± 1.3, p = 0.03). Term placentas from 3100 m had less glucose and NAD+, greater lactate and hydroxybutyrate and more glutathione (GSH) than those from sea level. In comparison to first trimester tissue, placentas from 3100 m had less glucose, greater hydroxybutyrate and GSH. The ATP/ADP ratio was lower at 3100 m as compared to sea level and first trimester placentas (3100:1.22 ± 0.8, sea level: 1.79 ± 0.11; trimester: 1.56 ± .14).

Conclusions. Early gestation placentas respond to low oxygenation by utilizing vestigial polyol metabolic pathways to successfully maintain ATP concentrations and redox potential, yet mature placentas exhibit evidence of glycolysis, lowered ATP/ADP ratio and antioxidant production.
**P8.01**

**FIRST TRIMESTER MATERNAL SERUM HORMONES TO PREDICT PREGNANCY OUTCOME IN THREATENED MISCARRIAGE.**

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**Objectives:** To investigate whether levels of inhibin A, activin A, follistatin, hCG and PAPP-A in women in their first trimester threatened miscarriage (TM) are related to pregnancy outcome and to study the relationship between these hormones in TM compared with gestation matched controls.

**Methods:** Serum samples were taken from 122 women presenting with TM and an ongoing pregnancy and 33 gestation matched controls taken from women undergoing therapeutic evacuation of the uterus of a normal pregnancy. Inhibin A, activin A and follistatin were measured using a two-site in-house ELISA. PAPP-A and hCG were measured using commercial ELISA kits. The data were log transformed and analysed using students t-test and logistic regression.

**Results:** Inhibin A levels were lower in pregnancies that subsequently miscarried when compared with term births (p=0.0007) and controls (p=0.0146). hCG levels were also lower in miscarriages when compared with term births (p=0.0001) and controls (p=0.0187) but significantly higher in term and preterm births when compared with controls (p=0.0001 and p=0.0317 respectively). Logistic regression analysis of inhibin A, hCG and a combination of the two for predicting the likelihood of subsequent miscarriage showed the area under the ROC curve of 0.6916, 0.6088 and 0.6810 respectively.

**Conclusion:** Inhibin A and hCG are lower in cases of TM that subsequently miscarried. Inhibin A levels could be useful in predicting those women who are more likely to miscarry and therefore require follow up. Higher hCG levels in successful pregnancies could represent more efficient adaptation to placental stress associated with TM.

**P8.02**

**MACROPHAGE INHIBITORY CYTOKINE-1 IN FIRST TRIMESTER MATERNAL SERUM OF WOMEN WITH RECURRENT MISCARRIAGE.**

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**Objectives:** It has been previously shown that maternal serum levels of MIC-1 in early pregnancy are significantly lower in women who subsequently have a miscarriage compared to those with normal pregnancies. In this study we have explored whether first trimester levels of MIC-1 in women with a history of recurrent miscarriage are predictive of outcome.

**Methods:** Maternal serum samples collected at 5-7 weeks gestation from women with history of unexplained recurrent miscarriage were assayed for MIC-1, using an in-house assay. Women were grouped according to pregnancy outcomes into livebirths (n=25) and further miscarriages (n=16).

**Results:** Overall the mean/SEM MIC-1 level in those women with a subsequent livebirth was significantly higher than those in those who had a further miscarriage (4036±638pg/mL vs 1559±415pg/mL; p=0.015). Expressed as multiples of median (MoMs), at a cut-off of ≤0.7MoM 81% of miscarriages would be correctly identified but 24% of viable pregnancies also identified.

**Conclusions:** These data suggest that in unexplained recurrent miscarriage placental and/or decidual production of MIC-1 is decreased prior to pregnancy loss. This supports a potential diagnostic role for MIC-1 in this setting.

**P8.03**

**UNCOMPLICATED PREGNANCY AND SPONTANEOUS MISCARRIAGE: THE ROLE OF PECAM-1 IN THE PLACENTA AND PLACENTAL BED.**

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**Objectives:** Both preeclampsia and spontaneous miscarriage (SM) are linked to foiled cytotoxic cell damage (CTB) invasion. It has been suggested that CTB which invade spiral arteries mimic the cell adhesion molecule phenotype of endothelial cells and that this feils in preeclampsia (PE). We previously showed that PECAM-1 expression was similar in normal pregnancy and PE. In this study we hypothesised that PECAM-1 is abnormally expressed in cases of SM.

**Methods:** Placenta and placental bed samples were obtained at Yorkhill and Garnetval Hospitals, Glasgow. Western blotting was used to determine the specificity of the PECAM-1 antibody. Immunohistochemistry was performed using the ABC method.

**Results:** PECAM-1 was expressed on villous and uterine endothelial cells at all gestations. CTB in the cytotrophoblast/syncytiotrophoblast, cell islands or cell columns were negative for PECAM-1 throughout gestation. Within the placental bed, intramural, intralaminar, perivascular and interstitial CTB were also PECAM-1 negative both within decidua and myometrium throughout pregnancy. Examination of CTB in cases of SM showed that invasive CTB remained PECAM-1 negative.

**Conclusions:** We have found no evidence that CTB express PECAM-1 either as they form cell columns, or as they invade the decidua and myometrium. No changes in expression of PECAM-1 were noted in cases of SM. These data do not support a role for PECAM in normal human CTB invasion or in foiled invasion linked to spontaneous miscarriage. Sponsored by Action Research.

**P8.04**

**RECURRENT SPONTANEOUS ABORTION WITH ANTIPHOSPHOLIPID ANTIBODIES: INCIDENCE AND THERAPEUTIC OUTCOME.**

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**Introduction:** Antiphospholipid antibodies (APAs), especially the IgG isotype, have been associated with thrombotic and cytotoxic events of placental vessels and trophoblast, which could lead to pregnancy loss, but there have been a few reports about IgM isotype. Moreover, the therapeutic principles for the treatment of patients suffering recurrent spontaneous abortion with APAs has not been fully clarified.

**Objectives:** 1) To investigate the incidence of IgG and IgM isotypes of APAs including anticardiolipin (CL), anti-phosphatidyl ethanolamine (PE), and anti-phosphatidyl serin (PS) in RSA patients. 2) To explore the effects of the therapy for RSA with these APAs through measurement of the titres of antibodies.

**Method:** 237 women with the history of RSA registered at Jikei University Hospital were examined retrospectively. 1) Prior to treatment and conception, APAs were detected. 2) Patients with any APAs were treated with herb-medicine, aspirin and/or heparin.

**Results:** 1) Incidence of antibodies to CL-IgG–IgM, PE-IgG–IgM, and PS-IgG–IgM were 19/219(8.7%), 60/219(27.4%), 34/267(16.4%), 4/195(2.1%), 12/189(6.3%) and 52/191(27.2%), respectively. 65 out of total 332 samples(19.6%) for IgG, and 116 out of total 313 samples(37.1%) for IgM isotype were recognized to be positive. 2) Pregnancy was successfully maintained in 75/81(92.6%) of RSA patients with APAs through anti-coagulation therapy. 40 out of 43(93.0%) with both of IgG and IgM–positive patients and 35 out of 38(92.1%) with only IgM-positive patients demonstrated successful outcome. 3) 16 out of 16(100%) patients with low to medium titre of IgM and 12 out of 13(92.3%) patients with low titre of IgG demonstrated successful outcome through only aspirin therapy.

**Conclusions:** 1) APAs were greatly involved in RSA, and a high incidence was found of PE-IgG, CL-IgM and PS-IgM antibodies. The incidence of IgM antibodies was significantly higher than that of IgG. 2) Anti-coagulation therapy to RSA patients with APAs was effective, and the isotypes of APAs did not influence the therapeutic outcome. The titre of APAs might be important in the choice of therapy.
**P8.05**

**EXPRESSION OF HLA-G AND HLA-E IN RECURRENT MISCARRIAGE AND NORMAL PREGNANCY.**

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**Objectives:** Recurrent miscarriage may be defined as the loss of three or more pregnancies in the first trimester. Recurrent miscarriage is estimated to affect 1% of women. In approximately 50% of cases there is no identifiable cause for the miscarriages but immunological factors may play a role in some cases of recurrent miscarriage. The non-classical class I human leucocyte antigen (HLA) proteins HLA-G and HLA-E are thought to be involved in helping extra villous trophoblasts evade destruction by the maternal immune system. We undertook this study to determine whether there was a difference in the expression of HLA-G or HLA-E between women with recurrent miscarriage and normal pregnancy.

**Methods:** Ultrasound-guided placental bed biopsies were obtained from 45 women with recurrent miscarriage and 17 women with gestation-matched normal pregnancies. Villous placental tissue was also obtained from most of the women. The biopsies were snap frozen and serial thin sections were cut using a cryostat. The sections were stained for HLA-G and human leucocyte antigen (HLA) proteins HLA-G and HLA-E.

**Results:** HLA-G was expressed only by extravillous trophoblasts while HLA-E had a more widespread expression pattern. Two women with recurrent miscarriage, as well as two women with normal pregnancies appeared to have some trophoblast columns that did not express HLA-G. There was no overall difference in the expression of either HLA-G or HLA-E between women with normal pregnancy and women with recurrent miscarriage.

**Conclusion:** Differences in the expression of HLA-G and HLA-E do not explain recurrent miscarriage.

**P8.06**

**ULTRASOUND SCREENING FOR MOLAR PREGNANCY IN MISSED MISCARRIAGE.**

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**Objective:** To examine the relationship between ultrasound and histological features when screening for molar change in missed miscarriage.

**Methods:** A prospective cohort study was conducted on all missed miscarriages, with features suspicious of molar pregnancy, on ultrasound and/or on histological examination over a five year period. All cases of molar pregnancy diagnosed histologically were examined and cross referenced with cases diagnosed on ultrasound. When available maternal serum p human chorionic gonadotrophin (MShCG) levels were recorded and compared with MShCG levels in gestation-matched non-molar miscarriages.

**Results:** Fifty-one cases of suspected molar pregnancy were referred to the regional centre for further opinion and follow-up, five cases were excluded because of the diagnosis of hydropic abortion (HA). In 33 cases, molar pregnancy was suspected at the initial scan. Of these 26 (78.8%) were confirmed on histology resulting in a 56% detection rate using ultrasound alone. In 15 cases MShCG results were available, of which nine were greater than two multiples of the median. In all cases MShCG levels in missed miscarriages were considerably lower than in normal and molar pregnancies.

**Conclusions:** The diagnosis of both complete (CHM) and partial mole (PHM) in first trimester miscarriage is more difficult because ultrasound and histological appearances are less pronounced than later in pregnancy. MShCG is significantly higher in both CHM and PHM and in conjunction with transvaginal ultrasound could provide the screening test required to enable us to counsel women more confidently towards non-surgical management of their miscarriage, where histopathological examination is not available.

**P8.07**

**TUMOR NECROSIS FACTOR-ALPHA CONVERTING ENZYME IN THE HUMAN PLACENTA FROM NORMAL PREGNANCY AND PREGNANCIES COMPLICATED BY CHORIOAMNIONITIS.**

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**Objectives:** Tumor necrosis factor-alpha (TNF-α) plays a pivotal role in the induction of cytokine response in intrauterine infection-related preterm delivery. TNF-α converting enzyme (TACE) is essential for the release of TNF-α. The objectives of this study were: (1) to characterize the expression of TACE mRNA and protein in the human placenta throughout pregnancy; (2) to determine the role of TACE in pregnancies complicated by chorioamnionitis; and (3) to investigate the effects of lipopolysaccharide (LPS) on the expression of TACE.

**Methods:** Placental samples were collected from early (7-12 wk), mid (13-28 wk), and late (32-40 wk) pregnancy. TACE protein and mRNA were studied by immunohistochemistry, Western blot and real-time quantitative PCR. We further compared the immuno-reactivity of TACE in normal term placentas to that in placentas with chorioamnionitis. The effects of LPS on the expression of TACE were assessed by using an in vitro villous culture system.

**Results:** TACE was mainly localized at the syncytiotrophoblast and cytotrophoblasts in villous tissues of early gestation. It also appeared in the stromal and endothelial cells in later pregnancy. Both TACE mRNA and protein levels were lower in placentas from mid pregnancy compared to those from early or late pregnancy. Placentas with chorioamnionitis had stronger immunostaining of TACE compared to normal term placentas. Furthermore, LPS caused a significant increase in the levels of mRNA and immunoreactivity of TACE.

**Conclusions:** These findings implicate a role of TACE in placental development and in the pathogenesis of pregnancies complicated by chorioamnionitis.

**P8.08**

**CHRONIC UTEROPLACENTAL INSUFFICIENCY ASSOCIATED WITH NORMAL BIRTH WEIGHT - A STEROELOGICALLY DISTINCT ENTITY.**

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**Objectives:** Chronic uteroplacental insufficiency (CUPI) causes accelerated villous maturation (AVM). In 800 low risk pregnancies, 79 had AVM, in whom pre-eclampsia (PET) and thrombophilia were excluded. Of these 53 were associated with normal infant birth weights (CUPINBW), and 17 with IUGR (CUPIIUGR).

**Methods:** 10 Placentas were assessed from each of the following groups: normal pregnancy and birth weight (NBW), CUPI-NBW, CUPI-IUGR and PET with IUGR (PET-IUGR). The placental disc volume was measured followed by uniform random sampling of 10 full thickness biopsies. 5 fields were examined from coded H&E stained sections. Stereology comprised star volume and surface area measurements of terminal villi and capillaries. Two-dimensional counts of syncytiotrophoblast knots were also performed.

**Results:** The CUPI-NBW had significantly reduced capillary star volume and surface area, but had a normal villous surface area compared to NEW. This contrasted with CUPI-IUGR in which all the parameters, including surface area, were reduced similar to PET-IUGR. The PET-IUGR capillary star volume was partially reduced compared to PET. The CUPI-NBW required surgical/instrumental delivery compared to 53% in CUPI-IUGR and 48% in NEW.

**Conclusions:** The normal total villous surface area in CUPI-NBW compared to NEW and reduced terminal villous vascular volume similar to PET-IUGR provides an understanding of previously unexplained intrauterine hypoxia.
**P8.09**

**CHRONIC UTEROPLACENTAL INSUFFICIENCY (CUPI) CAUSING IUGR IN ONE OF DICHOIRAL TWINS: EVIDENCE OF A PRIMARY DISORDER OF THE NON-VILLOUS TROPHOBLAST**

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**Objectives:** Placentas from 8 dichorial twin pairs (gestation 36-40 weeks) with discordant birth weights showed accelerated villous maturation in the smaller twin, suggesting CUPI, while the second placenta was normal. This setting suggests impaired maternal physiological vascular adaption in one twin, reflecting a primary trophoblast disorder in one twin only.

**Methods:** Stereology was used to compare 8 twin placentas with 8 gestation matched placentas from (i) normal pregnancy and birth weights (NBW) and (ii) pre-eclamptic gestations with IUGR (PET-IUGR). The volume of each placenta was measured, followed by uniform random sampling of 10 full thickness biopsies. 5 fields were examined from coded H&E stained sections. The stereological examination comprised star volume and surface area of terminal villi and their capillaries. 2-dimensional counts of syncytial knots were also performed.

**Results:** Placental villi from the IUGR twins had significantly reduced total surface area and star volume compared to NBW and the normal sized co-twin, while having no significant difference from the PET-IUGR group. The villous capillary space also demonstrated a significant reduction in area and star volume in parallel with the villous measurements above. The 2-dimensional syncytial knot count was significantly elevated in the IUGR twin and PET-IUGR, while the count in the NEW twin was similar to that in the normal control.

**Conclusion:** As PET or thrombophilia of necessity should effect both twin placentas, this study strongly suggests that this pathogenesis is unique to only one of the twins, indicating a primary disorder of the non-villous trophoblast is involved in some cases of CUPI.

**P8.10**

**PLACENTAL ORIGIN OF PARADOXICAL RENIN ANGIOTENSIN SYSTEM ACTIVATION IN RECIPIENT FETUSES WITH TWIN-TWIN TRANSFUSION SYNDROME.**

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**Objective:** Twin-twin transfusion syndrome (TTTS) occurs only in twin pregnancies that share a monochorionic placenta, and is attributed to net transfusional imbalance through placental vessel anastomoses, causing hypervolaemia and cardiac failure in the recipient and hypovolaemia and growth restriction in the donor. Simple transfusion cannot explain all phenotypic manifestations. Renin expression is increased only in donor kidneys, implicating activation of the renin-angiotensin system (RAS) in the donor phenotype. The recipient phenotype suggests it might also be exposed to an activated RAS, despite low renal renin expression. We tested the hypothesis that RAS effectors are elevated in the recipient and this results from placental renin production.

**Method:** Fetal blood, placenta and kidney were obtained from TTTSs (n=10) and normal (twin and singleton) (n=10) pregnancies. Renin and angiotensin n levels in fetal blood were determined by ELISA. Placental and renal renin were investigated by immunohistochemistry and RT-PCR, and expression of other vasoregulatory genes evaluated by targeted microarray.

**Results:** Renin and angiotensin n were similar in recipient and donor blood, and both were elevated compared to control levels (p=0.001). Placental mRNA for renin was raised in recipient compared to donors and controls, whereas renin expression was increased in donor kidneys. Placental mRNAs for angiotensinogen, AT1 and AT2 receptors were increased in recipient compared with donors and controls.

**Conclusions:** Despite down-regulation of renal renin, the recipient is also exposed to increased RAS effectors, but unlike the donor, this is due to paracrine and endocrine effects of the placental RAS system.

**P8.11**

**EFFECTS OF SULFASALAZINE ON KYNURENINE- POTENTIAL THERAPEUTIC AGENT**

P Patel, N Tetlow, C Albrecht, L Lakasing, S Soumian, M Sullivan, K Nicolaides, C Williamson. Institute of Reproductive & Developmental Biology, Imperial College London, King’s College London.

**Objective:** ABCA1 is an ATP-binding cassette transporter that facilitates the transfer of choline-phospholipid and cholesterol complexes from peripheral tissues to circulating apo-A1 to form high-density lipoproteins. ABCA1 also plays a role in phosphatidyl serine externalisation in lipid membranes, a process central to macrophage engulfment of senescent and apoptotic cells. ABCA1 null mice have malformed placentas and a high incidence of pregnancies compromised by severe embryo growth restriction, fetal loss and neonatal death. We hypothesised that ABCA1 may play a role in the aetiology of abnormal placenta in human pregnancies complicated by pre-eclampsia and antiphospholipid syndrome (APS).

**Method:** Placental specimens were obtained from pregnancies complicated by pre-eclampsia (n=12), APS (n=7, 3 with superimposed pre-eclampsia) and from 12 controls, and fixed or frozen immediately after collection. Messenger RNA was localised by in situ hybridisation, and ABCA1 protein by immunohistochemistry. ABCA1 protein levels were determined by Western blots of frozen tissue extracts.

**Results:** Riboprobes localised ABCA1 to endothelium and syncytiotrophoblast, and this was confirmed by immunohistochemistry. Western blot analysis revealed a lower level of ABCA1 protein in placentas from women with APS compared with controls. However, there were no differences in placentas from women with pre-eclampsia.

**Conclusion:** ABCA1 protein was localised to cells implicated in placental transport, namely syncytiotrophoblast and villous vascular epithelium. In addition, ABCA1 protein in APS placentas was decreased, suggesting that altered lipid handling may contribute to the overall pathology.

**P8.12**

**PLACENTAL ABCA1 PROTEIN AND MESSAGE IN ANTIPHOSPHOLIPID SYNDROME.**

P Patel, N Tetlow, C Albrecht, L Lakasing, S Soumian, M Sullivan, K Nicolaides, C Williamson. Institute of Reproductive & Developmental Biology, Imperial College London, King’s College London.

**Objective:** ABCA1 is an ATP-binding cassette transporter that facilitates the transfer of choline-phospholipid and cholesterol complexes from peripheral tissues to circulating apo-A1 to form high-density lipoproteins. ABCA1 also plays a role in phosphatidyl serine externalisation in lipid membranes, a process central to macrophage engulfment of senescent and apoptotic cells. ABCA1 null mice have malformed placentas and a high incidence of pregnancies complicated by severe embryo growth restriction, fetal loss and neonatal death. We hypothesised that ABCA1 may play a role in the aetiology of abnormal placenta in human pregnancies complicated by pre-eclampsia and antiphospholipid syndrome (APS).

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**Conclusion:** ABCA1 protein was localised to cells implicated in placental transport, namely syncytiotrophoblast and villous vascular epithelium. In addition, ABCA1 protein in APS placentas was decreased, suggesting that altered lipid handling may contribute to the overall pathology.
**P8.13**

**EXPRESSION OF INDOLEAMINE 2,3-DIOXYGENASE (IDO) IN NEOPLASTIC TROPHOBLASTS OF GESTATIONAL TROPHOBLASTIC NEOPLASIA**

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Objectives: Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme that catalyzes the initial and rate-limiting step in tryptophan degradation. Recent studies have shown that IDO is expressed in placentoblastic trophoblasts and macrophages during pregnancy and blocks the proliferation of alloreactive T-cells by depleting tryptophan locally to prevent rejection of the fetus by maternal T-cells. The present study examined IDO expression in human gestational trophoblastic neoplasia (GTN).

Methods: Immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections including 3 hydatidiform moles, 8 choriocarcinomas, 3 placental site trophoblastic tumors (PSTT) and 11 normal placentas was conducted using the anti-IDO monoclonal antibody. Western blotting and FACS analyses were performed to detect IDO protein expression in choriocarcinoma cell lines (JEG3, JAR, CC-4) and tissues as well as in a human extravillous trophoblast cell line, HTR-8/svneo (generously provided by Dr. Charles Graham).

Results: IDO protein was detected as 42-kDa bands in both normal placenta and choriocarcinoma tissues on Western blot analysis. Intracellular IDO expression was detected in choriocarcinoma cell lines and HTR-8 cells on FACS analysis. Immunohistochemical studies showed that IDO was strongly expressed in neoplastic trophoblasts of choriocarcinoma and PSTT.

Conclusions: IDO is expressed not only in normal placentoblastic trophoblasts, but also in neoplastic trophoblasts of GTNs, which are allogeneic or semi-allogeneic tumors possessing paternal genes. These results support our hypothesis that IDO is involved in protection of GTN from attack by host T-cells.

**P8.14**

**PLACENTAL MESENCHYMAL DYSPLASIA: A REPORT OF CASE WITH DIFFERENTIATION FROM PARTIAL HYDATIDIFORM MOLE**

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Placental mesenchymal dysplasia (PMD) is a rare recently recognised placental vascular malformation. We report a case of a 23-year-old woman, gravid 1, para 0 with premature labor at 28 weeks gestation. She had first routine antenatal ultrasound scan at 22 weeks which revealed thickened placenta with multicystic changes („swiss cheese“ pattern). Amniocentesis showed a normal 46XX female karyotype, u-fetoprotein was 5.7 mg/l. Premature labor was spontaneous and she delivered a 930 gm female infant vaginally. There were no obvious external dysmorphic features. After the following 8 months, the baby was found well with normal development and no features of Beckwith-Wiedemann syndrome were found. The placenta weighed 363 gm (over 90 percentile) with dimension of 160 mm x 140 mm and up to 35 mm in thickness. The cord was eccentrically inserted and contained three vessels. On the maternal plate of the placenta there were grape-like, cystic vesicles measuring up to 20 mm in diameter. Histology of the placenta showed marked hydropic swelling of the stem villi with cisternal formation. Some stem and terminal villi had focal marked choriongiomatoid changes. The extramedullary haemopoiesis was focally present in villi, too. PMD is most easily confused with a partial hydatidiform mole, clinically and pathologically. Postnatally, careful gross and microscopic examination of the placenta is required to distinguish these two conditions. The present case report highlights the importance to distinguish PMD from partial mole, as a management is entirely different. The need for detailed pathological examination of all abnormal placentas cannot be overemphasised.

**P8.15**

**ACARDIAC-TWIN PREGNANCY - PLACENTAL PATHOLOGY. ACARDIUS AND NEUROPATHOLOGY OF SECOND TWIN: A CASE REPORT**

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Acardiac twinning is a rare complication of multiple pregnancy. We present a case 24-year-old woman, with labour at 26 weeks of gestation. The normal-looking female newborn (weight 900 gm) and the acardius were delivered by Cesarean section. The placenta weighing 740 gm was diagnostically-monochorionic. The umbilical cord (of normal twin) was eccentrically inserted, hypertwisted and oedematous. The second cord (of acardius) had vascular malformation. Histology of the placenta showed dysmaturity of terminal villi, their swelling and presence of numerous Hofbauer cells within stroma. The acardiac twin, weighing 1540 gm, was a grossly round cystic mass. Autopsy revealed the absence of thoracic organs, liver, spleen, pancreas and adrenals. There was omphalocele with stomach. A cytogenetic study revealed 46, XX karyotype. The normal-looking female twin died three days after delivery. An autopsy revealed lung with hyaline membrane disease, obstructive hypertrophic cardiomyopathy and hepatomegaly. Neuroradiological examination showed numerous hypoxic-ischaemic lesions dispersed in the cortex and white matter. Foci of necrosis in the cerebral cortex, brainstem and cerebellum were seen. A small foci of hemorrhages were in periventricular germinal matrix.

Changes in the normal twin described above leads to the recommendation that autopsy and neuropathological examination should be carried out of all fetuses and newborns from acardiac-twin pregnancies, including those which appear normal. The monitoring of neonates of such pregnancies for changes in the central nervous system is also advisable.

**P8.16**

**INFLUENCE OF THROMBOPHILIA ON PLACENTAL VILLUS STRUCTURE: A STEREOLGICAL STUDY.**

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Objectives: Stereological assessment of placental vili in thrombophilia.

Methods: A prospective study of both acquired (AT) and inherited (IT) thrombophilia in 800 low-risk prima gravid pregnancies provided four clinical cohorts for this study. 10 placentas were sampled from each of the following groups: pre-eclampsia with IUGR (PET-IUGR); normal birth weight controls (NBW); AT and IT. The volume of each placental disc was measured followed by uniform random sampling of 10 full thickness biopsies. 5 fields were examined from coded haematoyxin and eosin stained sections. Stereological assessment comprised star volume and surface area measurements of terminal villi and of their capillaries. Two-dimension enumeration of syncitial knots was also performed in each case.

Results: The results showed a statistically significant reduction of both star volume and surface area of terminal villi in AT and IT compared to NEW. While the surface area of capillaries was significantly reduced in AT, IT and PET-IUGR, capillary star volume was only significantly lower in AT and IT when compared to controls (the lack of difference in PET-IUGR is possibly related to antihypertensive therapy in these mothers). Syncitial knots were increased in each test group when compared to NBW.

Conclusions: These findings demonstrate that both AT and IT clearly affect the placenta in a way which is very similar to PET-IUGR.
P8.17

REPLICATION OF HUMAN CYTOMEGALOVIRUS IN THE THIRD TRIMESTER HUMAN PLACENTA.

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Objectives: Since only 40% of pregnant women with primary human cytomegalovirus (HCMV) infections give birth to infected infants, an effective fetal barrier must exist. The placental cells should be considered as both the most important site of the fetus protection and reservoir of HCMV. In this study, we estimated the susceptibility of trophoblasts and placentac macrophages, as well as explants of chorionic villi and deciduas isolated from third trimester placentas to HCMV infection in vitro.

Methods: 15 human placentas were obtained immediately after delivery. The explants of third-trimester chorionic villi and deciduas, as well as isolated cells (trophoblasts and macrophages) have been exposed to infection with laboratory strain of HCMV AD169. Accumulation of infectious HCMV in cell supernatants and cell lysates were assayed on MRC-5 cells and by PCR method.

Results: The infection of organ cultures occurred at higher level of released virions after 2-3 days of incubation. Trophoblasts and placentac macrophages were permissively infected with HCMV in vitro. However, virus loads in infected trophoblasts were lower than in macrophages isolated from fetal and maternal part of placenta. Infectious virus produced by trophoblasts was predominantly cell associated.

Conclusions: Presented results show that placentac cells can be permissively infected with HCMV, but virus replication in trophoblasts is slow and less effective than in macrophages. This work was supported by the State Committee for Scientific Research, grant no 3 POSE 00125.

P8.18

APPLICATION OF QUANTITATIVE REAL-TIME PCR FOR DETECTION OF HUMAN CYTOMEGALOVIRUS INFECTION IN PLACENTAL CELLS.

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Objectives: Prenatal human cytomegalovirus (HCMV) infection may cause pregnancy complications such as intrauterine growth restriction and birth defects. Congential HCMV infections are commonly associated with chronic villitis. In the present study, we used a quantitative real-time PCR and qualitative PCR to test for viral DNA in placental cells after termination of pregnancies.

Methods: Twenty third-trimester placentas were obtained from pregnant women with HCMV infection. Placental cells were isolated from both deciduas and chorionic villi by enzymatic digestion and examined separately. Isolates of DNA were examined for the presence of HCMV DNA by PCR detecting UL55, UL144 and US28 genes, the viral DNA loads were measured by quantitative real-time PCR.

Results: HCMV DNA was detected in about 90% of placental samples. Viral infection was transmitted to chorionic villi in about 83% of organs. Placental virus load ranged from 10^6 to 10^10 copies per 5 x 10^6 cells. However, only in six placentas (32%) the number of HCMV DNA copies was > 5 x 10^1.

Conclusions: Quantitative PCR can be useful for assessment of virus load in placental samples and study of HCMV transmission to chorionic villi. However, the relation between the severity of illness and quantity of virus in placenta is uncertain. This work was supported by the State Committee for Scientific Research, grant no 3 POSE 00125.
LOCALISATION OF EPIDERMAL GROWTH FACTOR AND ITS RECEPTOR IN HYDATIDIFORM MOLE AND HYDROPIC ABORTION - DECREASED EXPRESSION OF EGF RECEPTOR INDICATES A HYPERPROLIFERATIVE ACTIVITY AND ENHANCED INVASIVE POTENTIAL.

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Objectives: The EGF/EGFR signalling network has been shown to play an important role in cell division, proliferation, differentiation, adhesion, invasion and tumour cell metastasis. Hydatidiform mole complete (CHM) and partial (PHM) as well as hydropic abortion are considered as disorders of placental development, maturation and trophoblast proliferation. In recent studies we have investigated the different trophoblastic proliferation potential and cell-matrix-interactions in CHM and PHM. Therefore, aim of this study was to examine the expression of EGF and EGFR in hydatidiform mole compared to hydropic abortion.

Methods: Immunohistochemical analysis of EGF and EGFR was performed in 4 CHM, 4 PHM, 8 hydropic abortions and 4 non-molar abortions of peristatic reasons (controls), between 7 and 14 weeks of gestation.

Results: Most striking was a strongly enhanced expression of EGF and a markedly decreased or missing immunoreactivity of EGFR in hyperproliferative intermediate trophoblast of CHM.

Conclusions: Our results suggest an enhanced invasive ability of trophoblast cells in complete hydatidiform mole that might explain its potential to transform into invasive mole or choriocarcinoma.
**P9.01**

**HUMAN PLACENTAL GROWTH HORMONE RELEASE FROM TROPHOBLAST CELLS SHOW A BIPHASIC RESPONSE TO GLUCOSE.**

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Human placental growth hormone (pGH) is released by the syncytiotrophoblast into the maternal circulation, starting at ~6 weeks of pregnancy and increasing towards term. pGH appears to be involved in conditioning maternal metabolism in pregnancy. Previous research has shown an association between pGH and fetal growth. The aim of this study was to investigate the control of pGH release in response to changes in glucose concentration and glucoregulatory factors.

**Methods:** BeWo cells were incubated under standard, serum-free conditions for 24 hrs, after which the media was changed to serum-free medium containing 1-20 mM glucose for an additional 24 hrs. Cells were also incubated in IGF-I, leptin (100 ng/ml) or insulin (50 nM). Cell supernatants were assayed for pGH by a specific ELISA; cell proliferation was measured using a fluorescent DNA-binding assay.

**Results:** pGH in culture supernatants averaged 63 ± 7 ng/ml (n=3). Incubation with increasing glucose concentrations showed a ~2 fold increase in pGH secretion between 1 and 10 mM glucose (n=3), pGH release peaked at approximately 10 mM glucose and decreased thereafter. pGH release was not affected by IGF-I or leptin; there was a trend towards decreased pGH production (32 ± 14%; p=0.15) following exposure to insulin.

**Conclusion:** These results show that pGH release has a biphasic response to glucose concentration. At lower concentration (<10 mM), pGH is positively regulated by glucose; above 10 mM, pGH release is progressively suppressed by increasing glucose concentration. This biphasic response may explain the contradictory results obtained previously and is consistent with studies showing an association between pGH and increased fetal growth. (Supported by NIH R01 HD06982).

**P9.02**

**EXPRESSION OF C-REACTIVE PROTEIN IN TERM HUMAN PLACENTAL TISSUE.**

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**Objective:** C-reactive protein (CRP) is a marker of systemic inflammation. Recently, it has been shown that CRP is present in amniotic fluid, and fetal urine and that elevated levels are associated with adverse pregnancy outcome. However, the precise source of amniotic fluid CRP, its regulation, and function during pregnancy is still a matter of debate. The present in vivo and in vitro studies were designed to investigate the production of CRP in human placental tissues.

**Material and Methods:** Ten paired blood samples from peripheral maternal vein (MV), from the umbilical cord artery (UA) and umbilical vein (UV) were collected from women with elective caesarean-sections at term. The placental protein accumulation capacity of hCG, hPL, leptin and CRP was compared with the dual in vitro perfusion method of an isolated cotyledon of human term placenta and quantified by ELISA. Values for accumulation (release) were calculated as total accumulation of maternal and fetal circuits normalized for tissue weight and duration of perfusion. For gene expression, RNA was extracted from placental tissue and reverse transcribed. RT-PCR and real time PCR were performed using specific primers.

**Results:** The median (range) CRP level was significantly different between UA and UV [50.1 ng/ml (12.1-684.6) vs. 61 ng/ml (16.9-708.1)]. The median (range) difference between UV and UA was 9.3 ng/ml (2.2-31.6). A significant correlation was found between MV CRP and both UA and UV CRP levels. Median (range) MV CRP levels [2649 ng/ml (260.1-8299)] were 61.2 (6.5-96.8) fold higher than in the fetus. In vitro, the total accumulation rates (mean±SD) were 31±1 (mU/g/min, hCG), 1.16±0.19 (µg/g/min, hPL), 4.71±1.91 (ng/g/min, CRP), and 259±118 (pg/g/min, leptin). mRNA for hCG, hPL and leptin was detectable using conventional RT/PCR, while CRP mRNA could only be demonstrated by applying real time RT/PCR. In the perfused tissue the transcript levels for the four proteins were comparable to those detected in the native control tissue.

**Conclusion:** Our results demonstrate that the human placenta produces and releases CRP mainly into the maternal circulation similarly to other analyzed placental proteins under in vitro conditions. Further studies are needed to explore the exact role of placental CRP during pregnancy.

**P9.03**

**EFFECT OF BETAMETHASONE ON PLACENTAL QUINOLINIC ACID.**

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**Objective:** Our studies show that inflammation increases placental quinolinic acid (quin) output and that elevated quin levels increase the risk of fetal cerebral injury. Glucocorticoids given to women with PROM resulting from infection may increase quin output through elevated enzyme levels/activity. Increased quin in the peripheral fetal circulation may damage endothelial cells, facilitating its entry into the fetal brain. We studied the effects of betamethasone on placental enzymes TDO/IDO required for quin synthesis, and effects of quin on endothelial cell tight junction proteins and oxidative damage.

**Methods:** Term placental explants were treated with betamethasone (100ng/ml, n=6) and HUVECs with 10-40U/mL quin (n=4), for up to 72h. Isoprostanate measured through EIA was used to assess the extent of oxidative damage. Changes in mRNA expression and protein of TDO/IDO, tight junction molecules occludin and zona occludin (ZO)-1 were assessed by qRT-PCR and Western hybridization respectively. Data was analysed by Mann-Whitney U test.

**Results:** Compared to controls betamethasone treatment significantly decreased IDO/ TDO mRNA expression and protein levels (p<0.01). Quin treatment (40U/mL) significantly decreased ZO-1 and occludin mRNA expression and protein levels (p<0.01) while 20U/mL quin significantly increased isoprostane production by HUVECs (mean±SD, 127±177pg/ml, p<0.02).

**Conclusion:** Unlike hepatic TDO, placental TDO is not induced by betamethasone but, the anti-inflammatory effects of betamethasone may be beneficial in temporarily reducing placental quin output through decreased TDO/IDO. However, chronic exposure to elevated quin levels may compromise the fetal blood brain barrier leading to an accumulation in the fetal brain and neuronal damage.

**P9.04**

**TOCOLOGY INCREASES THE RELEASE OF PLACENTAL PROTEIN 13 FROM PLACENTA INTO MATERNAL BLOOD.**

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**Objective:** Placental protein 13 (PP13) is expressed already very early in the syncytiotrophoblast. It is a galecin binding annexin as well as a mild lypoxygenase phospholipase A that is involved in calcium mobilization, liberation of long chain fatty acids (linoleic and arachidonic acid) and their turnover into prostaglandins. We have analyzed the amount of PP13 in venous serum samples from pregnant women undergoing tocolysis.

**Methods:** Serum samples from peripheral venous blood were collected throughout pregnancy. PP13 was quantified in these samples utilizing a PP13 specific sandwich ELISA. PP13 concentrations were correlated to onset and duration of the tocolysis therapy.

**Results:** In control pregnancies the amount of PP13 in maternal blood stays low during the first and second trimester and only increases during the third trimester. In cases with preterm labour and thus tocolysis medication, this therapy showed a direct effect and lead to an increase in the amount of PP13 in maternal blood independent on the start of the medication during pregnancy.

**Conclusions:** Increased release of PP13 from the placenta is known to take place in pathologies with placental degeneration such as preclampsia. Tocolysis medications are known to affect placenta and such therapies are contra-indicated with placental insufficiencies. Our results support the hypothesis that tocolysis therapies may have an adverse effect on trophoblast integrity.
**P9.05**

**EFFECTS OF PENTAERITHRITYL TETRANITRATE IN HUMAN PLACENTAE DURING DUALY RECIRCULATING PERFUSION IN VITRO**

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**Objectives:** During the last years organic nitrates were applied in obstetrics for acute uterine relaxation including tocolysis. Diseases with decreasing blood circulation of the utero-placental vessel system, e.g. preeclampsia could be possibly influenced by NO donors because of their vasodilatory effects. Disadvantages of glycerol trinitrate, which is used for tocolysis, are developed tolerance and nitrate headache. One possible alternative is pentaerithritol tetranitrate PETN, but there is no experience about use in gynecology yet. Possible side effects on placental function play an important role to assess the safety for the fetus. The purpose of this study was to determine the influence on the placental metabolism and pharmacokinetics of PETN across perfused placenta.

**Methods:** Separated lobules from term human placenta were dually perfused in vitro as described by Schneider and Huch. After the control period of 2 hours, the medium was replaced and maternal site perfused with PETN-containing medium for further 4 hours. Samples from fetal and maternal circulation were collected every 30 minutes. Effects on placental carbohydrate and oxygen metabolism were assessed in both circulations. Additionally, β-HCG release into the maternal circulation was measured. Perfusion pressure of the fetal circulation was analysed. PETN and metabolites were determined via chromatographical methods.

**Results:** No changes in carbohydrate and oxygen metabolism were detected but β-HCG secretion decreased. After application of PETN into the maternal circulation, a slight decrease of fetal perfusion pressure was observed.

**Conclusions:** The observed perfusion pressure reduction reflects the vasodilatory NO donor effects of PETN.

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**P9.06**

**PRODUCTION OF PLACENTAL PROTEINS (PP13, PP17, PP23, PP25) DURING EX VIVO PERFUSION OF HUMAN PLACENTAL TISSUES**

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**Objectives:** PPs were isolated from human term placenta and physicochemically characterized two decades ago. Later, molecular biological investigations shed new lights on their structural/functional characteristics and oncodevelopmental importance. According to earlier data, one human term placenta contains 3.7 mg of PP13/gaelectin-13, 2.5 mg of PP17/Sandrin, 3 mg of PP23/HEBP2 and 5 mg of PP25/HSPC034. Now, we examined the effect of ex vivo perfusion of tissue levels of these PPs.

**Methods:** In all perfusions (n = 5, 7h), media (NCTC-Earls buffer 1:1) were changed after 1h and 3h of perfusion. Media were equilibrated for protein content and run on SDS-PAGE. Western blots were carried out with monospecific anti-PP antibodies and densitometrically analyzed.

**Results:** Compared to the initial point, after 7h of perfusion, tissue levels of PP17 were noticeably changed. A decrease in levels of PP17c (p<0.02) was seen, while increased levels of PP17a (ns), PP17b (p<0.03), PP25 (p<0.03) and PP13 (ns) were detected. However, after normalizing the values to gram tissue, significant change for PP17c (p<0.003) could be seen.

**Conclusions:** Our preliminary ex vivo perfusion results suggest that production of PPs involved in cell-cell/matrix interactions (PP13/gaelectin-13), lipid metabolism/apoptosis (PP17/Sandrin), oxidative processes (PP23/HEBP2) or cell cycle regulation (PP25/HSPC034) might be in vivo significantly altered due to variable placental conditions.

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**P9.07**

**HUMAN TROPHOBLAST PRODUCES TRANSTHYRETIN**

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**Objectives:** The transfer of maternal thyroid hormone to the fetus is crucial for normal fetal development. Our laboratory has previously reported evidence that in the isolated perfused human placenta trophoblastic type III iodothyronine deiodinase (D3) prevents thyroxine (T4) transfer. We hypothesised a cytosol thyroid hormone binding protein is present in trophoblast and modulates availability of T4 to D3.

**Methods:** Placental cytosol was assayed for 53I-T4 binding affinity. Western blotting was used to examine the presence of serum thyroid hormone binding proteins; thyroxine binding globulin (TBG), transthyretin (TTR) and albumin (Alb). Trophoblast synthesis was examined with RT-PCR and immunohistochemistry. Additional thyroid hormone binding proteins were purified by column chromatography. Effect on deiodination was assessed by inhibiting protein binding with mefenamic acid.

**Results:** High affinity binding was identified in placental cytosol. Western blotting identified all 3 serum thyroid hormone binding proteins (although TBG expression was weak). RT-PCR confirmed TTR and Alb mRNA in placenta but not TBG. Immunohistochemistry localised TTR to the apical compartment of syncytiotrophoblasts while albumin was demonstrated on the apical cell membrane. Purification procedures identified TTR as the protein with the highest affinity for thyroid hormone. Addition of mefenamic acid, a potent inhibitor of T4-TTR binding, to placental homogenates significantly increased deiodination.

**Conclusions:** Human trophoblasts synthesise TTR which may modulate T4 deiodination and hence transfer to the fetus.

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**P9.08**

**PATTERN OF EXPRESSION OF ENDOCANNABINOID RECEPTORS (CB1 AND CB2) IN RAT UTERINE TISSUES DURING PREGNANCY**

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Reports of adverse effects of exocannabinoid exposure upon human pregnancy are consistent with the presence of an active cannabinoid system in pregnancy i.e. endocannabinoid synthesis and their receptors, CB1 and CB2. This suggests that the endogenous cannabinoid system may have an important physiological role in pregnancy. Indeed, studies in animal models and human suggest a critical role for embryo implantation and maintenance of pregnancy. However no studies have been performed during the period of placental development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development. In order to gain insight into the role of endocannabinoids in the development of fetoplacental unit we have investigated the temporal and spatial pattern of expression of cannabinoid receptors, CB1 and CB2, during the period of placentation development.
ANTI-HYPERTENSIVE DRUGS CLONIDINE, DIAZOXIDE, HYDRAZONE AND FURESEMIDE, REGULATE CYTOKINE PRODUCTION FROM PLACENTAS AND PBMCs IN NORMAL PREGNANCY

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Objectives: Anti-hypertensive drugs such as clonidine, diazoxide, hydralazine and furosemide are used in the hypertensive disorders during pregnancy to control blood pressure, but it is not clear if they modulate placental or circulating cytokine production. The aim of this study is to examine the effect of pharmaceutical doses of anti-hypertensive drugs on placental tissue and peripheral blood mononuclear cells (PBMC) production of cytokines IL-10, TNF-α and IL-6 in normal pregnancy.

Methods: Placental biopsies were taken from the decidual surface of normal term placentas (n=6) and PBMC (n=7) were separated from the whole blood of normal term pregnant women. Both villous explants and PBMC were cultured with increasing concentrations of anti-hypertensive drugs. The dose effect of drugs on placental and circulating cytokine production (IL-10, TNF-α and IL-6) were examined using ELISA.

Results: Placental IL-10 production was not affected by clonidine, but significantly decreased after diazoxide, hydralazine and furosemide incubation. There was significant increase of IL-10 production from PBMC with increasing concentrations of clonidine and at lower doses of hydralazine. There was a stepwise reduction of TNF-α and IL-6 with increasing doses of diazoxide, hydralazine and furosemide from placentas and PBMC of women with normal pregnancy.

Conclusions: Clonidine and hydralazine can stimulate circulating anti-inflammatory cytokine IL-10 production, while furosemide and diazoxide inhibit IL-10 and pro-inflammatory TNF-α and IL-6 production from placentas and PBMCs.
P10.01
HIGH MOBILITY GROUP BOX 1 (HMGB1) EXPRESSION BY HUMAN FETAL MEMBRANES AT TERM GESTATION IS RELATED TO LABOUR
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Objectives: Inflammatory cytokines are believed to play a significant role in the biome-

llectual mechanism leading to labour. HMGB1, previously termed Amphoterin, is a ubiqui-
tous and chromosomal protein that can be actively released by a wide range of immune and

non-immune cells and it is now classified as a non-classical pro-inflammatory cytokine.

Since labour is considered an inflammatory process, we investigated whether HMGB1 is

expressed in human fetal membranes and whether its presence could be correlated with

labour.

Methods: Human foetal membranes at term gestation obtained were obtained either after

vaginal delivery (V.D.) or during elective cesarean Section (C.S.). HMGB1 was detected by

Western Blot using a polyclonal antibody.

In some experiments tissues were incubated for 24 h at 37°C with sodium nitroprusside, a

nitric oxide (NO) donor.

Results: Human fetal membranes at term expressed HMGB1. HMGB1 expression in samples

obtained after V.D. is higher than in samples obtained during C.S. NO modulates HMGB1

expression.

Conclusions: HMGB1 could be involved in labor-related inflammatory events. An anti-

HMGB1 treatment could represent a therapeutic strategy to limit an exaggerated inflamma-
tory response, as in the case of pre-term labour in the setting of intrauterine infection.

P10.02
ASSOCIATION OF PAT FAMILY PROTEINS WITH LIPID BODIES IN TERM PLACENTAL MEMBRANES
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Objectives: As storage depots for neutral lipids, lipid bodies (LBs) accumulate with

advancing gestation and labor in placental membranes. Little is known about the pro-
teins associated with these structures. The perilipins 4/DIRP/TIP47 (PAT) proteins rep-

resent a unique family intimately associated with LB formation and function. We assessed

(1) the expression of PAT proteins in term human placental membranes and (2) the degree to which these proteins were associated with the LBs present therein.

Methods: With informed consent, placental membranes were collected from uncom-

promised singleton gestations at term (n=15). RT-PCR and immunoblotting were used to

assess PAT family member expression, and immunolocalization was performed on frozen

sections of membrane rolls.

Results: ADRP and TIP47 were expressed in both amnion and chorioideciudua (CD).

ADRP selectively localized to LBs stained with a fluorescent neutral lipid dye within

amnion epithelium and chorion trophoblasts, while TIP47 localized both to LBs and cytoplastic regions within these cells. Perilipin was detected more frequently in CD

than amnion. Among multiple immunoreactive bands, a ~61kDa protein (possibly rep-

resenting perilipin A) was inconsistently observed, and a ~50kDa protein (potentially rep-

resenting perilipin B) was predominant in CD. By immunofluorescence, perilipin

localized to amnion epithelial and chorionic trophoblast cells; however, this immunore-

activity was cytoplasmic rather than LB-specific.

Conclusions: ADRP and TIP47 are closely associated with LBs within cells of the placent-
al membranes. While apparently expressed, the association of perilipin with these

LBs (as suggested by a recent report) could not be corroborated.

P10.03
IN VITRO ANALYSIS OF VARIED O2 TENSIONS IN HUMAN GESTATIONAL TISSUE – AN ASSESSMENT OF TISSUE VIABILITY AND CELL DEATH
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‘Department of Physiology, University of Melbourne, Victoria, Australia. Translational Proteomics, Baker Medical Research Institute, Victoria, Australia.

Objective: To determine the effects of O2 tension (8%, 21% or 95% O2) on apoptosis in

placental and fetal membrane tissue incubated in vitro.

Methods: Placentae and fetal membranes were collected from normal pregnancies

at the time of Caesarean section. Tissues were incubated for 6 h in 8, 21 or 95% O2. Tissue

Casparase-3 & -9 protein content was assessed by Western blot analysis and M30
cytodeath marker used in this study (accumulation of lactate dehydrogenase (LDH).

The dye spot and surrounding areas were dissected. Similarly, membrane was dissected

before the insertion of the urinary catheter, a large, swab stick saturated with a non-toxic,

nitric oxide (NO) donor.

Results: Human fetal membranes at term expressed HMGB1. HMGB1 expression in samples

obtained after V.D. is higher than in samples obtained during C.S. NO modulates HMGB1

expression.

Conclusions: HMGB1 could be involved in labor-related inflammatory events. An anti-

HMGB1 treatment could represent a therapeutic strategy to limit an exaggerated inflamma-
tory response, as in the case of pre-term labour in the setting of intrauterine infection.

P10.04
ASSOCIATION OF PAT FAMILY PROTEINS WITH LIPID BODIES IN TERM PLACENTAL MEMBRANES: AN NOVEL PROSPECTIVE IDENTIFICATION OF
THE SUPRA-CERVICAL SITE
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Objective: To identify the putative site of rupture by assessing morphologic and apoptotic

features.

Methods: Fetal membranes (n=8) were collected from women at term, not in labour, with

intact membranes and undergoing Caesarean section. Prior to commencement of surgery,

before the insertion of the urinary catheter, a large, swab stick saturated with a non-toxic,

ethanol based dye, (flonkey’s blue) was inserted through the cervix to the fetal membrane. A

successful outcome was denoted by a blue mark, corresponding to the supra-cervical site.

The dye spot and surrounding areas were dissected. Similarly, membrane was dissected

from a distal site on the membrane. Fetal membrane sections were rinsed in PBS, rolled,
cut, fixed and then embedded in paraffin for immunohistochemical analysis. Sections were

stained with a mouse monoclonal antibody, M30 Cytodeath, which recognises a Caspa-

rase-3 cleavage site of cytokeratin 18 and with Haematoxylin and Eosin for morphological analy-

sis. Staining of M30 was quantified using Leica Qwin image analysis software.

Results: There was evidence of loss of integrity across the amnion and chorion and fewer

trophoblastic nuclei within the chorion laeve in the sections from the supra-cervical site

compared to the distal site. M30 staining in the supra-cervical site was 10-fold greater than that

in tissue observed in the supra-cervical site. (0.01% staining of whole tissue ±

0.001% compared to 0.001% ± 0.0007; Students t-test, p = 0.004).

Conclusion: Cell death and tissue remodeling were greater in tissue obtained from the supra-

cervical site compared to the distal site.
P10.05

EFFECTS OF LOW IN VITRO OXYGEN TENSION ON HUMAN FETAL MEMBRANES

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Objective: The relative hypoxia of the early human conceptus, and increases in maternal blood flow to the placenta at ~10 weeks of gestation are well established. In contrast, little information is available on fetal membranes. The first 10 weeks are presumed to be hypoxic, and few studies published indicate that relative hypoxia of fetal membranes continues through most of pregnancy. The aim of this study is to directly assess the effects of low in vitro oxygen tension on prostaglandin (PG) and interleukin (IL) output, and tissue structure of fetal membranes.

Method: Fetal membranes from elective term Caesarean deliveries were cultured at 37°C in defined media overnight, either in 5% CO2: 95% air, or in 5% CO2: 2% O2: 93% N2. Fresh media ± Ing IL-1β/ml was added and cultures continued for 2h, 4h, 6h and 24h. Media was frozen until assay for PGE2 and IL-6. Tissue explants were fixed, and kept in 70% ethanol until histology for overall structure and tissue structure of fetal membranes.

Results: Tissue structure and bacteria-positivity were not affected by the lower levels of oxygen. The output of PGE2, and of IL-6 were affected in subtle ways. Maximum PGE2 output was lower in lower levels of oxygen (by about 20%). In atmospheric oxygen, IL-1β increased IL-6 levels after 6h of culture, but 24h was needed to increase IL-6 output in the presence of lower levels of oxygen.

Conclusions: Decreased levels of oxygen during in vitro culture of fetal membranes seem to have subtle effects on inflammatory responses to IL-β, whereas gross effects (e.g. on membrane structure) were not found. These preliminary data suggest that further work on the physiological levels of oxygen to which fetal membranes are exposed, and the impact of these on tissue function are urgently needed.

P10.06

EFFECT OF MECHANICAL STRETCH ON THE EXPRESSION OF INTERLEUKIN-1BETA IN HUMAN UTERINE MYOCYTES.

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Pro-inflammatory cytokines such as interleukin-1beta (IL–1beta), IL–6, IL–8 and tumour necrosis factor-alpha as well as some prostaglandins (E2 and F2alpha) are known to increase within tissues of the labouring uterus. Mechanical stretch and incubation with IL-1beta have both been shown to increase the expression of IL-8 and the prostaglandins in uterine myocytes by activation of MAPK pathways. The effect of mechanical stretch could be secondary to an increase IL-1beta in the myometrium. Using cultured human myometrial cells we have studied the effect of mechanical stretch on IL-1beta expression. Primary human uterine myocytes (obtained from three different patient groups) (a) pregnant but not in labour, NL, (b) pregnant and in labour, L and (c) non pregnant, NP) were isolated and cultured in DMEM medium containing 7.5% FCS, 100 units/ml penicillin and 100 ng/ml streptomycin in 6-well flexible-bottomed culture plates precoated with collagen type I and subjected to a static stretch of 0, 6, 11 or 16% for 1h using a flexcell serum unit. At the end of the experiment mRNA for IL-1beta and GAPDH was quantified using the Rotor-Gene™ (Corbett Res).

Results: Mechanical stretch increased the expression of IL-1beta in a dose-dependent manner. The larger increases seen in NP group (138.1 ± 9.7, 207.7 ± 29.2 and 165.6 ± 27.0% increase at 6, 11 and 16% respectively; mean ± SEM; n=6; P=0.028, 0.018 and 0.028). Similarly non significant increases were seen at 6h stretch for the NL group. The increases of IL-1beta expression upon stretching of uterine myocytes could contribute to the elevation of this cytokine in vivo. The larger increases seen in NP myocytes reflect a lower basal level of IL-1beta in this tissue.

P10.07

HUMAN BETA-DEFENSINS 1 AND 2 ARE EXPRESSED IN HUMAN CHORION TROPHOBLAST

A.44

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Objectives: 30% of preterm births are complicated by infection. Human β-defensins (HBD) are natural antimicrobial peptides expressed in the female reproductive tract. Previous reports suggest that HBD1 and 2 are present in the pregnant uterus. The current study investigates HBD1 and 2 expression in human fetal membranes and the effects of betamethasone treatment and chorioamnionitis on localization of these peptides.

Methods: Fetal membranes were collected from women from six groups: (a) term, not in labour; (b) term, in labour; (c) preterm, no betamethasone; (d) preterm, betamethasone; (e) preterm, no betamethasone, chorioamnionitis; and (f) preterm, betamethasone, chorioamnionitis. Immunohistochemical (IHC) localization of HBD1 and 2 was performed using standard IHC protocols with antigen retrieval.

Results: HBD1 was localized in the chorion trophoblast (CT) layer of fetal membranes and was also present in decidua. Distribution was similar between groups. HBD2 was also present in CT with no differences between groups. This lack of change in localization is consistent with our studies of HBD2 expression in placental syncytiotrophoblast.

Conclusions: CT is likely to be a key barrier to the progression of infection from the lower genital tract into amniotic fluid and subsequently, to the fetus. Expression of HBD1 and 2 by these cells suggests that the innate immune system has an important role in the limitation of uterine infection during pregnancy.

P10.08

POLYMORPHISM IN TOLL RECEPTOR 4 (TLR4) IS ASSOCIATED WITH ELEVATED RISK OF PREGNANCY PATHOLOGIES

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Introduction: Genetic variations like Asp299Gly TLR4 polymorphism (A299G) are associated with elevated risk of diverse diseases. The role of TLR 4 during parturition is poorly understood, the presence and localization of TLR 4 in placenta and amnion is controversial.

Objectives: To test if Asp299Gly is associated with premature delivery (PD), premature rupture membranes (PPROM), toxiemia, and intrauterine growth restriction (IUGR). Methods: DNA from umbilical cords of 164 newborns was analyzed by PCR using mist matching primers. Immunocytochemistry and Western blotting was performed in chorioamnionitis from 5 PD and 5 (TP). Results: 17/164 newborns present Asp299Gly (p=0.04 chi square test). An immunopositive reaction to TLR4 was observed in all of the samples in the amniotic epithelium. Western blot confirmed the presence of the protein.

Conclusions: Patients with As299G show elevated risk of obstetric pathologies. The presence of TLR4 in the amnion suggests it plays a role in the inflammatory mechanisms that leads to delivery. Additional studies are necessary to confirm this hypothesis.
P10.09

PROSTAGLANDIN E 2 RECEPTORS IN HUMAN PLACENTA AND FETAL MEMBRANES THROUGHOUT GESTATION, WITH AND WITHOUT LABOR.
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Objective: Prostaglandin (PG) E2 levels in plasma and amniotic fluid increase with advancing gestation and labor. The effects of PGE2, are mediated through specific G-protein coupled EP receptors (EP1-4), acting via different second messengers. The presence of these receptors in the placenta and fetal membranes may indicate additional roles for PGs in the signaling pathways associated with placental function and labor. We determined the localization and expression of the PGE2 receptor isoforms in human placenta and fetal membranes throughout gestation and with labor.

Methods: Placentae and fetal membranes (n=5 each group) were collected following delivery at term or preterm, with or without labor. The localization and expression of EP1-4 receptors was determined by IHC and Western Blot.

Results: PGE2 receptors were localized to placental villous stroma, endothelial cells and fetal membranes; amnion epithelium, amnion fibroblasts and chorion trophoblasts. Intense staining for EP3 was observed in the syncytiotrophoblast layer. In the amnion epithelium, EP1 and EP4 showed a different cellular localization with labor, changing from intense basal membrane to punctate cytoplasmic staining, suggestive of a different cellular function. There were no changes in the expression of EP2 associated with gestational age, labor or when comparing the amnion to chorion. Three putative EP3 receptor splice variants were identified with varying expression between the amnion and chorion, indicating a possible shift in regulation of EP3 throughout gestation. Overall EP3 expression was greater in the chorion compared to the amnion.

Conclusions: PGE2 receptor isoforms were localized in placental villous stroma and endothelial cells and fetal membranes; amnion epithelium, amnion fibroblasts and chorion trophoblasts. The placenta at term expresses a wide range of TLRs, which could have a role in initiation and/or maintenance of labour both at term and preterm.

P10.10

EXPRESSION AND ACTIVITY OF TOLL LIKE RECEPTORS ‘1-9’ IN THE HUMAN TERM PLACENTA.
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Objectives – Toll like receptors (TLR) have emerged as key upstream mediators of inflammation at many tissue sites in humans. We hypothesize that TLRs are expressed in the placenta where they have an important role in the initiation of labour and in various pathological states of pregnancy such as infection associated preterm labour.

Methods: Placentas were collected after delivery in the absence of labour. Explants of placental tissue were cultured in vitro in the presence of ligands for TLR 1-9 and cytokine production (TNFa, IL-6, IL-8 & IL-10) into the culture supernatants measured using ELISA. Biopsies were taken for storage in RNA later for RT-PCR and quantitative PCR and snap frozen in liquid nitrogen for protein analysis.

Results: As reported elsewhere, placental explants responded to TLR4 ligand (Lipopolysaccharide) and TLR2 ligand (Peptidoglycan). The novel findings were robust cytokine responses to: TLR5 ligand (Flagellin) 100ng/ml; 21264+/+7534 pgTNFα/ml +/-SEM; TLR7/8 ligand (antiviral compound R848) 100ng/ml 9918 +/5067 pgTNFα/ml +/-SEM; LOX, 100ug/ml 14681+/+18445 pgIL-8/ml and TLR3 ligand (Poly I:C, 25ug/ml; 418596+/+82132pgIL-6/ml +/-SEM) by the term placenta. Immunohistochemistry and RT-PCR analysis will enable further characterisation of these TLRs.

Conclusions: The placenta at term expresses a wide range of TLRs, which could have a role in initiation and/or maintenance of labour both at term and preterm.

P10.11

NUCLEAR AND METABOLIC RETINOIDS PATHWAYS IN HUMAN FETAL AMNIOTIC MEMBRANES.
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Objectives: The placenta is a transitory but indispensable structure for harmonious gestation in mammalian species. If the molecular mechanisms are now well studied in the human placenta, curiously those of the linked amniotic membranes are poorly understood. Here we analyzed the metabolic and molecular pathways of the retinoids pathway in human amniotic fetal membranes.

Methods: Human fetal membranes were obtained from undergoing elective caesarean section. Tissues were frozen for RT-PCR and immunohistochemistry assays. Culture of primary amniotic and WISH cell line were conducted as previously described. The retinol metabolism was tested by a DR5-1kCAT reporter gene in primary culture cells.

Results: We established the presence of the metabolic actors and nuclear receptors of retinoids pathway in amniotic tissues and cells. The retinol activation into retinoic acid and retinol metabolism was tested by a DR5-1kCAT reporter gene in primary culture cells.

Conclusions: For the first time, the molecular and metabolic pathways of the retinoids were described in the human amniotic fetal membranes. Transcriptional activation of targets genes established the functionality of this retinoid pathway and its potential interest in the physiopathology of obstetrical pathology like premature rupture of membranes.
P11.01

ACUTE EFFECTS OF ALDOSTERONE ON THE ACTIVITY OF NA+/H+ EXCHANGER (NHE) ACROSS GESTATION IN THE HUMAN PLACENTAL SYNCYTIOTROPHOBLAST.

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Objectives: Epithelial NHE activity regulates sodium homeostasis and intracellular pH ([pH]i) and acute application of aldosterone increases the activity of NHE in renal tissue. We hypothesised that aldosterone modulates NHE activity in the syncytiotrophoblast across gestation.

Methods: Villous fragments from term and first trimester placentas were loaded with 1µM BCECF (pH sensitive fluorescent dye). The syncytiotrophoblast was acidified with a pre-pulse of 20mM NH4Cl and the Na+-dependent recovery of pH was taken as a measure of NHE activity ([pH]i units/sec, mean±SEM, n=number of placentas).

Results: There was no difference between resting pH, between term and first trimester syncytiotrophoblast (7.40±0.05, n=12 compared to 7.33±0.06, n=7 respectively). Aldosterone (10nM) significantly increased the rate of recovery to a similar extent in term (0.0099±0.0012 pH units/sec, n=12) and first trimester (0.0047±0.0005 pH units/sec, n=7, respectively). There was no difference in the control rate of recovery between first trimester and term syncytiotrophoblast (0.0046±0.0007 pH units/sec, n=12, 0.0047±0.0005 pH units/sec, n=7, respectively). Aldosterone (10nM) significantly increased the rate of recovery to a similar extent in term and first trimester syncytiotrophoblast (7.40±0.05, n=12 compared to 7.33±0.06, n=7 respectively).

Conclusion: These data, together with similar data for cortisol (Speake et al, 2004), show that corticosteroids acutely regulate syncytiotrophoblast NHE in early and late gestation, possibly part of a placental stress response.

Supported by The Wellcome Trust
Reference
Speake et al (2004a). Placenta. 25:A19

P11.02

PMCA1 AND PMCA4 EXPRESSION IN PLACENTA OF PTHrP-NULL MICE.

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Objectives: The final step in maternofetal Ca2+ transport is the active extrusion of Ca2+ against a concentration gradient mediated by placental isoforms 1 and 4 of plasma membrane Ca2+ ATPase (PMCA). Placental Ca2+ transport is altered in mice homozygous for the PTHrP-null mutation (NL) and in NL fetuses whole blood Ca2+ concentration is reduced. Here we examine whether the expression of PMCA 1 and 4 is altered in placentas of PTHrP-null mice.

Methods: Mice heterozygous (HZ) for the PTHrP-null mutation were mated (day 1) and litters harvested at day 18 of gestation. Litters selected had at least one wild-type (WT), HZ and NL fetus. Placental plasma membrane (PM) fractions were isolated and Western blots of PM protein probed for both PMCA1 and 4. Rat brain protein (RB) served as a positive control.

Results: Immunoreactive signals for PMCA1 and 4 were detected in all placentas as a triplet (158, 150 and 143kDa) or doublet (175 and 165kDa) respectively which co-migrated with signal in RB. Densitometric analysis (arbitrary units) of signal revealed protein expression was not significantly different in WT, HZ and NL groups for either PMCA1 (3.9±0.5, 3.5±0.4 and 3.8±0.5) or PMCA4 (2.4±0.5, 2.3±0.4 and 3.0±0.6, mean±SE, n=6 litters) respectively.

Conclusions: Placental expression of PMCA1 and 4 is unaltered in late gestation by deletion of the PTHrP gene.

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P11.03

MATERNAL PRE-PREGNANCY BODY COMPOSITION INFLUENCES PLACENTAL SYSTEM A ACTIVITY

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Objectives: Little is known about the influence of maternal nutritional status on placental function. As System A is an important mediator of placental amino acid transfer, we related maternal body composition before conception to placental System A activity.

Methods: In the presence or absence of NA+, uptake of 5µmol/l 14C-methylaminoisobutyric acid (a System A specific amino acid analogue) over 15 min at 37°C was measured in villous fragments from 104 term placentas from the Southampton Women's Survey. Uptake was adjusted for fragment protein content.

Results: Lower maternal BMI had a weak association with lower placental System A activity (P=0.12), but there were stronger relations between thinner pre-pregnancy upper arm circumference and arm muscle area and lower System A activity (P=0.04 and P=0.004 respectively). Independent of maternal arm muscle area, System A activity tended to be lower in those reporting strenuous exercise within the previous 3 months (P=0.07), but was not related to sum of skin folds, height, parity or smoking.

Conclusions: Placental System A activity was lower in women with a thinner arm muscle area and who exercised. Maternal nutrient availability is likely to be reduced in these groups, and lower placental System A activity could be an important adaptation to limit nutrient transfer to the fetus.

P11.04

ACTIVITY OF THE SYSTEM A AMINO ACID TRANSPORTER AS A MARKER OF IN UTERO GROWTH RESTRICTION: METHODOLOGICAL ASPECTS OF A SYSTEMATIC REVIEW

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Objective. In utero growth restriction (IUGR) is associated with relatively low activity of the System A amino acid transporter in the microvillous membrane of the syncytiotrophoblast. This study aimed to use the published literature to examine the utility of System A activity as a diagnostic marker of IUGR.

Methods. A standardized checklist for studies about diagnostic tests (STARD, BJOG 111:638-640) was used to scrutinize papers that report Na+-dependent uptake of methyl-AIB at 30 seconds in cases of IUGR and in infants with a birthweight appropriate for gestational age (AGA).

Results. Three papers, including data for a total of 45 cases of IUGR and 40 AGA controls drawn from three clinical populations, were available for analysis. Two papers included information about 8/25 items on the STARD checklist and one paper included 9/25 items. Items not included in any of the papers included: cut-off points for a normal value; numbers of misclassified cases and controls (needed to calculate sensitivity/specificity); measures of test-reproducibility between testers and populations.

Discussion. On the basis of the data provided in these papers it was not possible to explore whether System A activity could provide a diagnostic test for IUGR (although none of these papers specifically aimed to evaluate the diagnostic accuracy of System A activity). Information such as the degree of overlap in System A activity between cases of IUGR and AGA controls would provide a context for the experimental findings. Presenting such information in future work, or in systematic reviews of this topic, is likely to develop our understanding of how derangements in placental function relate to clinical outcome.
Uptake and transplacental transfer of fatty acids is essential for fetal development. In the placenta, it is a preferential uptake of fatty acids towards long-chain polyunsaturated fatty acids (LC-PUFA), especially arachidonic acid (AA) and docosahexaenoic acid (DHA). The lipid droplet associated protein adipophilin (human homologue of Adipo cyt y differenti ation-related protein, ADRP) is implicated in cellular fatty acid uptake in adipocytes. In addition to its high expression in adipose tissue, it is also highly expressed in placenta, but the role of ADRP in placenta is not known.

In this report we show that adipophilin mRNA and protein is regulated by fatty acids in a time- and dose-dependent fashion in the placental cell line BeWo, as well as in primary trophoblasts. Comparison of the effect of several fatty acids in the n-3 and n-6 class indicates that long-chain fatty acids (DHA, 22:6n-3, EPA, 20:5n-3, ARA, 20:4n-6) are more efficient than shorter fatty acids in stimulation of ADRP mRNA. This correlates well with the specific uptake and transport of long-chain fatty acids by the placenta to the fetus, and the importance of these fatty acids for the fetus for appropriate growth, neural and visual development. The fatty acid mediated increase was not related to differentiation state of the cells.

The peroxisome proliferator-activated receptors (PPARs) have been shown to be good candidates in fatty acid mediated gene activation. Our initial data indicate that this might be the case also in trophoblasts. Stimulation of cells with synthetic ligands for PPARs, 6 or y induces ADRP mRNA expression markedly. Furthermore, we show that stimulation of BeWo cells with the fatty acid DHA increases the fatty acid uptake in the cells, correlating with the increase in ADRP mRNA. We conclude that enhanced ADRP expression may contribute to fatty acid uptake by the placenta.

FUNCTIONAL DIFFERENCES IN PLACENTAL STEROID SULFATE UPTAKE OF ORGANIC ANION TRANSPORTER 4 (OAT4) AND ORGANIC ANION TRANSPORTING POLYPEPTIDE 2B1 (OATP2B1).

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Objectives: Human trophoblasts depend on the supply of external precursors such as DHEA-S and 16α-OH-DHEA-S for synthesis of estrogens. Recently, we have shown that uptake of steroid sulfates (SS) by trophoblasts is mediated by OATP2B1 and OAT4. Immuno- histochemistry results suggest a role of both carrier proteins inplacental uptake of SS from the fetal circuit (1). Characteristics and possible role of both transporters in the uptake of SS was unclear.

Methods: OAT4 or OATP2B1 were stably expressed in FlpIn-293-cell lines and the kinetic characteristics of DHEA-S and estrone-3-sulfate (E1S) uptake was determined.

Results: Uptake of E1S by OAT4- and OATP2B1-transfected cells was ~40-times higher, compared to control cells. DHEA-S uptake was highly stimulated in OAT-4 (40-times), but only weakly in OATP-B cells (3-times). Both, uptake of DHEA-S and E1S by OAT4 were partly (~50%) Na+-dependent, whereas uptake of E1S by OATP2B1 was totally Na+-independent. Kinetic analysis of the initial uptake rates of DHEA-S by OAT4 and OTAP2B1 and E1S by OAT4 revealed values of $K_m$ and $V_{max}$ of about 20-30µM and 600 pmol/mg prot/min. In contrast, the affinity of OATP2B1 towards DHEA-S was low ($K_m$=200µM).

Conclusions: The different substrate specificities of the two transporters suggest different physiological functions. OAT4 is involved in both, uptake of precursors for de novo synthesis of estrogens and removal of estrogen sulfates from fetal blood, whereas OATP2B1 is involved only in the last process.

FUNCTIONAL EXPRESSION OF TASK1 IN CULTURED HUMAN CYTOTROPHOBLAST CELLS

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Objectives The syncytiotrophoblast microvillous membrane potential is a determinant of nutrient transfer from mother to fetus. In non-placental epithelia, the K' channel TASK1 (TWIK-related acid-sensitive K' channel) has a role in setting the resting membrane potential and can be activated experimentally by raising extracellular pH. TASK1 mRNA is expressed in cytotrophoblast cells and villous tissue of human placenta. In this study, we investigated the localization and function of TASK1 in cultured cytotrophoblast cells. Methods Cytotrophoblast cells were isolated from normal term placentas (n=5), taken with LREC approval, and cultured to 66 h. TASK1 localization was determined with immunofluorescence using a TASK1 antibody. Functional assays were performed using $86Rb^+$ efflux as a marker for K' transport. Results TASK1 staining was clearly localized to the plasma membrane of multinucleated cytotrophoblast cells. Basal $86Rb^+$ efflux was stable at pH7.4 and increased by extra- cellular pH-8.0 in all experiments. Anandamide (10 µM), a TASK1 blocker, significantly reduced $86Rb^+$ efflux at both pH7.4 and pH8.0 (n=5 for both; p<0.05, ANOVA with Bonferroni).

Conclusion Localization of TASK1 to the membrane of multinucleate cytotrophoblast cells, the effect of pH and inhibition of basal $86Rb^+$ efflux by anandamide, suggests a role for TASK1 in maintaining the K' conductance and microvillus membrane potential of the syncytiotrophoblast.
PLACENTAL VITAMIN B₁₂ UPTAKE: CHARACTERIZATION IN HUMAN ISOLATED CYTOTROPHOBLASTS.

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Objectives: Vitamin B₁₂ (folic acid; FA) is critically important during pregnancy for normal fetal development. Cellular mechanisms involved in FA transport through the placental barrier, from maternal to fetal circulation, are not fully understood. The aim of this work was to characterize placental FA uptake using primary cultured human cytotrophoblasts (CTB) as a model.

Methods: Normal human term placentas were obtained from the Dept. Obstetrics, Hosp. São João-Porto, within half an hour after spontaneous delivery or elective cesarean section. CTB isolation method was adapted from Kliman et al. (1986). Cellular uptake of FA was tested by exposing cell monolayers to ³H-FA (10 nM).

Results: ³H-FA uptake was higher at pH 5.5 than at pH 7.5 and was Na⁺-independent. At pH 5.5, ³H-FA uptake was: a) inhibited by its analogs 5-methyltetrahydrofolate (5-MTHF) and methotrexate (MTX), b) inhibited by the anion transport inhibitors DIDS and SITS, c) not affected by the proton ionophore FCCP nor by the endocytosis inhibitors cytochalasin D and monensin. At physiological pH, ³H-FA uptake was: a) less inhibited by 5-MTHF and MTX, b) inhibited by DIDS, but not by SITS, and c) inhibited by FCCP and by monensin.

Conclusions: Placental FA uptake seems to be mediated by the reduced folate carrier-1 at pH 5.5, whereas at pH 7.5 folate receptors seem to be also involved. Kliman et al. (1986) Endocrinology 118:1567-82.

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HYPERGLYCEMIA IN EARLY PREGNANT RATS INCREASES FETAL WEIGHT AND DOWNSREGULATES SYSTEM A AT TERM.

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Objectives: In diabetes, glucose control is often suboptimal in early pregnancy and first trimester HbA₁c values correlate to birth weight. We tested the hypothesis that hyperglycemia in early pregnancy in rats affects placental transport and fetal weight at term.

Methods: Glucose (2g/kg, HG-hyperglycemia, blood-glucose 8-14 mm) or saline (control) was given i.p. 3 times/day on gestation day (GD) 10 and 11. Group 1: Rats were euthanized at GD 21 and placentas homogenized for western blot. Group 2: Placental transport was measured on GD 21 in chronically catheterised animals using methyl-³H-glucose and ¹⁴C-methyl-aminosuberic acid (MeAIB).

Results: Placental System A protein expression (SNAT 2 isoform) was 33% lower in HG rats (n=6) than in controls (n=7, p<0.05), and GLUT1 and GLUT3 were unchanged. Placental MeAIB transport was 33% lower in HG rats (n=7) compared to controls (n=8, p<0.05), whereas placental glucose transport was unaltered. Fetal and placental weights were increased by 8% and 9%, respectively, in HG rats (n=11) compared to controls (n=10, p<0.05).

Conclusions: Hyperglycemia in early pregnancy increases fetal growth, which in part is explained by a larger placenta. Whereas placental glucose transport capacity was unchanged, system A activity and expression was down-regulated at term. Early metabolic perturbations may program placental growth and transport for the remainder of pregnancy.

HIGH GLUCOSE ACTIVATES CALCINEURIN/NFAT SIGNALING VIA Na⁺-DEPENDENT GLUCOSE TRANSPORTER IN HUMAN TROPHOBLAST CELLS

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Objectives: Glucose is a major energy source for trophoblast cell growth. The uptake of glucose through glucose transporters (GLUT) has been extensively studied under physiology and pathophysiological conditions, such as normal development and diabetics. However, the functional role of sodium-dependent glucose transporters (SGLT) within placenta under normal and abnormal conditions is poorly studied.

Methods: To address this issue, we used RT-PCR and western blot analysis to determine the expression pattern of SGLT in the human trophoblast cells. Next, we used transfection assay to determine if the glucose-mediated calcineurin(Cn)/NFAT activation. Finally, specific inhibitor was used to determine if glucose-mediated Cn/NFAT activation is through SGLT.

Results: We showed that SGLT are expressed in the human trophoblast cells. Subsequently using RT-PCR we identified SGLT2 and SGLT3 but not SGLT1 predominately expressed in these cells. Furthermore, we demonstrated that calcineurin/NFAT signaling was activated in response to high concentration of glucose (15 mM to 30mM) but not low concentrations of glucose (4mM to 10mM). More intriguingly, we found that high glucose-mediated NFAT activation was blocked by SGLT specific inhibitor, phlorizin, but not the GLUT inhibitor, phloretin.

Conclusions: This study directly indicated that high-glucose-mediated NFAT activation is through activation of the SGLT transporter but not the GLUT transporter. This finding implies that SGLT-mediated calcineurin/NFAT activation may contribute to the pathophysiological change in the placenta under diabetic conditions.
P11.13

PROTEIN EXPRESSION FOR THE SNAT4 ISOFORM OF SYSTEM A IN HUMAN PLACENTA IS GESTATIONALLY REGULATED

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Objectives: The System A amino acid transporter is encoded by three members of the SLC38 gene family. The SNAT4 isoform is thought to have unique tissue distribution to the liver. However, we have detected SNAT4 mRNA in human placenta using qPCR and shown expression is significantly higher in first trimester compared to term. We therefore aimed to identify SNAT4 protein in placenta and tested the hypothesis that expression would be gestationally regulated.

Methods: Membrane enriched tissue homogenates were prepared from first trimester (7-13 weeks) and term (38-40 weeks) placentas. SNAT4 protein expression was determined by Western Blotting (WB) and densitometry performed to compare expression between the two gestations. Immunohistochemistry (IHC) was used to examine localisation.

Results: WB revealed a signal at ~60kDa in all placental samples comparable to the signal seen in liver, used as a positive control. Expression at term is significantly higher than first trimester (n=4 for both, p<0.05, 2-tailed unpaired t-test). Preabsorbing antibody with SNAT4 peptide abolished the signal, confirming specificity. IHC shows SNAT4 is localised to the syncytiotrophoblast and fetal endothelium at both stages of gestation.

Conclusion: The SNAT4 isoform of System A is expressed in placental syncytiotrophoblast from first trimester onwards and shows complex gestational regulation: mRNA decreases whilst protein increases towards term.

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P11.14

ENVIRONMENTAL EXPOSURE AND FETAL HEALTH: THE IMPORTANCE OF PLACENTAL TRANSFER

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Objectives: The fetal radiation dose following exposure of the mother has previously been assumed equivalent to that received by the uterus. Placental transfer and fetal uptake of radioactive material may result in a higher radiation dose to fetal organs than predicted.

Methods: Detailed maternal and fetal biodistribution of 131-sodium iodide (I-131) and 85-strontium chloride (Sr-85) was compared during mid (stage 2, 4-6 weeks) and late (stage 3, 7-9 weeks). Pregnant guinea pigs received an oral bolus of the radionuclide of known activity in water administered at time-points up to 14 days prior to sacrifice at the end of mid or late pregnancy. In order to determine biodistribution and placental transfer, maternal and fetal organs were dissected and individual organ activity measured on an automatic gamma counter.

Results: Both I-131 and Sr-85 rapidly cross the placenta with fetal organ uptake evident within an hour of maternal exposure. Fetal I-131 concentrations were higher than maternal in most tissues, particularly thyroid (6-fold). Sr-85 accumulated extensively in fetal bones.

Conclusions: Active uptake of these radionuclides by the fetus will contribute significantly to the fetal radiation dose and may have health risk implications dependent upon duration and timing of maternal exposure.

P11.15

FETAL CALCIUM ACCRETION IN PTHrP-NULL MICE.

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OBJECTIVES: Skeletal development and mineral deposition are abnormal in PTHrP-null (NL) fetuses. We have previously shown a raised unidirectional maternofetal calcium clearance across the placenta of NL fetuses compared with wild-type (WT) or heterozygote (HZ) mice in late pregnancy. Here we examine how fetal blood calcium and skeletal calcium content are altered over late gestation in NL fetuses.

METHODS: Fetuses were harvested from mice at 17, 18 and 19 days of pregnancy (dp) and a tail tip taken for genotyping. At 18 and 19 dp, ionised calcium concentration ([Ca2+]i) was measured in fetal blood. At 17, 18 and 19 dp fetuses were ashed and total calcium content determined. Data (mean ± SEM) were analysed with ANOVA/Tukeys post hoc test.

RESULTS: At 18 dp fetal [Ca2+]i (mM) in NL fetuses (1.25 ± 0.07) was significantly reduced compared to both HZ (1.46 ± 0.02) and WT (1.45 ± 0.02) groups (p<0.001; n~45 fetuses). A similar trend in fetal [Ca2+]i was observed at 19 dp: 1.37 ± 0.04, 1.42 ± 0.02 and 1.51 ± 0.03 mM in NL, HZ and WT respectively (p<0.01; n~70). Fetal calcium content (mmol/kg; n=9-40 fetuses) at 19 dp was significantly higher in NL (0.085 ± 0.004) than WT (0.079 ± 0.003) or HZ (0.082 ± 0.003; p<0.005) but at 17 and 18 dp was only significantly different between NL and WT (0.025 ± 0.001 and 0.022 ± 0.001; 0.046 ± 0.002 and 0.042 ± 0.002 at 17 and 18 dp respectively; p<0.005).

CONCLUSIONS: Fetal blood [Ca2+]i is significantly lower in NL compared to HZ and WT at 18 and 19 dp. Fetal calcium content is higher in NL at 19 dp compared to both WT and HZ, but only WT at 17 and 18 dp. We suggest that maternofetal calcium clearance may be upreglated in NL fetuses to meet the increased requirement for skeletal calcium deposition.

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P11.16

THE PLACENTAL SCAVENGER RECEPTOR CLASS B TYPE-I (SR-BI) UNDERGOES SPATIO-DEVELOPMENTAL CHANGES IN HUMAN PREGNANCY.

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Objectives: Placenta and fetus require considerable amounts of cholesterol (cholesterol) for various synthesis functions. This demand is mainly covered from maternal lipoproteins (Lp). Little is known about placental uptake and transport of high-density Lp. Recently, one key player in the Lp receptor family, SR-BI was found in the human placenta. Here the hypothesis was tested that the increasing fetal demand for cholestanol with advancing gestation is associated with developmental changes in location and expression levels of placental SR-BI.

Methods: Human first trimester and term placental tissue as well as isolated trophoblasts (first trimester FT, term TT) and endothelial cells (EC) were analyzed by immunohistochemistry and western blotting as well as by RT-PCR.

RESULTS: In the first trimester SR-BI was exclusively located on the microvillus syncytiotrophoblast membrane (mmv), whereas at term an additional staining was found around feto-placental vessels regardless of their calibre. At term tissue levels of SR-BI protein were 2.5-fold higher (p<0.05) than in first trimester. SR-BI mRNA levels were similar in FT and EC and about 50% lower (n.s.) than in TT. In contrast, protein levels were higher (p<0.05) by 70% in FT than in TT. EC levels were 40% lower than those in TT. Conclusion: At the end of gestation SR-BI is also expressed on the placental surface facing the fetal circulation suggesting its contribution to feto-placental choles transport. The additional expression site at the placental endothelium may account for the increasing protein levels in placental tissue between first trimester and term of gestation. The location of SR-BI on the mvm suggests no direct involvement in choles efflux out of the human trophoblast. (European Commission PERILIP grant QLRT-2001-00138; ÖNE 10055)
P11.17

EXPRESSION AND ACTIVITY OF ABC TRANSPORTER PROTEINS IN CULTURED TROPHOBLAST, JAR AND BEWO CELLS.

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Introduction: Human placenta expresses ABC efflux transporters such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and multidrug resistance proteins (MRPs). We have examined expression and activity of these proteins in BeWo, Jar and primary cytotrophoblasts in vitro to evaluate models of placental drug efflux activity.

Methods: Cytotrophoblasts derived from trypsin digestion of term placenta were cultured for 3-4 days to syncytiotize. Jar and BeWo cells were cultured under standard conditions. Real-time PCR and immunoblotting were used for analysis of mRNA and protein expression, respectively. Functional activity was measured using selective inhibitors (5-10 µM) of efflux of P-gp, BCRP and MRP-1 fluorescent substrates, calcein AM (Pg-p and MRPI) and Hoechst 33342 (BCRP).

Results: Expression of mRNA for all four genes was confirmed in all cell types by PCR (BCRP > MRP-2 > P-gp > MRP-1). BCRP and P-gp proteins were readily detectable in all cells, whereas MRP-1 and -2 were barely detectable. Increased intracellular accumulation of calcein AM in response to MK 571 was marked in Jar cells, was also evident in BeWo cells, but absent in trophoblast cells. Pgp-4008 enhanced accumulation of calcein AM in Jar and cytotrophoblast cells, and to a lesser extent BeWo cells. Fumitremorgin C significantly increased accumulation of Hoechst 33342 in all cell types, confirming functional BCRP activity.

Conclusions: The present data compares for the first time functional expression of ABC efflux pumps in primary trophoblasts and trophoblast-like cell lines. Both cell lines may be useful models for studying various aspects of placental ABC transporter expression and function.

P11.18

IN VITRO PLACENTAL TRANSFER MODELS OF FETAL EXPOSURE TO ENVIRONMENTAL AGENTS

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Objectives: Transfer of organochlorine pesticides to the fetus may be implicated in the development of adverse health effects in childhood such as allergy and leukemia. Human placental transfer was investigated using a perfused placenta model.

Methods: Placentas were obtained from normal C-section deliveries at term. Open dual circuit perfusions were established and the transfer of radio-labelled pesticides determined. A cell culture model was also established using the BeWo choriocarcinoma cell line.

Results: Dichlorobenzene (DCB) transferred most rapidly, reaching a plateau within 10 minutes, followed by polychlorinated biphenyl-77 (PCB) and then DDT and DDE with a clearance index of 0.98, 0.73, 0.61 and 0.61 respectively. All the agents with the exception of DCB showed extensive accumulation in placental tissue. Maternal to fetal and fetal to maternal transfer gave similar values. Preliminary work in the cell culture model demonstrated transfer of antipyrine in a reproducible manner.

Conclusions: These pesticides transferred by passive diffusion in an order predicted by their lipophilic properties. The perfusion model provides a useful basis for interpretation of clinical findings and the cell culture model may have potential as a rapid throughput model for screening and reduction of animal numbers. Further investigation and comparison with in vivo data is required.

P11.19

SYSTEM A AMINO ACID TRANSPORTER GENE EXPRESSION AND ACTIVITY IN FETAL T LYMPHOCYTES AND PLATELETS.

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Objectives: System A (Sys A) transporter activity is mediated by three members of the sodium-coupled neutral amino acid transporter (SNAT) family, SNAT1, 2 and 4. Sys A activity is reduced in the syncytiotrophoblast microvillous membrane from intrauterine growth restricted (IUGR) pregnancies. As a prelude to examining whether Sys A activity is altered in other fetal cell types in IUGR, our aim was to characterize SNAT mRNA expression and activity in fetal T lymphocytes (FTL) and platelets (FPL).

Methods: Pure, viable FTL were isolated from cord blood from normal term pregnancies at birth. FPL were isolated from platelet-rich cord plasma. Gene expression for SNAT1 and 2 in both FTL and FPL suggest these isoforms mediate Sys A activity in these fetal cell types. Supported by the Wellcome Trust.
P12.01

IRREGULAR PLACENTAL HAEMODYNAMICS IN THE PATHOPHYSIOLOGY OF PRE-ECLAMPSIA

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Objectives: Pre-eclampsia (PE) is characterized by maternal endothelium dysfunction, which is potentially induced by the elevated shedding of the placental syncytiotrophoblast (ST). The trigger for this shedding is unknown. We hypothesised that ST could be liberated by irregular haemodynamics within the intervillous space (WS) as a result of retained vascular resistance and incomplete transformation of myometrial spiral arteries.

Methods: In pilot studies, placental lobules from term healthy human pregnancies were dually perfused in open-circuit (n=2). The IYS was perfused at 14ml/min and then 55ml/min, mimicking blood turbulences in both health and PE, respectively. Insoluble fractions of maternal venous perfusates were concentrated following centrifugation. Temporal control perfusions, at 14ml/min throughout, were additionally performed to permit histological comparisons. Alkaline phosphatase was used as a marker of ST. The impact of collected material on HUVEC cultures was assessed by MTT assay.

Results: At 14 and 55ml/min, maternal arterial hydrostatic pressures were 33.5±0.93 mmHg and 128.4±8.2 mmHg, respectively. Correspondingly, alkaline phosphatase in the collected venous perfusates were 94.7±7.3 and 458.8±106.6 U/L. Low pressurised villi showed minimal damage, whereas high pressures caused severe tissue disruption, typically ST shedding and generalised oedema. These features were localised to cannula insertions. Increased maternal pressures reduced HUVEC survival, with low and high pressure venous perfusates causing 10.6±9.7% and 32.9±2.6% inhibition, respectively.

Conclusions: These preliminary findings support the concept that haemodynamic forces liberate ST and that this material may initiate the endothelial dysfunction of PE.

P12.02

EXPRESSION OF VEGF IN PLACENTA FROM PATIENTS WITH PREGNANCY INDUCED HYPERTENSION

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Objectives: Extensive angiogenesis followed by organized placental vasculature and invasion of maternal decidua by trophoblasts are essential for successful placentation. Abnormal placental development is associated with pregnancy induced hypertension (PIH) due to failure of angiogenesis and trophoblast function. Vascular endothelial growth factor (VEGF) is a potent angiogenic inducer of blood vessel formation and maintains integrity of endothelium. Little is known about their involvement in pathogenesis of PIH. Here, we have examined the expression pattern of VEGF in placentae from pregnancies complicated with PIH patients and compared with normotensive placentae.

Methods: Forty placental tissue samples from pregnancies complicated with PIH (n=20) and gestational matched normotensive pregnancies (n=20) were collected from the Department of Obstetrics and Gynaecology, Safdarjang Hospital, New Delhi. The location and intensity of VEGF staining were determined by immunohistochemistry. Data were analysed statistically using SPSS10.0.

Results: Immunolocalization of VEGF in PIH group as well as in normotensive group was found in mainly syncytiotrophoblast and endothelial cells with diffuse staining in the villous core. Intensity of VEGF immunostaining was found to be significantly reduced in PIH group as compared with normotensive group.

Conclusions: These results suggest that down-regulation of VEGF expression in PIH patients may contribute to the disturbances in the trophoblasts function, which may result in impaired placental vasculature, maternal endothelial cell function and thus pathogenesis of PIH.

P12.03

ENDOGENOUS ANTI-OXIDANT PRODUCTION AND PRE-ECLAMPSIA

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Objectives: The aim of these studies was to measure the levels of endogenous antioxidants; superoxide dismutase (SOD), glutathione peroxidase (GPx), thioredoxin reductase (TRxR) and thioredoxin (TRx) in pre-eclamptic and normal tissues. Protein carbonyls (PCO) and lipid peroxides (LPO) were also measured.

Methods: Placental samples were collected from preeclamptic patients (n=20) and gestationally aged matched controls (n=18). All samples were frozen at –80°C prior to being homogenised, centrifuged and the supernatant was collected for biochemical analysis. The activities of GPx TRxR and SOD were measured by enzymatic assay. Thioredoxin was measured by ELIZA. The levels of LPO and PCO were determined using commercially available kits. All measurements were standardized against extracted protein concentrations.

Results: The mean activity of GPx in pre-eclamptic placental extracts was 11.50nmol/min/mg which was significantly lower than 17.33nmol/min/mg observed in the normal group (p<0.005). The activity of thioredoxin reductase was significantly lower, 13.02 U/mg for the pre-eclamptic group versus 19.25 U/mg for the normal group (p<0.02). The levels of TRx was also decreased in PET, 91.12ng/mg versus 107 ng/mg for the normal group (p<0.02). The level of SOD activity was 2.02±0.51 U/mg for pre-eclampsics compared to a mean activity of 2.48±0.6 U/mg for normal controls (P<0.02). There were significantly higher concentrations of lipid peroxides and protein carbonyls in the placental tissues.

Conclusions: These results provide biochemical evidence of decreased anti-oxidant activity and increased biological oxidation (ie oxidative stress) in pre-eclamptic placental tissues.

P12.04

ALTERED PLACENTAL SYNCTIN AND ITS RECEPTOR-ASCT2 EXPRESSION IN PLACENTAL DEVELOPMENT AND PRE-ECLAMPSIA

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Objectives: To investigate the alterations of synctin, a fucosogenic membrane protein involved in syncytiotrophoblastic layer formation, and its receptor-ASCT2 expression in placental development and pre-eclampsia, as well as in the cytrophoblasts cultured in normoxic and hypoxic conditions.

Methods: The quantitative real-time PCR was used to study the synctin and ASCT2 mRNA expression during placental development in 35 placentas without pre-eclampsia (ranged from 5 to 40 weeks of gestation), and the alterations of pre-eclamptic placentas (n=16) compared with gestational age-matched controls (n=16). The hypoxic effect on trophoblastic synctin and ASCT2 expression was further studied in cytrophoblasts in cultured placental development and pre-eclampsia, as well as in the cytrophoblasts cultured in normoxic and hypoxic conditions.

Results: The level of synctin mRNA expression increased significantly since the first trimester of pregnancy until 37 weeks of gestation, when the level of synctin expression was reduced. The ASCT2 mRNA expression was decreased significantly since the first trimester and was relatively stable since then to 40 weeks of gestation. Furthermore, a significant reduction in synctin mRNA was observed in pre-eclamptic placentas and cytrophoblasts cultured in hypoxia, but not ASCT2 mRNA expression.

Conclusions: A reduced placental expression of synctin but not ASCT2 may contribute to altered cytrophoblastic cell fusion processes and disturbed placental function in pre-eclampsia. Correspondingly, hypoxia decreases synctin but not ASCT2 gene expression in cultured cytrophoblasts.
P12.05

PROTEOMIC ANALYSIS OF THE FETAL EFFLUENT OF PERFUSED PLACENTAL COTYLEDONS FROM NORMAL AND PRE- ECLAMPTIC PREGNANCIES.
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Objectives: Changes in the rate of release of proteins during in vitro perfusion of human placental cotyledons from pathological pregnancies compared to normal may reflect changes in placental cellular function. In the present study we used proteomics to compare the effluent of pre-eclamptic (PE) and normal placentas.

Methods: Effluent samples from the fetal circulation of 4 normal and 4 PE placentas were collected during bilateral perfusion with Kreb's solution. Concentrated, pooled samples were analyzed by 2D PAGE and Coomassie blue stained protein spot patterns were matched using PDQuest software (BioRad). Excised spots were identified by LC/MS/MS.

Results: Two proteins were identified that were increased to >680% in the PE samples. They were carbonic anhydrase I (Swiss-Prot accession no. P00915), an enzyme that catalyzes the reversible hydration of CO2 and plasmagmin (P00747), a proenzyme of the fibriolytic system. A further two proteins were identified that were decreased to <14% in the PE samples. They were cystatin C [precursor] (P01034), an inhibitor of cysteine proteinases and apolipoprotein A-I [precursor] (P00647), a major protein of plasma HDL.

Conclusion: Previous published data describes the expression of all four of the identified proteins in human placenta. Thus, this study demonstrates that proteomic analysis of fetal effluent of perfused cotyledons can identify differentially expressed placental proteins between normal and pathological pregnancies. The diverse nature of the released proteins suggests a range of changes in placental cell functions associated with PE.

P12.06

SEQUENCE VARIANTS IDENTIFIED IN THE TAC AND TACR3 GENES IN PATIENTS WITH PRE- ECLAMPSIA.
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Objectives: There is speculation that the observed placental hyperperfusion in pre-eclampsia leads to secretion of placental factors like Neurokinin B (NKb). The role of NKb in pre-eclampsia per se, has been controversial. This study is the first to examine the human genes encoding NKb (TAC3) and its receptor (TAC3R) in pre-eclampsia.

Methods: Mutation analysis was performed on DNA samples from 20 primigravidas with early onset, severe pre-eclampsia and 10 healthy controls.

Results: In the TAC3 gene, an intronic G-T transversion (IVS3-53) was identified in three patients, a C-T transition in the 3' UTR of a single patient and a 5' UTR variant (C-T change at position -25) in 6 patients. In the TAC3R gene, a common variant T1471C was identified in the 3' UTR region and a A857G transition in exon 3 in a single patient.

Conclusions: Of the five sequence variants identified, two are relatively common. The TAC3-25C/T variant may influence transcription of the gene, while the T1471C (3' UTR) variant in the TAC3R gene may alter stability of the transcript. Functional studies are required to investigate these proposed effects and a correlation between genetic variant and NKB levels will be sought.

P12.07

REGULATORY LOOPS BETWEEN CYTOKINES AND HISTAMINE IN THE HUMAN PLACENTA.
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Objective: Pre-eclampsia has been linked to increased inflammatory responses in the maternal circulation, including higher levels of cytokines and histamine. Increased microvillous loss from the placenta into the maternal circulation has been implicated in this inflammatory process. We have investigated whether placental cytokines and histamine may also be involved.

Method: Placental explants from normal term pregnancies were incubated in serum-free medium containing cytokines (interleukin (IL)-1, IL-10 or interferon-γ (IFNγ)), or histamine, for periods up to 24 hours. Tissue and media were frozen until further analysis by RT-PCR & ELISA.

Results: All three cytokines regulated histamine production time-dependently. The greatest effects (3-4 fold increase) were observed after 2h of culture. Histamine dose-dependently increased the levels of IFNγ (Th1 cytokine) after 24h of culture, and there was a parallel decrease in levels of the IL-10 (Th2 cytokine), leading to a net increase in the Th1:Th2 ratio.

Conclusion: We find evidence of a positive feed-forward loop in human placental explants between histamine and cytokines. This is true particularly for IFNγ, which is increased by the addition of histamine to placenta, and will itself stimulate histamine production. These data are consistent with progressive activation of placental inflammatory responses in pre-eclampsia, which may contribute to the overall pathology.

P12.08

MATERNAL CONCENTRATIONS OF PENTRAxin-3 (PTX3) IN PREGNANCIES COMPPLICATED BY PREECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION.
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Objectives: PentraxinS (PTX3), a recently described acute phase protein, appears significantly elevated in acute cardiovascular diseases. We investigated maternal PTX3 in pregnancies complicated by preclampsia (PE) and intrauterine growth restriction (IUGR).

Methods: PTX3 was determined by ELISA in maternal blood of 17 PE pregnancies, 17 IUGR pregnancies and 17 normal pregnancies during the HI trimester. It was also tested in I (n=8) and n trimester (n=ll) of normal pregnancies. In I and n trimester PTX3 levels were 3.1±0.7 and 1.9±0.1 ng/ml respectively. Preeclamptic and IUGR pregnancies significantly higher values of PTX3 which significantly lower than normal which significantly higher values of PTX3 which significantly lower than normal which significantly higher values of PTX3 which significantly lower than normal.

Results: PE pregnancies showed significantly higher PTX3 levels vs normal pregnancies of the HI trimester: 24.0±6.8 vs 2.7±0.6 ng/ml respectively (p<0.01). PTX3 levels were 10.0±4 ng/ml in IUGR group, not significantly different from levels in normal pregnancies. In I and n trimester PTX3 levels were 3.1±0.7 and 1.9±0.1 ng/ml respectively, similar to IUGR level pregnancy normal pregnancy levels. Preclamptic and IUGR pregnancies delivered earlier and their fetal and placental weights were significantly lower than normal pregnancies (p<0.001).

Conclusion: PTX3 levels do not change significantly in normal pregnancies among the three trimesters. Preclamptic patients show significantly higher values of PTX3 which may represent a marker of altered endothelial function typical of preclampsia.
P12.09

TNF-α AND PLATELET ACTIVATING FACTOR (PAF) INCREASE NEUTROPHIL ADHESION TO TROPHOBLAST CELLS

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Objectives: Preeclamptic women have elevated circulating levels of inflammatory mediators and signs of neutrophil (PMN) activation. It is not known if activated PMNs bind to placental trophoblasts at the maternal-fetal interface. We tested the hypothesis that PMNs adhere to trophoblasts following exposure to TNF-α or PAF, two mediators increased in the blood of women with preeclampsia.

Methods: Human trophoblast cells (HTR-8/SVneo) were cultured in 6-well plates until confluent and then pre-treated for 24 hrs with TNF-α or PAF (both at 10 ng/ml). Human PMNs were isolated from peripheral blood and labeled with a fluorescent dye that stains living cells (Cell Tracker). Labeled PMNs (1 X 10⁵/well) were incubated with trophoblast monolayers for 30 min at 37°C. Following incubation, the plates were washed twice, and PMN adhesion was examined using fluorescent microscopy.

Results: Counts by an observer unaware of group assignments showed an average of 16.8 +/- 1.1 SD adherent PMNs per field on control trophoblasts. PMN adhesion increased in the presence of TNF-α (81.8 +/- 10.1 SD cells, n=3, p<0.001) or PAF (39.9 +/-12.9 cells, n=3, p<0.01). Preincubation with a blocking antibody against β1 integrins decreased TNF-α or PAF-induced adhesion by over 85%. Because preeclampsia is associated with compromised uteroplacental blood flow and placental hypoxia, we also examined TNF-α and PAF-induced adhesion under hypoxic conditions (2% O₂, 5% CO₂, 93% nitrogen) and found similar results.

Conclusions: These experiments suggest that PMNs can adhere to placental trophoblasts. Adhesion is enhanced by TNF-α and PAF, two mediators increased in the blood of preeclamptic women, and is dependent, in part, on (β1) integrin adhesion molecules (NTH grant KO8-A049238).

P12.11

IN VIVO EXPRESSION OF HEPATOCYTE GROWTH FACTOR ACTIVATOR INHIBITOR TYPE I (HAI-1) IN PREECLAMPTIC TERM HUMAN PLACENTA

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Objectives: HAI-1 is an integral membrane Kunitz-type serine proteinase inhibitor. Recently, Kataoka et al. reported that HAI-1 was expressed on villous cytotrophoblast (CTB) in human placenta (Histochim Cell Biol 114: 469,2000). The aim of this study was to find out whether differences in the in vivo expression of HAI-1 of CTB occurred in preeclampsia compared with normal pregnancies by immunofluorescence microscopy.

Method: Human full-term placentas were obtained from women in uncomplicated normal and preeclamptic pregnancies. Villous tissue samples of the preeclamptic placenta were collected from areas with and without infarction. The samples were fixed with 4% paraformaldehyde. Five μm-thick cryostat sections were made and then immunostained with anti-HAI-1 antibody (clone C76/18). Fluorescence intensities showing HAI-1 expression were analyzed by confocal fluorescent microscopy.

Results: Counts by an observer unaware of group assignments showed an average of 16.8 +/- 1.1 SD adherent PMNs per field on control trophoblasts. PMN adhesion increased in the presence of TNF-α (81.8 +/- 10.1 SD cells, n=3, p<0.001) or PAF (39.9 +/-12.9 cells, n=3, p<0.01). Preincubation with a blocking antibody against β1 integrins decreased TNF-α or PAF-induced adhesion by over 85%. Because preeclampsia is associated with compromised uteroplacental blood flow and placental hypoxia, we also examined TNF-α and PAF-induced adhesion under hypoxic conditions (2% O₂, 5% CO₂, 93% nitrogen) and found similar results.

Conclusions: These experiments suggest that PMNs can adhere to placental trophoblasts. Adhesion is enhanced by TNF-α and PAF, two mediators increased in the blood of preeclamptic women, and is dependent, in part, on (β1) integrin adhesion molecules (NTH grant KO8-A049238).

P12.12

MATERNAL SERUM LEVELS OF PP-13 CAN BE USED TO DIFFERENTIATE EARLY-ONSET FROM LATE-ONSET PREECLAMPSIA PRIOR TO ONSET OF CLINICAL SYMPTOMS.

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Objectives: Last year we have shown placental protein 13 (PP13) is an effective serum marker to predict early-onset preeclampsia. Here we wanted to follow the amount of PP13 in maternal venous serum throughout gestation in normal pregnant women and those suffering from late-onset preeclampsia.

Methods: Serum samples from peripheral venous blood were collected longitudinally throughout pregnancy and until a few weeks after delivery. PP13 was determined in these samples utilizing a PP13 specific sandwich ELISA and concentrations were compared between cases and controls.

Results: In control pregnancies the amount of PP13 in maternal blood stays low during the first and second trimester and only increases during the third trimester. After delivery PP13 rapidly disappeared from maternal blood. In cases with late-onset preeclampsia the amount of PP13 in maternal serum displays similar dynamics and rises only in the third trimester.

Conclusions: A clear increase in the release of PP13 from the placenta during the second trimester can be visualized in early-onset preeclampsia. This is not true for cases with late-onset preeclampsia. Hence with this PP-13 ELISA it is possible to distinguish early-onset from late-onset preeclampsia prior to the onset of clinical symptoms.
P12.13

SHEDDING OF PLACENTAL DEBRIS IN LABOUR - NORMAL PREGNANCY AND PREECLAMPSIA COMPARED

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Background: Syncytiotrophoblast microparticles (STBM) are shed into the maternal cir-
culation in normal pregnancy. Increased shedding in pre-eclampsia is believed to cause
endothelial dysfunction. Placental corticotorpin releasing hormone (CRH) is similarly
increased in pre-eclampsia. We tested the hypothesis that labour/delivery may increase
STBM shedding and release of CRH.

Methods: To assess the effects of placental delivery, samples were taken from 10 normal
pregnant (NP) and 10 pre-eclamptic (PE) women undergoing elective caesarean section
pre-delivery, at placental delivery and 10 min, 60 min and 24h after. To assess the effects
of labour, samples were taken from 10 NP and 10 PE women pre-labour, at full dilation,
at placental delivery and 24h after. STBM were measured by ELISA and CRH measured
by RIA in peripheral venous plasma.

Results: During caesarean section, the pattern of STBM shedding was the same for NP
and PE, with a rise at placental separation which declined significantly by 24h. In NP
labour, there were no changes in STBM levels (86ng/ml at full dilatation) until a signifi-
cant decline at 24h. In contrast, STBM levels in labouring PE women rose significantly
from 81ng/ml pre-labour to 115ng/ml at full dilatation, followed by a significant decline
to 30ng/ml 24h post delivery. Plasma CRH levels declined rapidly with placental delivery
caeasarean section in NP and PE pregnancies. There was an insignificant rise in CRH
levels during labour in NP (1592pg/ml pre-labour to 1814pg/ml at full dilatation) and PE
(3104pg/ml to 3621pg/ml), followed by a rapid decline to <500pg/ml post delivery.

Conclusion: Labour enhanced STBM shedding but not the release of CRH. An increase
in STBM shedding in labour could be a trigger for postpartum pre-eclampsia in some
cases.

P12.14

CASPAPE ACTIVATION REGULATES STABILITY OF THE MYELOID CELL
LEUKEMIA (MCL-1L) PROTEIN THEREBY MODULATING TROPHOBLAST
CELL DEATH IN PREECLAMPRIA

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Objective: Preeclamptic placentae (PE) are characterized by aberrant oxygenation and
increased trophoblast cell death. Increased caspase activity has been previously detected
in PE, however molecular targets of these enzymes are presently unclear. The aim herein
was to examine the profile of Mcl-1 isoform expression in normal and PE tissues.

Methods: Total RNA and protein lysates were prepared from 1st, 3rd trimester and PE
placentae. Mcl-1 expression was quantified using isospecific qRT-PCR and Western
blotting. Explant culture was performed using 1st trimester placentae under various oxy-
genation conditions. Caspase activity was pharmacologically blocked using a pan caspase
inhibitor (zVAD-FMK).

Results: Placentae obtained from patients with severe early onset preeclampsia demon-
strated a dramatic switch in Mcl-1 isoform expression. Elevated levels of alternatively
spliced pro-apoptotic Mcl-1 S protein were detected by qRT-PCR as well as by Western
blotting in PE relative to control tissues. Concomitantly, this was accompanied by appear-
ance of a truncated product of Mcl-1L, named Mcl-1 Lc. The expression of Mcl-1 Lc can
be modulated by oxygen in vitro and likely results from caspase cleavage, as caspase
inhibition diminished expression Mcl-1Lc in explants and restored accumulation of the
anti-apoptotic Mcl-1L protein.

Conclusions: Aberrant oxygenation leads to excessive caspase activation, cleavage of
pro-survival Mcl-1 isoform and switch in Mcl-1 splicing, thus tilting the trophoblast
apoptotic rheostat towards a death pathway. Supported by the Canadian Institutes of
Health Research.

P12.15

OXIDATIVE STRESS, ANTIOXIDANTS AND SECRETION OF SOLUBLE FMS-
LIKE TYROSINE KINASE 1 (SFLT-1) BY HUMAN PLACENTAL EXPLANTS

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Objective: To investigate the role of oxidative stress in regulating secretion of soluble
fms-like tyrosine kinase 1 (sflt-1).

Methods: Placental samples were collected with consent from uncomplicated elective
caesarean deliveries, into medium equilibrated with 5% O2/5% CO2. One set were either
subjected to hypoxia/reoxygenation (I/R) by incubation under 0.5% O2 for 1 hr and reox-
genation at 10% O2 for 6 or 15 hr, or maintained under 10% O2 throughout. Another set
were incubated under 10% O2 with or without 1 mM H2O2. In both sets the p38 inhibitor
PD169316 was tested at 10 μM, and ascorbic acid and Trolox at 2 mM and 1 mM respec-
tively. Samples were snap-frozen for Western blots or fixed for immunohistochemistry
(IHC). Supernatants were collected for ELISA analysis.

Results: Both forms of oxidative stress increased concentration of placental sflt-1 as
determined by Western blots, and increased secretion into the supernatant. IHC localised
the sflt-1 primarily to the syncytiotrophoblast. Both the p38 inhibitor and vitamins C and
E significantly reduced tissue concentrations of sflt-1, and secretion. They also reduced
concentrations of markers of oxidative stress.

Conclusion: Oxidative stress is associated with increased secretion of sflt-1 by syncyti-
trophoblast. Vitamins C and E suppress these effects, and so may have a protective effect
in pregnancies at high risk of preeclampsia. Supported by The Wellcome Trust.

P12.16

EXPRESSION OF ADRENOMEDULLIN AND ITS RECEPTOR IN THIRD
TRIMESTER PLACENTAL BED DECIDUA FROM NORMAL AND PRE-
ECLAMPTIC PREGNANCIES

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Objective: The aim of this study was to determine whether changes in the expression
of adrenomedullin (AM) and its receptor (a heterodimer of calcitonin receptor-like receptor
(CL) and receptor-activity-modifying proteins (RAMPs)) in the placental bed (decidua
basalis) are indicated in the poor placentation that characterises pre-eclampsia.

Methods: Placental bed decidual samples were obtained by suction of the uterine wall
at the placental site, after removal of the placenta, during caesarean section from 10
normal and 10 pre-eclamptic pregnancies and snap frozen in liquid nitrogen. Northern
blotting was performed to compare expression of AM, CL, RAMP1 and 2 mRNAs. Deglycosylation
and immunoblotting experiments were carried out to determine the glycosylation state and
expression of CL protein. Double immunofluorescence was performed to assess CL localisation in vivo.

Results: Expression of AM, CL, RAMP1 and 2 mRNAs is unaltered in pre-eclamptic
compared to normal decidua. The CL receptor exists predominantly as a ~53KD (RAMP-
associated) mature glycoprotein and expression is unchanged in pre-eclamptic deciduas.
Both endothelial (CD34-positive) and trophoblast (cytokeratin 7-positive) cells, which
line decidual blood vessels, express CL in normal and pre-eclamptic deciduas.

Conclusions: No differences in AM and its receptor expression were found in near term
placental bed deciduas from normal and pre-eclamptic pregnancies. However, this does
not preclude a role for AM in the early stages of placental development in pre-eclampsia.
P12.17

EXPRESSION OF ONCOPROTEINS P53, BAK AND P21 IN NORMAL PREGNANCIES AND THOSE COMPLICATED BY PRE-ECLAMPSIA

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Objectives: The increased rate of apoptosis observed in placentae from pregnancies complicated by pre-eclampsia (PE) is accompanied by an increase in p53 expression within villous trophoblast. We investigated whether this is associated with an increase in expression of downstream effector oncoproteins, BAK and p21.

Methods: Fresh placental tissue was collected from 6 normal pregnancies and 6 pregnancies complicated by PE. Tissue was homogenised for Western blotting and fixed for immunohistochemistry. Sybyr green I qPCR was performed on extracted total RNA using primers for p53 and p21.

Results: Expression of p53 and p21 was increased in pregnancies complicated by PE. There was a strong correlation between the quantity of p53 and p21 mRNA (p < 0.001).

Conclusions: The tight correlation between p53 and p21 transcription indicates a transcription-dependent effect of p53. This is not the case with BAK, which is a transcriptionally independent effect of p53 in other tissues. Therefore, within villous trophoblast in pregnancies complicated by PE p53 could promote apoptosis through cell cycle arrest and increased mitochondrial membrane permeability.

P12.18

P2X4 RECEPTOR NITRATION IN HUMAN PLACENTA IS ALTERED IN PREECLAMPSIA

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Purinergic receptor activation by extracellular ATP raises intracellular calcium to alter cell function. In preeclampsia (PE) there is a greater potential for ATP release from increased hypoxia, oxidative or nitrative stress and cell death. We have previously shown upregulation of the P2X4 subtype in placentae from PE pregnancies, however, nitrination of this subtype has not been examined.

Objective: To determine whether P2X4 is a nitrated protein in the human placenta and whether there is altered nitrination in PE.

Methods: Samples were obtained from three groups; term PE, pre-term PE and term control placentae (n=5). Membrane protein homogenate was immunoprecipitated with anti-nitrotyrosine antibody and samples were Western blotted with P2X4 antibody. Protein band intensity was determined using scanning densitometry.

Results: Immunoprecipitation shows that the P2X4 receptor subtype is nitrated in human placenta. This P2X4 nitration is significantly increased in term PE samples vs. normal age-matched controls (p=0.01) and in pre-term PE vs. normal term controls (p=0.005) with no significant difference between term PE and pre-term PE (t-test).

Conclusion: The increase in P2X4 nitration in PE may simply reflect the increased P2X4 protein expression we have previously seen. Our results suggest that nitrination is involved with P2X4 signalling, although the exact role of this modification in altering P2X4 function has yet to be established.

P12.19

REGULATION OF THE PLACENTAL TRANSCRIPT PROFILE IN LONG LABOUR: PARALLELS WITH CHANGES SEEN IN PREECLAMPSIA.

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Objective: To investigate changes in the placental transcript profile resulting from the oxidative stress due to long labour.

Methods: Placental material was collected from women undergoing an elective caesarean section (n=10) and those delivered vaginally after long labour (≥ 10 hr, n=7). Tissues were harvested rapidly (< 10 min) and total RNA isolated. cDNA was prepared and labelled with Cy3 or Cy5 (aminoallyl labelling) and hybridized to custom-made human cDNA arrays, containing 15937 cDNAs probes in duplicate. All raw data were normalized within and between arrays using Linear Models for Microarray Data (Limma) before statistical analysis with CyberT. The false positive discovery rate was 6%.

Results: We identified 51 transcripts that were down-regulated including MAO-A, SOD1, PIGF and osteopontin, and 52 that were up-regulated such as Lep, sfll, MT2A, NDRG1, RhabA, MKK5, THBS1 and S100A8(MRP8). Up-regulation of leptin and sfll and down-regulation of PIGF, SOD1 and MAO-A have been shown in preeclamptic placental tissue. This suggests an acute ischaemia-reperfusion type insult that induces oxidative stress may activate similar mechanisms to those found in with preeclampsia. Increases in transcripts encoding calcium binding proteins S100A8, NDRG1 and Rab1 suggest perturbation of intracellular calcium regulation.

Conclusion: Acute ischaemia-reperfusion resulting from long labour can be used as an accessible model for studying some of the changes caused by oxidative stress which mirror some of those seen in preeclamptic placentas. The pattern of gene expression suggests that the placental oxidative stress may not only come from mitochondria; ER stress may also play a role. Supported by The Wellcome Trust.

P12.20

MATERNAL CIRCULATING LEVELS OF ADVANCED GLYCEMIC END PRODUCTS AND PRESENCE OF “ACUTE ATHEROSIS” IN PREECLAMPSIA

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Background: Advanced glycemic end products (AGEs) are reactive, cross-linking molecules formed from the non-enzymatical glycation of reducing sugars and proteins, lipids, and nucleic acids. AGEs increase with age and are elevated in diabetes and renal failure. AGE is considered a marker of oxidative stress. Preeclampsia (PE) is a pregnancy disorder associated with dyslipidemia, oxidative stress and endothelial dysfunction. A prominent finding in PE is the focal lipid deposition in the spiral artery walls in the utero-placental circulation, a phenomenon known as “acute atherosis”. We wanted to explore whether AGE levels in maternal circulation is elevated in preeclampsia, and whether elevated AGE is associated with the presence of “acute atherosis”.

Methods: Decidual tissue was harvested at cesarean delivery by vacuum suction of the placental bed in 81 patients. Paraffin embedded decidual tissue was immunostained with CD68, actin and cytokeratine. Acute atherosis was defined as the presence of foamy CD68 positive cells in spiral artery walls with fibroid necrosis. AGE was measured using ELISA immunassay for “total AGE” and CML (carboxymethyllysine).

Results: Presence of spiral arteries was demonstrated in vacuum harvested tissue in 62 of 81 patients (77%). Acute atherosis was present in 42% of the preeclamptic patients and 17% of the control patients. We found no statistical significant difference in levels of AGE or CML between the control pregnant and the preeclampsia group, nor between preeclamptic patients with or without acute atherosis.

Conclusion: Presence of acute atherosis in spiral arteries was not associated with elevated AGEs in preeclampsia.
THE PATHOPHYSIOLOGICAL ROLE OF ANGIOTENSIN II RECEPTOR ACTIVATING AUTOANTIBODIES IN PREECLAMPSIA
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Objectives: Pre-eclampsia (PE) is a gestation-specific syndrome with a high incidence of mother and infant morbidity and mortality. The triggering factors in the pathogenesis of the disease remain elusive. Recently we and other labs identify the angiotensin receptor activating autoantibody (AT1-AA) present in preeclamptic patients’ serum. However, the biological function is not well studied.

Methods: Using multiple culture systems to identify the biological function of AT1-AA in PE.

Results: Using cultured neonatal rat cardiac myocytes, we have shown that AT1-AA interact with AT1Rs resulting in increased contraction rates. Using cultured human mesangial cells, we have shown that AT1-AA stimulate PAI-1 production, which may cause kidney damage resulting in proteinuria, a hallmark manifestation of preeclampsia.

Conclusions: Taken together, our results provide strong evidence that AT1-AA associated with PE activate AT1Rs on a variety of cells and can be responsible for many features of the disease. This hypothesis has the ability to explain many features of this complex clinical condition and immediately suggests diagnostic and therapeutic opportunities. Prevention or successful treatment of PE has the potential to greatly reduce birth defects resulting from the preterm deliveries associated with PE.

DIFFERENTIAL ACTIVITY OF HISTIDINE DECARBOXYLASE IN NORMAL AND PRE-ECLAMPTIC PLACENTAE
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Objective: Maternal endothelial dysregulation and heightened circulatory inflammatory response is persistently observed in preeclampsia. Histamine, a potent activator of endothelial dysregulation and key mediator of inflammatory response is observed at high concentrations in blood from preeclamptic women. In all the tissue the rate of histamine production is dependent on the level of activity of histidine decarboxylase (HDC), which is the rate-determining step in histamine synthesis. We have studied the levels of HDC activity in normal and pre-eclamptic placentae.

Method: Placental tissues from 10 normal term pregnancies (delivered by Caesarean section) and 10 third trimester pregnancies complicated by pre-eclampsia were frozen within 30 minutes of delivery. Homogenates of these samples were tested for maximal histamine production, which was expressed as ng/mg tissue/hr.

Result: HDC activity was present in all placental homogenates. Mean histamine production from pre-eclamptic tissues was 10-fold higher than from normal placentae [2.0±0.38; 0.13±0.05, mean±SEM respectively p=0.0003 by Bonferroni/Dunn test].

Conclusion: HDC activity in pre-eclamptic placenta was raised. The finding suggests that increased maternal blood histamine may be related to de novo histamine synthesis from the placenta but further study is required to link the increased HDC activity to histaminemia of pregnancy.
P13.01

TRANSCRIPTIO NAL REPRESSION OF THE HUMAN METALLOPROTEINASE-9 PROMOTER BY WILD-TYPE P53 IN HUMAN CYTOTROPHOBLASTIC CELLS
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Objectives: The matrix metalloproteinase (MMP) family is known to play a key role in time remodelling during embryonic development and in pathological conditions. It has previously been shown that p53 regulates positively or negatively the expression of different MMPs. Because of similarities in the promoter sequence between MMP-9 and MMP-1 genes, we hypothesize that expression of MMP-9 could be also regulated by wild type (wt) p53.

Methods: In order to examine the effect of wt-p53 on MMP-9 expression, transcription assays were performed with chloramphenicol acetyl transferase reporter driven by -670, -531 and -90 promoter of the hMMP-9 gene cotransfected with expression vector of p53, in JH1080 and in primary cultures of human cytrophoblastic cells.

Results: The results demonstrate that wt-p53 down-regulates the -670 but not the -531 and -90 promoter activity of hMMP-9 and in a dose-dependent manner. One of the two p53 mutants tested lost this repressive activity. Whereas we determined a potential mechanism of p53 repression of MMP-9 in CTB and HT1080 cells by detection experiments, it seems that endogenous p53 is not involved in MMP-9 expression in these cells. In contrast, endogenous expression of MMP-9 is under control of p53 in MCF-7 cells.

Conclusion: Taken together, these observations suggest that the hMMP-9 gene could be a p53 target gene and that its expression could be subjected to p53 repression.

P13.02

CAFFEINE ENHANCES THE mRNA EXPRESSION OF THYROID HORMONE RECEPTOR β1, TYPE III IODOTHYRONINE DEIODINASE, AND c-FOS IN BE WO CELL CULTURE AND RAT PLACENTA.
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Objectives: Chronic caffeine exposure during pregnancy has been shown to affect intrauterine fetal growth. Placenta may be an important determinant of maternal imprinting events through the control of the nutrient supply to the fetus and hormonal interchanges between fetal and maternal tissues. We employed cDNA microarray technology to screen if caffeine alters gene expression in a human cytrophoblast-like cell line, BeWo, and found that the expression of thyroid hormone receptor β1 (TRβ1) and type III iodothyronine deiodinase (DIO3) genes was up-regulated by caffeine treatment. We further expanded this study to investigate the gene expression of the proto-oncogene c-fos, and discussed the caffeine’s effect to the placental thyroid status.

Methods: We isolated RNAs from the placenta of pregnant rats fed a diet supplemented with caffeine until day 20 of gestation, and analyzed the gene expression using LightCycler-based quantitative real-time PCR technology. Statistical analysis was done with nonparametric two-tail Mann-Whitney U-test.

Results: Significantly elevated gene expressions of the TRβ1, DIO3, and c-fos were shown in the caffeine-treated placentae.

Conclusions: It is conceivable that caffeine influences on both placental and fetal thyroid status, resulting in fetal growth retardation.

P13.03

mTOR: A PLACENTAL GROWTH SIGNALING
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Objectives: The proliferation and differentiation of trophoblast cells is under the control of a variety of hormones and growth factors and is influenced by nutrient availability. The intracellular signaling pathways acting downstream of these mitogenic factors and nutrients to regulate trophoblast proliferation and placental development are poorly understood.

Methods: Immortalized human trophoblast cells were used (HTR-8/SVneo) to investigate trophoblast proliferation in response to angiopeitin-2 (Ang-2), a major angiogenic factor and glucose (a major nutrient).

Results: Trophoblast cell proliferation was induced through activation of the phosphotyrosine-3 (PI-3) kinase and the mammalian target of rapamycin (mTOR) signaling pathway. mTOR signaling was inhibited by PD98059 and Wortmannin.

Conclusions: Overall the results show that growth factor and nutrient signaling converge at tuberin, an upstream regulator of mTOR and that mTOR functions as an important placental growth signaling sensor. These results are the first to link mTOR with GFAT metabolites as nutrient sensors for trophoblast cell proliferation.

P13.04

FUNCTIONAL ROLE OF LEPTIN SIGNALING PATHWAYS IN HUMAN ENDOMETRIAL EPITHELIAL CELLS
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Objectives: Despite the importance of leptin in endometrial biology as an essential factor in the cascade of events leading to the acquisition of endometrial receptivity, the signaling pathways activated by leptin in endometrial cells are largely unknown. The aim of this work was to determine which signaling pathways are activated by leptin in malignant and non-malignant endometrial cell lines, and which ones are obligatory to leptin induced VEGF.

Methods: Non-malignant (HES) and malignant (Ishikawa) endometrial cell lines were incubated with different concentrations of leptin and inhibitors for JAK2/STAT3, MAPKERK1,2, PI-3K/Akt-1 pathways. Leptin-induced effects were assessed by Western blots using specific antibodies to non-phosphorylated and phosphorylated STAT3, ERK1/2 and Akt-1. An ELISA was used to determine leptin-induced VEGF secretion.

Results: Leptin increased the levels of phosphorylated ERK1/2, STAT3 and Akt-1 in a dose-dependent manner. The levels of phosphorylated STAT3 and Akt-1 were higher in HES when compared to Ishikawa cells. Leptin increased the levels of VEGF secreted by Ishikawa cells in a dose-response dependent manner. The increase was inhibited by PD98059 and Wortmannin.

Conclusions: Leptin activates JAK2/STAT3, MAPKERK1,2 and PI-3K pathways in endometrial cells. Further our inhibitor studies suggest that ERK1/2 and Akt-1 are required only for leptin-induced VEGF in the malignant cells.

This work was supported in part by CIICCR, a program of CONRAD, Eastern Virginia Medical School Grant CIG-02-87.
P13.05

NITROSYLATION OF PROTEIN KINASE C REGULATES TROPHOBLAST APOPTOSIS.

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Pro-invasive RNA (siRNA) against inhibitor JEG-3 cells.

Methods and Results: Apoptosis was monitored in both first primary trimester extravillous trophoblasts and the extravillous trophoblast derived cell line SGHPL-4 using time-lapse microscopy and caspase activity assays. Treatment of cells with TNFa, TRAIL or a CD95 activating antibody resulted in negligible cell death. However, inhibition of basal NO synthesis with the nitric oxide synthase inhibitor L-NAME significantly increased the amount of apoptosis following treatment with the death inducing ligands. Nitrosylated proteins were isolated from trophoblasts using the biotin-switch method and proteins were identified by western blot analysis. Protein kinase C-epsilon (PKCepsilon) was one of the proteins identified, with nitrosylation of PKC known to result in inhibition of protein function. PKC has been identified as a regulator of the anti-apoptotic protein c-FLIP with phosphorylation of c-FLIP by PKC preventing its recruitment to the death receptors thus inhibiting apoptosis.

Conclusion: We suggest that trophoblast resistance to death inducing ligands is regulated by the NO mediated inhibition of PKC which prevents c-FLIP phosphorylation and allows it to be recruited to the death receptors thus inhibiting apoptosis.

P13.06

LEPTIN AND ADIPOGENIN STIMULATE THE RELEASE OF PRO-INFLAMMATORY CYTOKINES AND PROSTAGLANDINS FROM HUMAN PLACENTA VIA NF-κB, PPAR-α AND ERK1/2

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Objectives: Beyond their effects on central metabolic functions, leptin, resistin and adiponectin have profound effects on other physiological processes, including immune function and inflammation. Although these hormones are produced within the intrauterine environment, their immuno-regulatory actions in placenta are not known. The aim of this study was to determine the effect of leptin, resistin and adiponectin on pro-inflammatory mediators from human placenta.

Methods: Samples were obtained from normal pregnancies at the time of Caesarean section. Tissue explants (n = 5) were incubated in the absence (basal control) or presence of a leptin (1, 10 and 100 ng/ml), resistin (1, 10, and 100 ng/ml) and adiponectin (0.1 and 0.5 µg/ml). After 6 h incubation, the medium was collected and the release of IL-1β, IL-6, TNF-α, PGE2 and PGF2α was quantified by ELISA.

Results: There was no effect of resistin on pro-inflammatory cytokine or prostaglandin release; however leptin at 100 ng/ml and adiponectin at 0.1 and/or 0.5 µg/ml significantly increased the release of IL-1β, IL-6, TNF-α, PGE2 and PGF2α (p<0.05). Furthermore, this leptin and adiponectin induced pro-inflammatory response could be abrogated by treatment with the anti-inflammatory ERK1/2 MAPK inhibitor U0126, the PPAR-α ligand troglitazone and the NF-κB inhibitor BAY 11-7082 (p<0.05).

Conclusions: These data indicate that leptin and adiponectin activate pro-inflammatory cytokine release and phospholipid metabolism in human placenta, and anti-inflammatory agents can abrogate leptin- and adiponectin-induced inflammation.

P13.07

NUCLEAR FACTOR KAPPA B PATHWAY REGULATES PRO-LABOUR MEDIATORS IN HUMAN PLACENTAL JEG-3 CELLS

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Objectives: Nuclear factor kappa B (NF-κB) is activated in response to a number of inflammatory mediators, and regulates pro-labour mediators in human gestational tissues. The aim of this study was to assess the effects of (i) TNF-α and LPS and (ii) short interfering RNA (siRNA) against inhibitor κB kinase (IKK)-β on NF-κB activation, and NF-κB regulated gene products in JEG-3 cells.

Methods: JEG-3 cells (n = 3) were (i) incubated in the presence or absence of 10 µg/ml lipopolysaccharide (LPS) or 20 ng/ml TNF-α, or (ii) transfected with 100 nM IKK-β siRNA. After 48 h incubation, the cells were collected and cytoplasmic and nuclear protein extracts, and incubation media collected for analysis of IL-6 and PGE2 release by ELISA. Western blot analysis was used to determine the expression of COX-2, IKK-β, IκBα and phosphorylated IκBα in cytoplasmic extracts, and NF-κB subunits in nuclear extracts.

Results: Incubation of JEG-3 cells with LPS and TNF-α increased the expression of cytoplasmic IKK-β and phosphorylated IκBα, and the nuclear expression of the NF-κB proteins p50 and p65. This was associated with an increase in COX-2 protein, and release of IL-6 and PGE2 (p<0.05). Treatment of cells with BAY 11-7082 at 50 µM significantly inhibited both basal, LPS and TNF-α induced NF-κB and COX-2 protein expression, and IL-6 and PGE2 release (p<0.05). Transfection of JEG-3 cells with IKK-β siRNA resulted in a 40% and 48% reduction in IL-6 and PGE2 release (p<0.05).

Conclusions: Pro-inflammatory mediators regulate the NF-κB pathway in JEG-3 cells, and the IKK-β/NF-κB pathway is a regulator of inflammatory mediators in placental JEG-3 cells.

P13.08

REGULATION OF PRO-INFLAMMATORY MEDIATORS IN HUMAN PLACENTA BY ERK, p38 MAPK AND JNK

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Objectives: Pro-inflammatory cytokines and prostaglandins are implicated in the initiation and progression of human labour and delivery, particularly in relation to infection-induced preterm labour. We have previously demonstrated that bacterial endotoxin and pro-inflammatory cytokines up-regulate pro-inflammatory mediators in human gestational tissues. The aim of this study was to determine whether or not LPS and pro-inflammatory cytokines increase pro-labour mediators via the MAPK pathway in human placenta.

Methods: Human placental tissues (n = 5 separate experiments) were incubated in the presence or absence of ERK 1/2 inhibitors U0126 (10 µM) and PD98059 (10 µM); p38 MAPK inhibitors SB203580 (10 µM) and SB202190 (10 µM); and JNK inhibitor SP600125 (10 µM) under basal, 10 µg/ml LPS and 20 ng/ml TNF-α stimulated conditions. After 6 h incubation, tissues were collected and ERK 1/2, p38 MAPK, and JNK protein expression assessed by Western blotting. The incubation medium was collected and TNF-α, IL-6, -β, and PGE2 release quantified by ELISA.

Results: Treatment of placental tissues with LPS, TNF-α and IL-β increased the activation of the MAPK cascade. U0126, PD98059, SB203580, SB202190 and SP600125 inhibited basal, LPS and TNF-α stimulated IL-6, and PGE2 release (p<0.05). Only U0126 decreased basal and LPS stimulated IL-β release. U0126, PD98059, SB203580, SB202190 and SP600125 inhibited LPS stimulated TNF-α release.

Conclusions: These data support the involvement of the MAPK pathway in mediating placental response to LPS and pro-inflammatory cytokines.
P13.09

**BENZo(a)PYRENE, A CIGARETTE SMOKE TOXICANT, INDUCES CELL CYCLE ARREST AND p53 ACTIVATION IN HUMAN UTERINE ENDOMETRIAL CELLS: PROTECTION BY VITAMIN E.**

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Maternal cigarette smoking is known to produce infertility and disrupt placental function. The polyaromatic hydrocarbon benz[a]pyrene (BaP) is a major toxicant in cigarette smoke that has been shown to alter placental cell function. This study investigated the effects of BaP, a ligand for the aryl hydrocarbon (Ah) receptor, on proliferation and cell cycle progression in the human uterine endometrial RL95-2 cell line. BaP significantly inhibited proliferation in a dose-dependent manner characterized by G2/M cell cycle phase arrest. No evidence of apoptosis was detected following BaP exposure. Although BaP had no effect on total cellular p53 levels, phosphorylation of p53 at serine 15 (p53-Ser15phos) was markedly increased. In this regard, ATM, a serine/threonine kinase, is known to function as a primary sensor of DNA damage in cells through activation of p53 phosphorylation. The presence of Wortmannin, an inhibitor of ATM kinase, decreased BaP-induced p53-Ser15phos. In addition, the presence of the antioxidant vitamin E significantly decreased BaP-induced p53 phosphorylation and CYP1A1 protein expression. Vitamin E further suppressed BaP-induced G2/M arrest. Thus, the anti-proliferative effect of BaP involves activation of a p53-dependent pathway involving cell cycle arrest at G2/M, providing evidence of oxidative stress and activation of a DNA damage response pathway in endometrial RL95-2 cells. The results of the present study further indicate that the nutritional antioxidant vitamin E may help to reduce the reproductive toxicity of an environmental exposure such as cigarette smoking. (Supported by NIH ES07375)

P13.10

**ACTIVATION OF P38 AND NF-kB PATHWAYS IN HUMAN PLACENTAL TISSUES DURING LABOUR**

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**Objective:** To identify signalling pathways activated in placental tissues during acute ischaemia-reperfusion in vivo.

**Methods:** Placental samples were collected with consent from uncomplicated pregnancies delivered by elective caesarean section (CS) or vaginally after long labour (LL) (≥10 hr). Samples were obtained rapidly (≤10 min), and snap-frozen for Western blot analysis or fixed for immunohistochemistry (IHC).

**Results:** CS samples showed increased phosphorylation of p38 compared to CS samples. Total protein levels of p38 remained unchanged, as did phosphorylation of SAKP, ERK1 and 2, and Akt. Placentas subjected to LL showed increased phosphorylation and degradation of c-EBP compared to CS. Oxidative stress was greater in LL than CS placentas, as determined by Western blot analysis for hydroxynoemal, heat shock protein 27, cyclooxygenase-2 and hypoxia-inducible factor-1α. IHC localised these changes principally to trophoblast and endothelial cells. Concentrations of activated caspase-3, caspase-9, and cleaved PARP were significantly increased in LL, and localised by IHC to syncytiotrophoblast. LL placentas also showed increased trophoblastic concentrations of IL1β and TNFα, as confirmed by Western blot and IHC.

**Conclusion:** Labour causes activation of the p38 and NFκB signalling pathways, and is associated with increased placental oxidative stress, apoptosis and secretion of inflammatory cytokines. Supported by Tommy’s the Baby Charity.

P13.11

**INVoLvEMEnT OF MAPK PATHWAY IN TNF-α INDUCED MMP-9 EXPRESSION IN hUMAN CYTOTROPHOBlastic CELLS**

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**Objectives:** The aim of this paper is the investigation of signalling pathways involved in metalloproteinase-9 (MMP-9) expression induced by tumour necrosis factor (TNF-α) in first trimester cytotrophoblastic cells.

**Methods:** Primary culture or cell line (HIPEC 65) of first trimester cytotrophoblastic cells were incubated with TNF-α in presence or absence of mitogen-activated protein kinase (MAPK) inhibitors. Cell lysates and supernatants were then subjected to Western blots, MMP ELISA and zymography.

**Results:** TNF-α-induced MMP-9 expression, secretion and activity were completely blocked by SAKP/JNK and Erk inhibitors (SP600 125 and U0126 respectively) but not by p38 MAPK inhibitors (SB203 580 and SB202 190). Stimulation of HIPEC 65 cells with TNF-α caused phosphorylation of JNK and Erk1/2 with a peak after 20 minutes of treatment. Transcription factors NF-κB and AP-1 binding sites were identified as the cis-elements involved in TNF-α activation as determined by electro-mobility shift assays. TNF-α-induced tranactivation of NF-κB was inhibited by U0126 whereas TNF-α-induced transactivation of AP-1 was inhibited by SP600 125.

**Conclusions:** Taken together, these results indicate that in cytotrophoblastic cells, TNF-α probably activates two different pathways leading to MMP-9 expression: a- Erk1/2 pathway which in turn initiates NF-κB activation and b- SAKP/JNK pathway that activates AP-1.

P13.12

**REGULATIoN OF TROPHOBlast HEmE oXYGENASE EXPRESSION BY nITRIC oXIDE AND CARBON MONoXIdE.**

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**Objectives:** The enzyme heme oxygenase (HO) metabolises heme to produce biliverdin, bilirubin and carbon monoxide (CO). The HO-1 isoform is inducible while HO-2 is generally constitutively expressed. HO and CO have recently been suggested to have regulatory roles in the placental environment. The localisation of HO expression has been well documented but the regulation of the HO/CO pathway has been little investigated. The purpose of this study was to increase our understanding of the role of HO in trophoblasts by investigating the mechanisms by which HO expression is regulated.

**Methods:** Expression of HO-1 and HO-2 was determined by Western blot analysis after stimulating trophoblasts (SGHPL-4 cells) in a time- and dose-dependent manner with hemin, hepatocyte growth factor (HGF), nitric oxide (NO)-donors, CO-donors and the HO inhibitor tin mesoporphyrin IX dichloride.

**Results:** Expression of HO-1 was significantly increased by hemin, and the CO- and NO-donor. HGF increased expression, although results were variable. The HO inhibitor also significantly increased HO-1 expression, possibly as a compensatory mechanism for decreased activity. HO-2 expression did not significantly change with any treatments used.

**Conclusions:** We have shown that trophoblast HO-1 expression can be induced by a wide range of stimuli such as hemin, CO, HGF and NO. The communication between the HO/CO and NO pathways are a novel interaction requiring further investigation.
THE PI3K/AKT PATHWAY INHIBITS TROPHOBLAST APOPTOSIS BY REGULATING FLIP, AND XIAP EXPRESSION

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Objectives: First trimester trophoblast cells express high levels of X-linked Inhibitor of Apoptosis (XIAP), which protects them from certain apoptotic stimuli by inhibiting caspase activation. We previously demonstrated that the inactivation of XIAP reactivates the caspase cascade and causes first trimester trophoblast cells to undergo apoptosis. The aim of this study was to determine how XIAP expression and inactivation is regulated in first trimester trophoblast cells.

Methods: Isolated primary human first trimester trophoblast cells and the 3A trophoblast cell line were studied. The expression and activation status of XIAP was evaluated by Western Blot analysis. Caspase activity was measured by a luminescent assay.

Results: Treatment with the PI3K/Akt (protein kinase B) inhibitor, LY-294,002, which inhibits the phosphorylated form of Akt (p-Akt), induced XIAP inactivation, down regulated the expression of the short form of Fllice-Like Inhibitory Protein (FLIPs) and resulted in a 2-fold increase in caspase-3 activity (p<0.001). In the presence of a pan caspase inhibitor, a decrease in p-Akt, FLIP, and XIAP expression, but not XIAP inactivation was still observed following LY-294,002 treatment.

Conclusion: In the present study, we demonstrate that the PI3K/Akt pathway plays a role in regulating FLIPS and XIAP expression in first trimester trophoblast cells. Since FLIPS is a potent caspase inhibitor and LY-294,002-induced XIAP inactivation is caspase dependent, the down regulation of FLIPS may provide a mechanism to inactivate XIAP.

MASH2 IS A POTENTIAL REGULATOR OF CELL DEATH IN MURINE TROPHOBLAST

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Objectives: Previously, we reported that withdrawal of FGF-4 in low oxygen conditions resulted in apoptosis of a subset of murine trophoblast stem (TS) cells. This is preceded by the HIF1 transcription factor, Mash2. In this study, we present data comparing apoptotic and caspase activities in Mash2-deficient TS cell lines and placenta.

Methods: Wildtype and KO TS cell lines were derived from blastocysts produced after mating heterozygous Mash2 mice. These were maintained under non-differentiating, standard TS cell conditions, in 20% oxygen. Cells were exposed to low oxygen conditions for 48hrs in the presence or absence of FGF-4; the percentage of cells exhibiting apoptotic morphology was recorded. In addition, d975 Mash2 KO and WT placenta were collected from heterozygous matings. Tissues and cells were assessed by TUNEL, and for the presence of various caspases.

Results: Mash2-deficient TS cells demonstrated lower caspase activity levels and cell death rates compared to WT TS cells in the absence of FGF-4. Consistent with this observation, Mash2 KO placenta displayed reduced numbers of TUNEL-positive cells and lower caspase activity levels in comparison to WT placenta.

Conclusion: It appears that Mash2 may, in part, be exerting its differentiation effects by regulating the cell death pathway in trophoblast cells.

IN VITRO TROPHOBLASTS UNDERGO PROLIFERATION OR DIFFERENTIATION DEPENDING ON ACTIVATION OF PI3 MAPK

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Objectives: It has been suggested that in vitro trophoblasts(CTS) do not undergo proliferation and are of limited use in studying potential causes of abnormal CT turnover in preeclampsia/IUGR. Previously we showed that inhibiting p38 MAPK inhibited CT differentiation. Here we tested the hypothesis that p38 inhibition increases CT proliferation.

Methods: CTS isolated from term human placentas were cultured with and without EGF(10ng/ml) and the p38 inhibitor SB203580(10uM)(SB). Differentiation after 5 days culture was measured by assaying secreted β-hCG and DAPI/desmoplakin fluorescence(IF). CTs treated with EGF and SB were subjected to immunoblotting and probed for cyclinD1, a kinase required for cell division, after 1&5 days of culture. A second marker of proliferation, BrDU incorporation, was also measured on days 1&5.

Results: Knock down of STAT3 reduced proliferation and invasion, whereas SOCS3 and STAT6. Small interfering RNA (siRNA) and scrambled oligonucleotides were self-designed. JEG-3 cells were stimulated with LIF, IL-4 and IL-6. Expression and phosphorylation of factors were analyzed by Western blots. Proliferation was measured by using a colorimetric assay, invasion by matrigel assays.

Conclusion: It appears that Mash2 may, in part, be exerting its differentiation effects by regulating the cell death pathway in trophoblast cells.

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3), SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) AND STAT6 REGULATE PROLIFERATION AND INVASION OF JEG-3 CHORIOCARCINOMA CELLS

A.60 Placenta (2005), Vol.26

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Problem: Invasion of trophoblast cells requires a fine tuning, which is fundamental for correct placentation. This tuning is regulated on intracellular level. Several signal transducers and their suppressors, known from former studies and tumor invasion, are expected to be involved.

Methods of Study: RNA interference (RNAi) was applied to JEG-3 cells to knock down STAT3, SOCS3 and STAT6. Small interfering RNA(siRNA) and scrambled oligonucleotide controls were self-designed. Jeg-3 cells were stimulated with LIF, IL-4 and IL-6. Expression and phosphorylation of factors were analyzed by Western blots. Proliferation was measured by using a colorimetric assay, invasion by matrigel assays.

Results: Knock down of STAT3 reduced proliferation and invasion, whereas SOCS3 knock down increased IL-6 induced TLR7/8 phosphorylation of STAT3 simultaneously with proliferation. STAT6 RNAi had no influence on invasion, but IL-4 induced proliferation was inhibited.

Conclusion: Expression and activation of STAT3, SOCS3 and STAT6 regulate proliferative and invasive capacities of JEG-3 choriocarcinoma cells, which may be regarded as a model for trophoblast cells.
Results: The expression of Ik6 in HTR-8/SVneo/Ik6 cells was confirmed by immunocytochemistry and Western blotting. There were no differences in proliferation ability for 96 hours and β-hCG concentration in either cell. Migration and invasion cells were reduced in HTR-8/SVneo/Ik6 by 64.1% and 66.1% of control, but not changed in mock-transfected cells.

Conclusions: Our results suggested that Ikx might participate in human placentation by promoting migration and invasion of EVTs.

mTOR MEDIATES PROLIFERATION IN BEWO CHORIOCARCINOMA CELLS.

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mTOR (mammalian Target Of Rapamycin) is a key component of a pathway that regulates growth in response to nutrients and growth factors. mTOR is a kinase which, when activated, phosphorylates downstream targets acting to regulate protein translation. This important pathway has not yet been mapped in human trophoblast. Our aim was to determine whether the mTOR pathway plays a role in the proliferation of BeWo choriocarcinoma cells.

Methods: BeWo cells were incubated under standard, serum-free conditions (DMEM/F12, 0.5% BSA) for 24 hrs, after which time the media was changed to 0.5% BSA, 2% or 10% FBS, in the presence or absence of 100 μM rapamycin for an additional 72 hrs. Cell proliferation was assessed using a fluorescent DNA-binding assay. Expression of phospho-p70S6K kinase (phospho-p70S6K) was measured by Western blotting in BeWo cell lysates using a polyclonal antibody specific for the phosphorylated form of p70S6K.

Results: Our results revealed that rapamycin decreased cell proliferation in cultures containing 2% FBS (22 ± 7%) or 10% FBS (34 ± 3%; mean ± SEM, n = 4, p < 0.05, t-test), but not in 0.5% BSA (11 ± 4%). In BeWo cell lysates, after 72 hrs of incubation, phosphorylation of p70S6K was increased by incubation in 10% FBS compared to 0.5% BSA. Inclusion of rapamycin decreased p70S6K phosphorylation in both the 0.5% BSA and 10% FBS incubations.

Conclusion: These results show that BeWo cell proliferation is mediated in part by the mTOR pathway. These data are supported by the finding that phosphorylation of p70S6K, a downstream target of mTOR, is altered in parallel with the changes in cell proliferation. It is probable that nutrients and growth factor acting through this pathway, will have similar effects on cell growth and proliferation. (Supported by NIH R01 HD46092).
**Stem Cells**

**P14.01**

**ISOLATION OF ‘EXTRAVILLOUS TROPHOBLAST PROGENITORS’ FROM FIRST TRimestER VILLI**

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**Objective:** A single pool of villous cytotrophoblast are widely believed to be precursors of both syncytiotrophoblast and extravillous trophoblast (EVT) in the first trimester of human pregnancy. We hypothesised that from at least 8 weeks of gestation syncytiotrophoblast and EVT arise from different villous cytotrophoblast progenitor populations. We have previously shown that the progenitors of EVT are localised to the, the multi-layered pockets of villous cytotrophoblasts in the tips of anchoring villi and that these cells can survive for prolonged periods in explant culture, whereas the majority of villous cytotrophoblasts die within a week in explant culture. This study was conducted to determine whether EVT progenitors could be isolated based on their extended survival in explant culture.

**Methods:** First trimester villous explants were cultured for 1 week. Cytotrophoblasts were then isolated from the explants following sequential digestion with trypsin. The cells from each digest were cultured on a thin layer of Matrigel. Non-adherent cells were removed by washing. Cell viability was confirmed by trypan blue exclusion and the cells were phenotyped by immunohistochemistry.

**Results:** Viable trophoblasts (>95% purity) were obtained from the 3rd and 4th trypsin digest which formed into clusters of proliferating cells, as confirmed by Ki67 expression. The proliferating trophoblasts expressed cytokeratin and εvβ6 integrin, but did not express the extracellular matrix protein tenasin.

**Conclusions:** The method of explant culture followed by trypsin digestion has allowed us to isolate a pool of proliferating trophoblast that express markers consistent with the cells we propose are EVT progenitors. This method will allow further characterisation of these cells.

**P14.02**

**CYTOTROPHOBLAST STEM CELL LINES DERIVED FROM HUMAN EMBRYONIC STEM CELLS MIMIC IMPLANTATION EVENTS.**

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Trophoblast cell stem lines are important tools to investigate early placentation. They have been derived from mouse embryos but not from human embryos so far. We aimed to generate cytotrophoblast stem (CTBS) cell lines from human embryonic stem cells (hESCs). In defined culture, embryoid bodies were generated from HESCs and selected for trophoblast enrichment by rounds of cellular aggregation and disaggregation. Human chorionic gonadotrophin (hCG) was used as a marker of trophoblast differentiation. Three CTBS lines were derived which could be maintained in the absence of feeder cells, extra-cellular matrix or residual HESCs; and displayed a variety of trophoblast lineage markers. Spheroid CTBS aggregates were generated and their interaction with luteal-phase endometrial stroma analysed by real-time image analysis. CTBS cells displayed typical cyto- and syncytiotrophoblast characteristics and exhibited further differentiation to the invasive endovascular phenotype involved in uterine blood vessel remodelling. Spheroid CTBS cells mimicked closely the early invasive stages of implantation when incubated with human endometrial stroma in vitro. These human CTBS cell lines are a significant new model for investigating human placentation and may also have considerable potential in cell therapies related to vasculogenesis.

**P14.03**

**TERM HUMAN AMNION– A POSSIBLE SOURCE OF PLURIPOTENT STEM CELLS**

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**Objectives:** The amnion is derived from embryonic epiblast cells that also gives rise to the three germ layers; hence may harbour pluripotent stem cells. We have investigated whether human term amnion contains markers of pluripotent stem cells, form teratomas and/or provide factors for neural cell regeneration.

**Methods:** Human amniotic epithelial cells (HAECs) were isolated using trypsin. Expression of mRNA and protein markers of pluripotent/neural stem cells was studied by RT-PCR and immunohistochemical analyses respectively (n=4). HAECs (5×10⁴) were palpable teratomas were not detected but HAECs formed vascularised masses and monolayers of the three germ layers; hence may harbour pluripotent stem cells. We have investigated whether human term amnion contains markers of pluripotent stem cells, form teratomas and/or provide factors for neural cell regeneration.

**Results:** Cells contained AC133, >95% had Nestin and late neural lineage specific transcription factors in 3-5% of cells. FACS and/or immunocytochemistry showed 75% of cells we propose are EVT progenitors. This method will allow further characterisation of these cells.

**Conclusion:** Term HAECs possess phenotypic characteristics of pluripotent stem cells and produce substances that enhance the regenerative capacity of neural cells. HAECs may be a novel source of stem cells for tissue engineering and cell transplantation therapy.

**P14.04**

**ISOLATION AND CHARACTERIZATION OF A NOVEL TELOMERASE-IMMORTALIZED HUMAN FIRST TRIMESTER TROPHOBLAST CELL LINE.**

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**Objectives:** Studies using first trimester trophoblast cells may be limited by the inability to obtain patient samples and adequate cell numbers. First trimester trophoblast cell lines have been generated by SV40 transformation or similar methods, however, several are known to exhibit abnormal phenotypes. The aim of this study was to isolate and characterize a human telomerase reverse transcriptase (hTERT)-immortalized first trimester trophoblast cell line, Swan-71.

**Methods:** Primary trophoblast cells isolated from 7-week normal placental villi were immortalized by hTERT, the catalytic subunit of telomerase. Telomerase activity was measured using a PCR-ELISA method and hCG secretion by an immunometric assay. Phenotypic marker expression was evaluated by immunocytochemistry and Western Blot analysis. Trophoblast cell viability was determined using a calcein assay and cytochrome profile by cytokine array.

**Results:** Swan-71 cells were positive for telomerase activity, hCG secretion and cytokeratin-7 expression, but did not express CD45, CD68 or vimentin. The expression of several cytokines was observed, including IL-8, MIP-1α, as well as the growth factor, VEGF. A 45% decrease in trophoblast cell viability (p<0.001) was observed following TNF-α treatment, but not with an agonistic anti-Fas mAb.

**Conclusions:** In the present study, we characterize a novel telomerase-immortalized human first trimester trophoblast cell line, which exhibits similar characteristics as primary trophoblast cells. This suggests that Swan-71 cells may represent a valuable model for future in vitro studies.
A SOURCE OF STEM CELLS FROM CANINE PLACENTA TO BE USED AS CELL THERAPY WITH GRMD (GOLDEN RETRIEVER MUSCULAR DYSTROPHY)

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Objective: The aim of this work is to characterize placental stem cells, as trophoblast cell or yolk sac from the GRMD (Golden Retriever Muscular Dystrophy dog) as a potential source for cell therapy. We first analyzed the morphology and quality of hemangio-blasts and their mononuclear cells produced during the pregnancy and after birth, thus, characterizing the canine yolk sac and its potentiality to produce stem cell.

Methods: Mongrel canine placentae from 20 to 63 days of pregnancy were perfused-fixed for histological investigation and casts were prepared for scanning electron microscopy (SEM). Fresh GRMD yolk sac obtained after birth was collected under sterile conditions and immersed in DMEM solution with supplements. Yolk sac preparation was broken up and pressed by net (200mesh), washed with the same explants solution 3 times and centrifuged. All solutions were analyzed by flow citometry to quantify the apoptotic cell process during the material collection.

Results: The observed value was 9.44% of viability. The yolk sac consisted of one large sacculation with inverted “T” shape and a great number of blood vessels since 25 days of pregnancy. Before that, a rounder structure was found in contact with the embryo, similar with other mammalian species such as cats, cows or humans. Hemangioblast cells were found in contact with the epithelium. These cells surround the vessels which are completely full of mononuclear cells stained by picrossirius reaction. After 55 days of pregnancy these vessels were found empty, suggesting that the mononuclear cells might have migrated to the fetus a few days before parturition.

Conclusion: After birth placenta (yolk sac) are poor in mononuclear cells and therefore if placenta and fetal membranes are used as a source of stem cells and alternative ways to expand them should be investigated.

WHY DOESN’T THE PLACENTA GET FAT?

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The developmental potential of the various cells that populate the placental villous mesenchyme with advancing gestation remains poorly defined.

Objective: Our aim was to develop a method for efficient isolation and propagation of mesenchymal cells that would allow analysis of their ability to differentiate in response to various environmental stimuli.

Methods: Cells were isolated from first trimester placenta after enzymatic removal of trophoblast. They were maintained in basal medium or transferred to differentiation media. Routine immunocytochemical characterization protocols were used in cultures and tissue sections.

Results: The stem cell markers SSEA-1, SSEA-4 and TRA-1-81 detected cells in vil-lous stroma. Vigorous stromal cultures of diverse morphology were obtained containing SSEA-4-positive cells. When cultures were transferred to medium containing insulin, phosphodiesterase inhibitor (3-isobutyl-1-methylxanthine), NSAID (indomethacin) and glucocorticoid (dexamethasone), cells appeared portraying an Oil Red O-positive vesicular morphology typical of adipocytes.

Conclusions: Progenitors present in placenta can develop along an adipogenic pathway, raising the question of why the placenta does not contain fatty tissue. We will examine the dependence of adipocyte differentiation on glucocorticoid concentration in order to test the hypothesis that placental 11β-hydroxysteroid dehydrogenase, which converts maternal cortisol to cortisone, may protect the placenta against adipocyte differentiation.

CHARACTERISATION OF A PRIMARY CELL CULTURE ISOLATED FROM THE BOVINE PLACENTAL CARUNCLE

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Objective: Feto-maternal contact in the bovine synepitheliochorial placenta is estab-

lished between maternal caruncular and fetal chorionic epithelium. So far, feto-maternal

communication in the bovine placenta has been studied by in vivo methods. To eluci-
date this interaction on a local cellular basis, we established a primary cell culture system

with predominantly caruncular epithelial cells.

Methods: Cells were isolated from caruncles from days 90-210 of pregnancy. During two cell passages, epitheloid cells were selected by trypsinization, cryo-conserved, recultivated and then examined by indirect immunofluorescence, detecting epithelial cytokeratin (CK) and zona occludens 1 (ZO-1) protein as well as mesodermal smooth muscle actin, desmin and vimentin. The results were compared with frozen sections from corresponding placentomes representing the status in vivo and confirmed by Western blot. Providing the fetus was male, the maternal origin of the cells was validated by fluorescence in situ hybridisation (FISH)-detecting the y-chromosome.

Results: Two cell populations corresponding to the status in vivo were differentiated: epi-
theloid cells expressing CK and ZO-1 and fibroblastoid cells expressing actin and desmin.

In contrast to its localisation in cells of mesodermal origin in tissue sections, vimentin was detected in both cultured cell types, indicating a beginning dedifferentiation. Y-chro-

mosomes were not detected in the cultured caruncular cells.

Conclusions: These results support the suitability of our in vitro model for primary bovine caruncular cells for further studies of the feto-maternal communication in the bovine placenta.
COMPARATIVE STUDY OF HCG EXPRESSION IN VILLOUS AND EXTRAVILLOUS TROPHOBLASTIC CELLS ISOLATED FROM FIRST TRIMESTER HUMAN PLACENTA

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Objective: The aim of this study was to characterise and quantify trophoblastic human chorionic gonadotropin hormone (hCG) production during the first trimester of pregnancy.

Methods: We thus isolated villous (VCT) and extravillous (EVCT) cytotrophoblasts from the same first trimester human chorionic villi and analysed gene expression and secretion of hCG and its two subunits α and β during in vitro cytotrophoblast differentiation. As previously established VCT were cultured on plastic dishes and fused to form a syncytiotrophoblast (ST) at 72 h, whereas EVCT were cultured on Matrigel-coated dishes and displayed an invasive phenotype from 24 to 48 h.

Results: We first showed by immunocytochemistry that invasive EVCT expressed hCG as VCT and ST did. We next quantified protein secretion (ELISA) and transcript levels (quantitative RT-PCR) in each cytotrophoblast subtypes and found that EVCT secreted substantial amounts of hCG compared with ST (180±25 vs 350±50 mU/L/µg DNA). However, analysis of hCG subunits showed that the ratio of free hCGs over β-subunits was 20 to 100 times weaker in EVCT compared to VCT or ST. Finally, we confirmed in situ by immunohistochemistry the presence of hCG in EVCT at most stages of their differentiation pathway i.e. in columns, in the decidua, and in uterine arteries.

Conclusion: Our data demonstrated that hCG is expressed and secreted by human EVCT but differently regulated compared to VCT and suggest a paracrine and/or autocrine role of extravillous hCG at the materno-fetal interface.

E.G F AND SIP ACTIVATE THE NA+/H+ EXCHANGER (NHE) IN TERM VILLOUS TROPHOBAST TISSUE

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Objectives: Placentas from pregnancies complicated by IUGR have abnormal trophoblast apoptosis rates and reduced NHE activity. In this study we investigated if there was any link between two known mediators of trophoblast apoptosis, EGF and SIP, and NHE activity in trophoblast tissue.

Methods: Villous fragments from term placentas were loaded with 1µM BCECF (a pH sensitive fluorescent dye). The syncytiotrophoblast was acidified with a pre-pulse of 20µM NH4Cl and the Na+-dependent recovery following administration of EGF (10ng/ml) or SIP(1µM) measured with or without the NHE inhibitors amiloride (NHE1,2&3), HOE (NHE1.2), and S3226 (NHE3). In vitro term trophoblasts were cultured for 24 hours with combinations of EGF, TNFα (pro-apoptotic 10ng/ml) and amiloride. Apoptosis rates were then assessed by comparing DAPI positive nuclei to total nuclei number.

Results: EGF stimulated an immediate increase in NHE activity (0.008±0.004pHu/sec versus control 0.003±0.001pHu/sec); stimulation was inhibited by amiloride, HOE and S3226, suggesting activation of NHE1.2&3. SIP stimulated an immediate rise in NHE activity (0.008±0.003pHu/sec versus control 0.003±0.001pHu/sec); stimulation was inhibited by amiloride and HOE suggesting activation of NHE1&2, but not NHE3. Amiloride increased apoptosis and inhibited the ability of EGF to inhibit apoptosis.

Conclusions: EGF and SIP acutely stimulated NHE activity in trophoblasts and may be involved in EGF’s anti-apoptotic response.

A PROTEOMIC APPROACH TO STUDYING TISSUE TRANSGLUTAMINASE FUNCTION IN PLACENTA.

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Tissue transglutaminase (tTG) catalyzes Ca2+-dependent acyl-transfer reactions, resulting in isopeptide bond formation. In addition it can hydrolyze GTP and has intrinsic kinase activity. It functions in programmed cell death, cell adhesion and differentiation and intracellular signalling. It is present in human placenta at the syncytial microvillus membrane (MVM) where its function remains undefined.

Objective: To use a proteomic approach to identify tTG target proteins in the MVM.

Methods: Low Mr biotinylated substrates were used for acyl transfer reactions catalysed by endogenous tTG in MVM vesicles isolated from term placenta. Probing blots with avidin-peroxidase confirmed the presence of biotinylated targets. Membranes were solubilised, biotinylated proteins selected chromatographically and visualised on SDS-PAGE. Bands were trypsinised and i.d. determined by LC-tandem MS. tTG and its target proteins were identified immunochromically in both MVM and primary trophoblast cultures. tTG activity was visualised in intact cells by biotinylation in situ.

Results: Target proteins in the MVM include actin, PLAP, annexin V and other members of the annexin family of phospholipid-binding proteins. In vitro tTG is seen in both syncytiotro- and cytotrophoblast, in association with cytoskeletal structures including peripher- al actin bundles and stress fibres. Its activity in situ is much more restricted, indicating the likelihood that control of intracellular cross-linking is by Ca2+ rather than enzyme abundance.

Conclusions: These data suggest a role for tTG in the organisation and turnover of trophoblast plasma membranes and associated cytoskeleton. Further work will look at the effect of blocking tTG activity on trophoblast differentiation and turnover.

POLYMORPHISMS IN THE MULTI-DRUG RESISTANCE GENE (MDR1): CORRELATION WITH FUNCTIONAL EXPRESSION OF P-GLYCOPROTEIN (P-GP) IN TROPHOBLAST CELL LINES.

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We have previously shown that JAr, BeWo and Jeg cells have different levels of functional expression of MDR1 P-gp and that this is closely related to their capacity to efflux xenobiotics i.e. JAr cells express abundant P-gp whilst BeWo and Jeg cells have little or no expression and this is reflected in the greater capacity of JAr cells to efflux vinblas- tine.

Here we tested the hypothesis that P-gp expression and function in the cell lines correlates with single nucleotide polymorphisms (SNPs) in the MDR1 gene. Initially five SNPs were screened using TaqMan®. These were ordered across the gene: intron 4 C/T rs2235074, exon 12 G/A rs2229190, exon13 C/T rs128503, intron14 C/T rs 2235035 and exon22 G/T/A rs2052582. The only difference found occurred in intron 14 where the JAr cells were homozygous for the T allele, whilst both the BeWo and Jeg cells were heterozygous C/T. This suggests that the C allele at this position is linked to reduced functional expression of P-gp. This intronic SNP is unlikely to be directly responsible for the reduced expression seen, but possibly tags a haplotype which results in lower levels of P-gp expression and function. Further work is needed to fully identify this haplotype since reduced levels of MDR1 expression in the placental trophoblast would result in increased xenobiotic accumulation in the fetoplacental unit.
P15.05

THE EFFECT OF ANTIPEILEPTIC DRUGS ON TROPHOBLAST FUNCTION

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Objectives: Women with epilepsy suffer poor obstetric outcomes including miscarriage, pre-eclampsia, intra-uterine growth retardation and stillbirth, a spectrum of disorders suggesting a problem with placentation. The cause of the poor placentation is unknown but hypotheses include the epilepsy itself or the effect of antiepileptic medication. The aim of this study was to investigate any direct effect of antiepileptic drugs on an established in vitro model of extravillous trophoblast function (EVT).

Methods: EVT were freshly isolated from the chorio-decidua of term placentae and cultured in the presence of phenytoin and sodium valproate at a range of concentrations with appropriate solvent controls for 48 hours. The formation of multinuclear giant cells was used as a marker of EVT differentiation and motility. All experiments were repeated on cells from three to eleven different placentae.

Results: We found that phenytoin inhibited EVT giant multinuclear cell formation in a dose dependent manner whereas valproate did not p=0.011.

Conclusions: Some antiepileptic medication can directly affect trophoblast function in vitro whereas others do not. This difference may have important clinical implications as to the optimal drug used for the management of epilepsy in pregnancy.

P15.08

ISOLATION OF SYNCYTIOTROPHOBLAST MICROVILLI FOR PROTEOMIC ANALYSIS

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Objectives: The dynamic range of protein expression in cells is thought to be 4-5 orders of magnitude. Therefore efficient proteomics analysis is best done on simplified fractions of cells or tissues. Thus reliable methods are needed for the preparation of highly enriched fractions. Our focus has been on isolating the microvilli from the syncytiotrophoblast (STB) of the human placenta. We have developed a method for altering the density of the apical portion of the STB so that it can be separated from other structures in the placenta in a highly enriched state.

Methods: We have modified the standard cationic colloidal silica labeling method to alter the density of the STB microvilli. Tissue homogenization followed by preparative and gradient centrifugation methods were used for sample preparation. Sample analysis has relied upon 1- and 2-dimensional gel electrophoresis and mass spectrometry, we have initiated an analysis of the proteome of these microvilli. The isolated microvilli are enriched in transformed trophoblast of GTDs (p<0.05). Both choriocarcinoma cell lines were positive for CEA, with granular deposits stained in JAr and JEG-3 cells, placental extract of 1st and 3rd trimester of pregnancy were identified by Western blot

Results: Using placental alkaline phosphatase (PLAP) as a marker, our preparations are routinely enriched 160- to 200-fold relative to the starting material. Using both 1- and 2-dimensional gel electrophoresis and mass spectroscopy, we have initiated an analysis of the proteome of these microvilli. The isolated microvilli are enriched in cytoskeletal proteins found in microvilli (e.g., moesin and ezrin) as well as proteins associated with the apical plasma membrane of the STB (e.g., PLAP, 5'-nucleotidase, and the transferrin receptor).

Conclusions: We have developed a method for the preparation of highly enriched microvilli from the STB suitable for proteomics analysis.

P15.07

INVESTIGATION OF GLYCOFORMS OF HCG SECRETED BY TRISOMY 21 VILLOUS TROPHOBLAST CELLS IN VITRO

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Objectives: In invasion by both normal and pathological trophoblast, cell adhesion molecules are important mediators of remodeling and modulators of cell proliferation and differentiation. Expression of carcinoembryonic antigen (CEA), a member of a divergent group of glycoproteins, in normal and transformed trophoblast of the gestational trophoblastic diseases (GTDs) and choriocarcinoma cell lines is presented here.

Methods: Immunocyto/histochemistry of normal placenta (n=18), invasive mole (n=8), choriocarcinoma (n=7), and JAR and JEG-3 cells was performed using rabbit anti-CEA antiserum and specific mouse monoclonal anti-CEA antibodies. Data were analysed with Mann-Whitney Test. CEA and CEA-related molecules in JAR and JEG-3 cells, placental extract of 1st and 2nd trimester of pregnancy were identified by Western blot.

Results: Data presented here demonstrate presence of CEA in normal placenta which is significantly increased in transformed trophoblast of GTDs (p<0.05). Both choriocarcinoma cell lines were positive for CEA, with granular deposits stained in JAr and JEG-3 cells, and marked membrane associated staining in JEG-3. Analysis of Western blots revealed CEA and several CEA-related molecules recognized by anti-CEA antibodies.

Conclusions: The results of immunocyto/histochemical staining and Western blot analysis presented here suggest that several CEA family members, including CEA, may be involved in trophoblast differentiation.

P15.06

CARCINOEMBRYONIC ANTIGEN AND RELATED MOLECULES IN NORMAL AND TRANSFORMED TROPHOBLAST

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Objectives: In invasion by both normal and pathological trophoblast, cell adhesion molecules are important mediators of remodeling and modulators of cell proliferation and differentiation. Expression of carcinoembryonic antigen (CEA), a member of a divergent group of glycoproteins, in normal and transformed trophoblast of the gestational trophoblastic diseases (GTDs) and choriocarcinoma cell lines is presented here.

Methods: Immunocyto/histochemistry of normal placenta (n=18), invasive mole (n=8), choriocarcinoma (n=7), and JAR and JEG-3 cells was performed using rabbit anti-CEA antiserum and specific mouse monoclonal anti-CEA antibodies. Data were analysed with Mann-Whitney Test. CEA and CEA-related molecules in JAR and JEG-3 cells, placental extract of 1st and 2nd trimester of pregnancy were identified by Western blot.

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Conclusions: The results of immunocyto/histochemical staining and Western blot analysis presented here suggest that several CEA family members, including CEA, may be involved in trophoblast differentiation.
MRJ is a co-chaperone protein that is widely expressed in adults, the embryo and trophoblast cells of the placenta. Mrj mutant mice die during mid-gestation due to a defect in chorioallantoic attachment during placental development. Tetraploid chimera experiments have shown that Mrj function is required in the trophoblast cell compartment but not the allantois. Recent studies have suggested that MRJ can associate with keratin (K) 18, a major intermediate filament protein in trophoblast cells of the placenta. To explore the role of MRJ in regulating the keratin cytoskeleton in trophoblast cells, we studied the phenotype of Mrj-deficient trophoblast cells in vivo and in vitro. In wildtype trophoblast cells, MRJ protein was detected in both the cytoplasm and nucleus. Using dual immunofluorescence in cultured cells, K18-containing filaments were not co-localized with MRJ. Yet strikingly, immunofluorescence of both K18 and its binding partner K8 in Mrj-deficient trophoblast cells revealed a collapsed keratin network instead of a normal, dense filamentous network. Histological sections of Mrj-deficient placentas revealed reduced immunoreactivity for K18 and the desmosomal protein desmoplakin in chorion trophoblast cells. Due to the keratin cytoskeleton defect observed, the expression of actin cytoskeletal proteins were assessed in Mrj mutant trophoblast cells. Remarkably, cortical actin filaments were absent and peri-nuclear actin aggregates were detected. In addition, the majority of actin-associated adhesion proteins E-cadherin and β-catenin were improperly presented on the cell membrane, remaining within the cytoplasm. These data are consistent with MRJ regulating the organization of both keratin and actin cytoskeleton.

**Conclusions:** The effects on placental function remain to be established.
P15.13

**EXPRESSION AND POTENTIAL FUNCTION OF CYSTEINE CATHEPSINS IN THE HUMAN PLACENTA**

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**Objectives:** Human cysteine cathepsins (CTSs) of the papain family are typically lysosomal proteases implicated in various cellular processes such as proliferation, differentiation, apoptosis and tumor progression. Evolution of a placenta-specific CTS family in rodents demonstrates the importance of these proteases in placental development. Here, we investigated the temporal and spatial expression pattern of the 11 human CTS family members during normal and abnormal development of the placenta.

**Methods:** RT-PCR and Northern blot hybridisations were performed using RNAs from normal human placentas [8-2 weeks (wks) - 16-5 wks, term] as well as preeclamptic placentas from 24 wks to 41+3 wks of gestation. Spatial expression patterns were analysed on paraffin-embedded normal placentas from 7+3 wks - term by in situ hybridisation, immunohistochemistry and immunofluorescence.

**Results:** All cysteine CTSs were expressed in the placenta. Expression levels differed between CTSs, with strongest expression observed for CTS B, CTS C, and CTS L in the Results: immunohistochemistry and immunofluorescence. Hence EVT lose their proliferative potential. P21 and p57 will maintain the cells in the cell cycle, but the parallel strong presence of p27 and p57 will arrest cells of anchoring villi and EVT cells, which had invaded the upper and deeper segments of the villous stroma. Additionally, PCNA, Ki67, cyclin B1 and cyclin A and p21, p27 and p57 were immunolocalised by single- or double-labeling in paraffin embedded tissue sections.

**Results:** PCNA, Ki67 and cyclin A were present in villous cytotrophoblasts and in cells of the villous stroma. Additionally, PCNA, Ki67, cyclin A and cyclin B1 were immunolocalized in proximal and distal extravillous cytotrophoblasts (EVT) of anchoring villi and in EVT in the superficial decidua, but only PCNA was expressed by EVT that had deeply invaded the decidua. Syncytiotrophoblast expression especially the cell cycle inhibitors p21 and p27 inhibitors, thereby ensuring cell cycle arrest. P27 and p57 were immunolocalized in villous cytotrophoblasts and stromal cells and in both proximal and distal EVT cells of anchoring villi and EVT cells, which had invaded the upper and deeper segments of the decidua.

**Conclusion:** PCNA, Ki67, cyclin A and cyclin B1 expression in EVT suggest that these cells are still in the cell cycle, but the parallel strong presence of p27 and p57 will arrest cell cycle. Hence EVT lose their proliferative potential. P21 and p57 will maintain the Syncytiotrophoblast in cell cycle arrest.

P15.15

**LOCATION OF CELL CYCLE PROMOTERS PCNA, Ki67, CYLIN B1, CYLIN A AND INHIBITORS P21, P27, AND P57 IN THE FIRST TRIMESTER PLACENTA AND DECIDUA**

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**Objectives:** Although placental development and implantation depend on the coordination of trophoblast proliferation, differentiation and invasion, little is known about the cell cycle regulators that are key in controlling these events.

**Methods:** Human placental villous and decidual tissues were obtained between weeks 9 and 12 post menstruation. PCNA, Ki 67, cyclin A, cyclin B1 and p21, p27 and p57 were immunolocalised by single- or double-labeling in paraffin embedded tissue sections.

**Results:** PCNA, Ki67 and cyclin A are present in villous cytotrophoblasts and in cells of the villous stroma. Additionally, PCNA, Ki67, cyclin A and cyclin B1 were immunolocalized in proximal and distal extravillous cytotrophoblasts (EVT) of anchoring villi and in EVT in the superficial decidua, but only PCNA was expressed by EVT that had deeply invaded the decidua. Syncytiotrophoblast expression especially the cell cycle inhibitors p21 and p27 inhibitors, thereby ensuring cell cycle arrest. P27 and p57 were immunolocalized in villous cytotrophoblasts and stromal cells and in both proximal and distal EVT cells of anchoring villi and EVT cells, which had invaded the upper and deeper segments of the decidua.

**Conclusion:** PCNA, Ki67, cyclin A and cyclin B1 expression in EVT suggest that these cells are still in the cell cycle, but the parallel strong presence of p27 and p57 will arrest cell cycle. Hence EVT lose their proliferative potential. P21 and p57 will maintain the Syncytiotrophoblast in cell cycle arrest.

P15.14

**ESSENTIAL ROLE OF CALCINEURIN SIGNALING IN NORMAL AND ABNORMAL PLACENTAL DEVELOPMENT**

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**Objective:** Calcineurin(Cn) is a protein phosphatase and a highly conserved cellular signal transducer that couples different extracellular signals to a variety of intracellular responses. We have previously shown that Cn/NFAT signaling plays an essential role in normal and abnormal placental development. Ca2+-dependent control of Cn activity is well recognized. However, the molecular mechanisms of Ca2+-independent regulation of Cn signaling are unclear.

**Methods:** To address this issue we designed a transfection bioassy to monitor NFAT activation in cultured human trophoblasts. A series of inhibitor and constitutive or dominant negative constructs of protein kinases have been used to determine the downstream candidates of MEKK3-mediated Cn/NFAT activation.

**Results:** We determined that MEKK3 was capable of stimulating Cn/NFAT signaling in human trophoblasts. We demonstrated that the MEKK3/MEK5/BMK1 pathway controls Cn activity through phosphorylation of MCIPI (modulatory calcineurin interacting protein 1) in human trophoblast cells. BMK1-phosphorylated MCIPI dissociates from Cn and binds to 14-3-3, a cytoplasmic phosphoprotein binding protein. The association of phosphorylated MCIPI with 14-3-3 dissociates phosphorylated NFAT from its docking site on 14-3-3, thereby allowing phosphorylated NFAT to serve as a substrate for the activated calcineurin. Using MEKK3-deficient mouse embryo fibroblasts we found that MEKK3 is essential angiotensin II-induced calcineurin-NFAT activation.

**Conclusions:** Our findings that the MAP kinase cascade (MEKK3/MEK5/BMK1) and 14-3-3 cooperate to modulate NFAT function provides insight into intracellular mechanisms of Ca2+-independent means regulating Cn signaling in placental development.
**P15.17**

**EFFECT OF CRYOPRESERVATION ON HUMAN CYTOTROPHOBLAST CELLS IN CULTURE: HCG and PALP EXPRESSION.**

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**Objectives:** Term villous cytotrophoblasts differentiate into syncytiotrophoblast during culture. Measurement of human chorionic gonadotropin (hCG) and placental alkaline phosphatase (PALP) is often used to assess viability and syncytialisation of cultured cells. This study aimed to investigate the effect of cryopreservation of isolated cytotrophoblast cells on HCG and PALP expression profiles during short-term culture.

**Methods:** Villous cytotrophoblasts were isolated from term placentae (n = 10) following elective caesarean section. Half of the cells from each placenta were cultured immediately, the other half were frozen in 10% DMSO/FBS and stored in liquid nitrogen prior to culture. Cells were cultured in identical conditions (5% CO2 in air) for 96h. Protein and DNA content of cells and HCG and PALP levels in culture medium were measured at 24 h intervals.

**Results:** Cell preparations were estimated, by cytokeratin/vimentin staining, to be >90% cytotrophoblasts both before and after freezing. Cryopreservation had no significant effect on the protein or DNA content of cultured cells but HCG levels in culture medium were significantly reduced after 72h (p = 0.025) compared to cultures of fresh cells. PALP levels were unchanged. After cryopreservation, cells adhered poorly to culture surfaces and syncytialisation appeared to be impaired in comparison to cultures of fresh cells.

**Conclusions:** Cryopreservation of isolated cytotrophoblasts prior to culture resulted in a significant decrease in basal hCG secretion, possibly caused by a failure or delay of the morphological and functional differentiation of cells. This suggests that cryopreservation of cytotrophoblast cells prior to culture may have an effect on some aspects of villous trophoblast behaviour in vitro.

**P15.18**

**ANTIBODY DEVELOPMENT AGAINST ADAM12**

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**Objectives:** ADAM12, a disintegrin and metalloprotease, is expressed in the human placenta in two splice variants: the ADAM12L long form and the ADAM12S soluble form. It comprises of a prodomain, a metalloprotease domain, a disintegrin domain, a transmembrane domain, and a cysteine rich domain. The ADAM12L has a transmembrane domain and a cytoplasmic tail. The ADAM12S contains an S-specific domain. Here we wanted to generate new antibodies against the different isoforms.

**Methods:** We have recombinantly expressed several parts of the protein in E.coli, using different systems. We used the isolated protein as immunogen in rabbits and chickens. The rabbits were used for the generation of polyclonal antisera and the chickens for the generation of monoclonal antibodies via phage display. We used the polyclonal antibodies for immunohistochemistry in placenta sections and western blots and we compared them with commercial antibodies and antibodies provided by other groups.

**Results:** ADAM12 is clearly localised in the trophoblast layer of the term placenta, while 1st trimester placenta very faintly stains with all antibodies. Antibodies specifically raised against the cytoplasmic domain stained the cytotrophoblast of term placenta villi. Western blots incubated with our antibodies on supernatants of cells transiently over expressing ADAM12S revealed high specificity and sensitivity.

**Conclusions:** With this new set of antibodies against various epitopes of the ADAM12 isoforms further elucidation of the localization and function of ADAM12 will be performed.

**P15.19**

**TRUNCATED SYNCYTIN PSEUDOTYPED MOLONEY MURINE LEUKEMIA VIRUS PARTICLES.**

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**Objectives:** Syncytin, derived from the envelope protein of a HERV-W virus, is expressed in syncytiotrophoblast. Apart from regulating fusion of trophoblast, syncytin may also play a role in placental defence against certain retroviruses. Syncytin also pseudotyped HIV-based viral particles. Therefore syncytin has a theoretical potential as an envelope in vectors for retroviral mediated gene therapy. We tested whether syncytin, or C-terminally truncated mutants thereof, can pseudotype the more commonly used Moloney murine leukemia virus (MLV)-based particles.

**Methods:** We generated a series of expression constructs encoding C-terminally truncated mutants of syncytin containing between 0 and 46 cytoplasmic residues. These mutants and the full length construct were cotransfected with an EGFP-expressing retroviral construct into Gp293 retroviral packaging cells, expressing the MLV gag and pol genes but no retroviral envelope. Viral particles in conditioned media were used to infect various target cell lines. Infection efficiencies were tested by flow cytometry.

**Results:** Virions pseudotyped with the two shortest syncytin mutants infected cells expressing receptor RDR with low efficiency, while virions pseudotyped with longer syncytin variants were unable to infect. Immunoblotting demonstrated that only the two shortest syncytin mutants were incorporated into MLV particles.

**Conclusions:** Syncytin truncated to encode only 0 or 7 cytoplasmic residues can pseudotype MLV particles.
P16.01

SOX15 PROMOTES TROPHOBLAST GIANT CELL DIFFERENTIATION BY INTERACTING WITH HAND1

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Objective: Members of Sox (SRY-type HMG box) gene family, which encode DNA binding proteins, may play important roles in regulating specific genes during development. This study aimed to isolate a Sox member expressed in placenta, and clarify its function in placental development.

Methods: The expression patterns of Sox15 mRNA in mouse placenta, mouse trophoblast stem (TS) cells and rat choriocarcinoma (Rcho-1) cells were studied by in situ hybridization and/or Northern blot analyses. Protein-protein interaction of Sox15 and Hand1 was investigated by in vitro and in vivo binding assays. The function of Sox15 was examined by trophoblast giant cell differentiation and luciferase reporter assays.

Results: Sox15 mRNA was expressed in mouse placenta, especially in trophoblast giant cells, and was gradually increased during the differentiation of TS cells. Coexpression of Sox15 and Hand1 in Rcho-1 cells promoted Hand1-driven differentiation into giant cells. We have also shown that Sox15 interacted with Hand1 in cotransfected 293T cells and that Hand1 bound to the HMG domain of Sox15 in vitro. The reporter assays indicated that the transcriptional activity of Sox15 was negatively regulated by Hand1, while that of Hand1 was enhanced by Sox15.

Conclusion: Sox15 may induce differentiation into trophoblast giant cells by raising the transcriptional activity of Hand1.

P16.02

CONNEXIN-40 MODULATES STRA-13 EXPRESSION IN EVT DIFFERENTIATION

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Marine TS cell differentiation is modulated by MHLH transcription factors (TF) whose function in human trophoblast is not fully understood. Our previous studies have shown a critical role for Connexin-40 (Cx40) in regulating the switch from a proliferative to invasive extra villous trophoblast (EVT) phenotype. We investigated the role of Cx40 in regulating MHLH TF during differentiation of human cytotrophoblast (CT) to EVT. Primary first trimester CT were seeded on collagen IV plates in supplemented defined KSFM and incubated at 8% P02 for 8 days. RNA was extracted every 2 days and reverse transcribed to cDNA before assessment of gene expression levels of trophoblast markers (HLA-G, PCNA and Cx40) and MHLH TF (HASS-2, STRA-13, HAND-1, GMC-1) by real-time PCR. By d4 of culture the CT cells had stopped proliferating, shown by a decrease in PCNA expression, and had begun to express EVT markers, HLA-G and Cx40. An upregulation of STRA-13 and GMC-1 between d4 and d6 of culture correlated with the increase of EVT markers. The CT-specific TF HASS-2 was only detectable at Day 0 of culture. Treatment on d4 of culture with Cx40 antisense oligonucleotides resulted in the downregulation of Cx40 and STRA-13; moreover, treatment with deciduall-conditioned medium, which results in the differentiation of the EVT to the invasive phenotype, resulted in the downregulation of CX40 and an upregulation of STRA-13. This data suggests that human trophoblast differentiation is mediated by similar transcription factors that control marine TS cell differentiation and that Cx40 signaling appears to act upstream of the STRA-13 transcription factor.

P16.03

PLACENTA-SPECIFIC INSULIN-LIKE GROWTH FACTOR 2 (P0) IS ASSOCIATED WITH PROLIFERATION AND DIFFERENTIATION IN THE JUNCTIONAL ZONE

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Objectives: Disruption of Igf2 (null) or the placenta-specific transcript of Igf2 (P0) is associated with fetal and placental growth restriction including reduction in junctional zone (Jz) volume. The null expresses no Igf2 whereas the P0 expresses Igf2 initially from spongiotrophoblast (Spt), then from glycogen cells (GC) and fetal endothelium. We counted cell numbers to investigate the relationship between Igf2 and Jz development.

Methods: The Optical Disector method was used to estimate numerical density on 20µm thick wax sections of E14 wildtype and mutant placentas from 3 litters of P0 and nulls. 7µm thick sections were stained with antibodies to classical EVT markers (PS, AnxA5) and annexin A5 in the fusion process. AnxA5 protein by 60% and intercellular fusion by 49.3%. These data support a role for AnxA5 in cell fusion. Cultures in forskolin alone or supplemented with anti-cytokeratin (negative control) exhibited maximum intercellular fusion: 79.2% [0.4] (mean [SD]) and 75.7% [0.4] nuclei in syncytial cells, respectively. Both monoclonal anti-PS (positive control) and AnxA5 antisense oligos decreased AnxA5 protein by 60% and intercellular fusion by 49.3%. These data support a role for annexin A5 in the fusion process.
P16.05
SYNCYTIN-A AND SYNCYTIN-B, TWO FUSOGENIC PLACENTAL ENVELOPE GENES OF RETROVIRAL ORIGIN CONSERVED IN MURIDAE
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Objectives: Recently, we and others have identified two human endogenous retroviruses that entered the primate lineage 25-40 Myr ago and that encode highly fusogenic envelope proteins (syncytin-1 and -2), possibly involved in the formation of the placenta syncytiotrophoblast layer. We then looked for retroviral envelope genes with syncytin-related properties in other species in which placenta trophoblast fusion takes place, such as rodents.

Methods and Results: An in silico search throughout mouse genome databases identified two fully coding envelope genes of retroviral origin, present as unique copies and that we named syncytin-A and -B. Quantitative RT-PCR demonstrates placenta-specific expression for both genes, and in situ hybridization further localizes these transcripts in the syncytiotrophoblast-containing labyrinthine zona. Consistently, we show that both genes can trigger cell-cell fusion in ex vivo transfection assays, with distinct cell type specificities suggesting different receptor usage. Genes orthologous to syncytin-A and -B -with a coding status- are found in all Muridae tested (mouse, rat, gerbil, vole and hamster), dating their entry into the rodent lineage about 20 Myr ago.

Conclusions: We discovered two new genes that are possible effectors of murine syncytiotrophoblast formation, and therefore unraveled a rather unique situation where two pairs of endogenous retroviruses, independently acquired by the primate and rodent lineages, would have been positively selected for a convergent physiological role.

P16.06
PL48, A NEW GROWTH REGULATORY GENE, INDUCES HUMAN PLACENTAL CYTOTROPHOBLAST DIFFERENTIATION
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Objectives: PL48 was cloned from a subtractive library of in vitro differentiating human cytotrophoblast cells. We wished to determine if PL48 contributes to regulation of differentiation of human cytotrophoblast cells in vitro.

Methods: Human term cytotrophoblast cells were extracted using 0.25% trypsin-DNase I and purified using CD9/HLA-I/HLA-II immunoelimination columns. PL48 expression rapidly (< 24 h), spontaneously increases in expression as cytotrophoblast differentiates into syncytiotrophoblast. We transfected an antisense PL48 construct in pcDNA3 vector, or empty vector control. 72 h after transfections (n=2), cells were fixed and stained for desmoplasmin to quantify syncytial unit formation. In situ hybridization of PL48 to first trimester and term placental sections to localize PL48 expression, and semi-quantitative PCR of PL48 expression in first trimester and term placenta were performed.

Results: PL48 was expressed exclusively in syncytiotrophoblast. Expression by in situ hybridization and PCR was weak in the first trimester but strong at term. There was a 19.9 +/- 1.1% decrease in syncytial unit formation in antisense-transfected cells compared to control.

Conclusion: PL48 is a multifunctional syncytial expressed gene that not only inhibits growth and induces apoptosis when in excess, but contributes to induction of differentiation of human cytotrophoblast cells.

P16.07
EXPRESSION OF hCG RECEPTOR DURING DIFFERENTIATION OF THE HUMAN VILLUS CYTOTROPHOBLAST
A.70
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Objectives: It has been shown that hCG, the human chorionic gonadotropin hormone, promotes the differentiation of cytotrophoblasts into syncytiotrophoblasts. The role of hCG on the trophoblast differentiation is mediated by the hCG/LH receptors. The aim of this study was to investigate the expression of this receptor in second trimester trophoblasts.

Methods: The immunohistochemical characterization of hCG/LH receptor in frozen sections from second trimester placentas was performed by using two monoclonal antibodies which recognize different epitopes of the hCG/LH receptor. We also used the primary cell culture model in which, villous cytotrophoblasts were isolated from second trimester placentas and cultured for three days to differentiate into syncytiotrophoblast.

Results: We confirmed by immunohistochemistry in situ and in cultured cells, the localisation of hCG/LH receptor in the villous cytotrophoblasts and syncytiotrophoblasts during the second trimester of pregnancy. hCG/LH receptor expression was also detected in perivascular cells in the villous core. By western blot analysis, we observed in cultured cells that a 70 KD band corresponding to the hCG/LH receptor as shown by its ability to bind specifically labelled hCG, was decreased in the syncytiotrophoblast compared to the cytotrophoblasts during the differentiation process.

Conclusion: hCG/LH receptor expression seems to be regulated during human villous trophoblasts. This regulation may involved a down-regulation of the receptor by its ligand.

P16.08
SINGLE CHAIN Fv ANTIBODIES AGAINST SYNCYTIN AND ITS RECEPTOR RDR
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Objectives: Syncytin and its receptor RDR are thought to play important roles in regulating trophoblast fusion. However, different laboratories, using different antibodies, have published partly contradictory data on the spatial and temporal expression of syncytin. At present, only one antibody against RDR has been described. We intended to enlarge the panel of available antibodies against these proteins by generating and selecting a series of specific chicken single chain Fv antibodies.

Methods: Chickens were immunized with recombinant proteins comprising sequences from the syncytin SU subunit or sequences comprising an intracellular and an extracellular loop of RDR. Phage display libraries were generated from chicken spleen RNA encoding scFvs. Phages reacting with their antigen were selected by panning. Specific scFv-encoding sequences were subcloned to a plasmid vector allowing expression of the scFvs as soluble proteins equipped with a (His)6 tag and a myc tag in E. coli. The scFvs were tested in ELISA, Western blotting, FACS analysis and immunohistochemistry. The His tag facilitates purification of the scFv by nickel affinity chromatography.

Results: We identified a series of scFvs against syncytin and RDR displaying differential reactivity and applicability in various types of assays.

Conclusions: These scFvs may prove to be invaluable tools in studying the roles of syncytin and its receptor in trophoblast fusion.
P16.09

A SYSTEMATIC STUDY ON THE ROLE OF CYTOPLASMIC RESIDUES OF SYNCYTIN IN INDUCING CELL-CELL FUSION

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Objectives: Syncytin is the envelope protein of an endogenous retrovirus of the HERV-W family. It plays an important role in regulating trophoblast fusion. Its ability to fuse and kill cells makes it an attractive candidate molecule in gene therapy against cancer. We intended to study the relevance of sequences in the cytoplasmic tail of syncytin for its ability to induce cell-cell fusion.

Methods: We generated a series of C-terminally truncated syncytin variants, truncated behind residues 469, 476, 483, 493, or 515. Constructs encoding these mutants and wild type syncytin were transfected into various model cell lines along with an EGFP-encoding construct. Cell-cell fusion was measured through counting the number of nuclei within syncyta - visible as uniformly green-fluorescent large areas of cytoplasm – as a fraction of all nuclei. Cell surface syncytin expression was measured by immunofluorescence and FACS analysis using a rabbit anti-syncytin antibody.

Results: We found that the first 8 to 14 cytoplasmic residues were necessary for displaying full fusogenicity. In contrast, the 23 most C-terminal residues of syncytin partially inhibited its fusogenicity. Cell surface expression of the most fusogenic syncytin variants was slightly enhanced compared to the less fusogenic variants.

Conclusions: Three variants of syncytin truncated behind residues 483, 493 and 515 were hyperfusogenic compared to wild type syncytin.

P16.10

ROLE OF GCM1 IN SYNCYTIAL FUSION OF HUMAN TROPHOBLAST

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The transcription factor glial cell missing-1 (Gcm1) is critical for syncytial formation in mice, and is expressed in an analogous manner in a subset of human cytotrophoblast (CT) cells. We demonstrated the critical role of Gcm1 for syncytial fusion using the technique of siRNA-mediated gene silencing in human-derived trophoblast cell line, BeWo. In our present studies, we have used our in-vitro floating villous explant model to study the behaviour of villous CT in their normal 3-dimensional environment under conditions that inhibit Gcm1-mediated cell differentiation. Enzymatically-denuded first trimester explants cultured in the presence of GCM1 antisense oligonucleotides did not regenerate their syncytio-trophoblast (SCT). The exposed CT remained viable and proliferative resulting in their accumulation on the basal membrane. Preliminary immuno-localization experiments indicate altered expression patterns for RDR (Syncytin receptor), Syncytin and connexin-43 – genes involved in the syncytialization process. We are presently using real-time-PCR in both floating explants and BeWo cells to study the post-Gcm1 pathways that regulate syncytial fusion and maintenance of the integrity of a healthy SCT. Post-Gcm1 pathways mediates syncytial differentiation may be altered in disease processes resulting in pathological syncytial shedding that trigger severe forms of pre-eclampsia.

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P16.11

ADAM12, A NEW CANDIDATE MOLECULE FOR REGULATING TROPHOBLAST FUSION

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ADAM12 is a catalytically active metallopeptase-disintegrin protein. It has been shown to be involved in fusion of myoblasts as well as in the formation of multinucleated giant cells and osteoclasts. Due to its importance for fusion of both myoblasts into muscle fibres and macrophages into osteoclasts, ADAM12 has attracted the attention as a candidate molecule for regulating trophoblast fusion in placenta. Its mRNA has been detected in placenta. Alternative splicing generates two isoforms: the ADAM12-L membrane anchored form and soluble ADAM12-S. There is evidence that ADAM12 mediates cell-cell/matrix adhesion, while ADAM12-S contains strong protease activity. Our work revealed the existence of both mRNA splice variants in placenta, the short splice variant encoding soluble ADAM12-S being dominant in term placenta. In parallel, immunohistochemistry revealed positive signals with various anti-ADAM12 antibodies in villous and extravillous cytotrophoblast cells and in syncytiotrophoblast, as well as in decidual cells. Which of the splice variants is expressed at which sub location is unknown at present. The role of the ADAM12 variants in placental function is also largely unknown. Which of the splice variants is expressed at which sub location is unknown at present.

Results: The smallest mutants demonstrated higher cellular expression than larger truncated syncytin variants. The differences were much more pronounced in transfected human 293 cells, containing syncytin receptor, than in hamster CHO cells, which are devoid of syncytin receptor. We hope to start a discussion on mechanisms important to syncytin maturation and trafficking, and the roles of the cytoplasmic tail of syncytin and the cellular background therein.

Methods: Constructs encoding C-terminally truncated syncytin variants or wild type syncytin were transfected into CHO or 293 cells. Cell lysates were stained with an anti-syncytin antibody after Western blotting. Proteins on cell surfaces and in cell lysates were treated with N-glycosidase F. Recombinant syncytin mRNA levels were quantitated by real-time RT-PCR.

Results: The smallest mutants demonstrated higher cellular expression than larger mutants and wild type syncytin. Recombinant syncytin mRNA levels did not differ significantly. In 293 cells, the differences in expression levels were huge compared to those in CHO cells. Deglycosylation experiments suggested that only a very small proportion of cellular syncytin was exposed on the cell membrane. Only wild type syncytin appeared to be efficiently cleaved to generate an SU subunit.

Conclusions: Cytoplasmic sequences and cellular environment influence syncytin maturation through mechanisms as yet not understood, but which may be linked to the levels of functional syncytin receptors present.

P16.12

CELL-TYPE SPECIFIC DIFFERENCES IN CELLULAR ACCUMULATION OF SYNCYTIN MUTANTS: UNKNOWN EFFECTS OF CYTOPLASMIC SEQUENCES AND CELLULAR ENVIRONMENT ON MATURATION AND TRAFFICKING?

Sascha Drewlo, Simone Leyting, and Andy J.G. Pötgens. Institute of Anatomy II, University Hospital RWTH Aachen, Aachen, Germany.

Objectives: We observed unexpected differences in cellular expression between C-terminally truncated syncytin variants. The differences were much more pronounced in transfected human 293 cells, containing syncytin receptor, than in hamster CHO cells, which are devoid of syncytin receptor. We hope to start a discussion on mechanisms important to syncytin maturation and trafficking, and the roles of the cytoplasmic tail of syncytin and the cellular background therein.

Methods: Constructs encoding C-terminally truncated syncytin variants or wild type syncytin were transfected into CHO or 293 cells. Cell lysates were stained with an anti-syncytin antibody after Western blotting. Proteins on cell surfaces and in cell lysates were treated with N-glycosidase F. Recombinant syncytin mRNA levels were quantitated by real-time RT-PCR.

Results: The smallest mutants demonstrated higher cellular expression than larger mutants and wild type syncytin. Recombinant syncytin mRNA levels did not differ significantly. In 293 cells, the differences in expression levels were huge compared to those in CHO cells. Deglycosylation experiments suggested that only a very small proportion of cellular syncytin was exposed on the cell membrane. Only wild type syncytin appeared to be efficiently cleaved to generate an SU subunit.

Conclusions: Cytoplasmic sequences and cellular environment influence syncytin maturation through mechanisms as yet not understood, but which may be linked to the levels of functional syncytin receptors present.
P16.13

EXPRESSION OF HERV-W ENV GLYCOPROTEIN AND D MAMMALIAN RETROVIRUS RECEPTOR IN THE HUMAN EXTRAVILLOUS TROPHOBLAST

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The product of the HERV-W envelope gene is a membrane glycoprotein (syncytin) that induces the formation of the human syncytiotrophoblast on interaction with the D mammalian retrovirus receptor (RDR). In the present study we investigated the expression of HERV-W Env glycoprotein and of RDR in the extravillous trophoblast of first trimester human placenta. The presence of HERV-W transcripts and Env glycoprotein was also investigated in cultured extravillous trophoblastic cells (EVTC) using an in vitro model of EVTC isolation and invasion.

Using monoclonal and polyclonal antibodies, the HERV-W glycoprotein was immunolocalized in all the cell types of the extravillous phenotype lineage: cytotrophoblastic cells of the column, interstitial trophoblastic cells, multinucleated giant cells and endovascular trophoblast. The presence of HERV-W transcripts and glycoprotein was also demonstrated in cultured extravillous trophoblast. Furthermore using a polyclonal antibody, the RDR was also localized in the various extravillous phenotypes. In conclusion HERV-W Env glycoprotein expressed in villous and extravillous trophoblast can be considered as a specific marker of human trophoblast. Furthermore the colocalization of HERV-W Env glycoprotein and its receptor in cells that do not fuse (trophoblastic cells of the column, interstitial and endovascular trophoblast) suggests that the trophoblastic cell fusion is a complex multifactorial process.

P16.14

CYTOTROPHOBLAST PROLIFERATION IS DEPENDENT ON THE PRESENCE OF AN OVERLYING SYNCYTIUM

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Objectives: In vivo, cytotrophoblast (CTB) is intimately associated with the overlying syncytiotrophoblast (STB). We hypothesise that intercellular interactions are important for maintaining proliferative potential as isolated human CTB exit from the cell cycle within 24h. We therefore examined proliferation of CTB in the presence or absence of syncytiotrophoblast (STB).

Methods: First trimester villous explant cultures were maintained in two different media for up to 11 days before analysis by histology, TEM or immunolabelling. BrdU incorporation was used to identify cells that had passed through S phase.

Results: Explants cultured in CMRL/FCS showed syncytial degeneration and then, by 72h, there was evidence of trophoblast differentiation as determined by measurement of hCG. At subsequent time points, a CTB multilayer, indicative of cellular proliferation, was observed in tissue cultured in either atmospheric or 3% oxygen, suggesting that this process is independent of oxygen concentration. When explants were cultured with serum free F12/DMEM, the syncytiotrophoblast (STB) was preserved and CTB proliferation (BrdU) was evident. However in explants pre-treated with trypsin (5%, 15min) to remove the syncytiotrophoblast, BrdU staining was absent until syncytial regeneration had occurred.

Conclusions: Once the syncytiotrophoblast is lost, CTB ceases proliferation and differentiates. However, a reserve population of CTBs is maintained that can respond to proliferative signals generated in concert with the formation of a new syncytiotrophoblast. These results may explain why CTBs in monoculture, which lack an overlying syncytiotrophoblast, fail to sustain proliferative activity.
P17.01

PLACENTAL GROWTH HORMONE STIMULATES HUMAN TROPHOBLAST INVASION
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Objectives: Hormones belonging to the growth hormone/prolactin family are expressed at the maternofetal interface. As they are involved in cell motility in various models, we examined the possible regulatory role of human placental growth hormone (hPGH) in extravillous cytotrophoblasts (EVCT) invasiveness.

Methods: EVCT were isolated and purified from human first-trimester chorionic villi and cell invasiveness studied in an in vitro invasion assay.

Results: We observed by immunocytochemistry with specific monoclonal antibodies, strong expression of hPGH and GH receptor (GHR) in EVCT when they invaded Matrigel and moved through the pores of the filter on which they were cultured. EVCT secreted hPGH in culture medium (235±90 pg/ml/2.5×10^5 cells) and expressed the full-length and truncated forms of the GH receptor as shown by PCR. The JAK2-Stat5 signaling pathway was activated in EVCT by hPGH treatment as demonstrated by transient transfection experiments. Human PGH but not prolactin stimulated EVCT invasiveness, and this effect was inhibited by a JAK2 inhibitor.

Conclusion: These results offer the first evidence for a placental role of hPGH, and suggest an autocrine/paracrine role of hPGH in the regulation of trophoblast invasion.

P17.02

ATORVASTATIN (LIPITOR) INHIBITS TROPHOBLAST MIGRATION AND MMP-9 ACTIVITY.
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Background: Anchoring villi facilitate placental attachment through extravillous trophoblastic (EVT) invasion of the uterine decidua, partly mediated by secretion of matrix metalloproteinase (MMP)-2 and -9. Atorvastatin (atorva), the most commonly prescribed cholesterol-lowering statin drug, is known to decrease expression of MMP-9 in human macrophages. Atorva exposure in pregnant rats has also been shown to reduce offspring survival by 45% through unknown mechanisms.

Goal: To investigate the effect of atorva on the differentiation of EVT and related MMP expression.

Methods: First-trimester human placental explants treated for 48 hours with atorva in decided conditioned media (DCM). The effect of atorva on cell cytotoxicity, trophoblast migration, markers of EVT development, and MMP-9 and MMP-2 expression were compared to atorva-free controls.

Results: At a drug concentration of 5 ng/mL, atorva visibly reduced DCM-induced EVT invasion in the explants, without evidence of cytotoxicity. Atorva displayed differential effects on the markers of proliferative and invasive EVT: there was no effect on α5 integrin, HLA-G, EGFR or ERB-2, but up-regulation of α6 integrin induced by DCM was strongly attenuated. Atorva significantly inhibited trophoblastic migration, to the same extent as exposure to a potent inhibitor of MMP-2 and -9. Zymography also demonstrated atorva’s inhibition of MMP-9 expression and activity in DCM treated explants.

Conclusion: Atorva may have a detrimental effect on early placental development by interfering with the matrix remodeling capabilities of the invading EVT during early formation of the utero-placental circulation.

P17.03

TROPHOBLAST INVASION CAN BE STUDIED IN VIVO IN THE SUBPLACENTA OF CAVIOMORPH ROODENTS.
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Objectives: Trophoblast invasion is usually studied in rather artificial in vitro systems which hardly reflect the in vivo situation. Different from other laboratory animals, trophoblast invasion in caviomorph rodents is phenotypically similar to that in humans. The present study aims to test whether (a) the subplacenta of caviomorph rodents is the source of trophoblast invasion, and (b) is a suitable model for the in vivo study of this process.

Methods: Pregnant degus (Octodon degus) (d 20 – d 50) and pregnant guinea pigs (Cavia porcellus) (d 22 – d 35) were injected with 1 to 3 doses of 50 to 200 µg weight, administered i.p. within 24 h, and killed 1 to 15 days after the administration. The distance of invasion covered daily in early gestation is about 300 µm.

Conclusions: The data suggest that the placentas of caviomorph rodents are suitable in vivo models to study the dynamics of trophoblast invasion. The lateral parts of the subplacenta in these species act as a kind of cell column.

P17.04

INHIBITION OF HUMAN TROPHOBLAST INVASIVENESS BY HIGH GLUCOSE CONCENTRATIONS.
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Trophoblast invasion of the uterus is regulated by local microenvironmental factors. Since certain conditions may affect uterine glucose levels during placentation, the aim of this study was to determine the effect of glucose concentration on trophoblast invasion. Compared with incubation in 0.2 mM and 2.5 mM glucose, a 24-h incubation in increasing glucose concentrations (5 mM and 10 mM) resulted in up to a 62% inhibition (P < 0.01) in the in vitro invasiveness of immortalised HTR-8/SVneo trophoblasts. This decreased invasiveness in 5 mM and 10 mM glucose was paralleled by inhibition of a plasminogen activator (PA) activity corresponding to active urokinase-type PA (uPA). Inhibition of pro-uPA binding to the uPA receptor (uPAR) decreased the invasiveness of cells incubated in 2.5 mM glucose down to levels observed in cells incubated in 0.2 mM glucose (P < 0.05). Gelatin zymography and Western blot analysis showed that the levels of matrix metalloproteinases-2 and -9, plasminogen activator inhibitor-1, and uPAR were unaffected by glucose. GLUT-1 glucose transporter levels were 26% and 34% higher in cells cultured in 2.5 mM and 0.2 mM glucose, respectively, versus 5 mM or 10 mM glucose (P < 0.05). In contrast, GLUT-3 levels were not affected by incubation in various glucose concentrations. These findings indicate that high glucose concentrations inhibit the invasiveness of HTR-8/SVneo cells by preventing uPA activation. Therefore, through its effects on uPA activity, glucose may be an important regulator of trophoblast invasiveness during implantation and placentation. (Supported by the Heart and Stroke Foundation of Ontario).
P17.05
INTERLEUKIN-10 PREVENTS MACROPHAGE-MEDIATED INHIBITION OF TROPHOBLAST INVASION.
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Objectives: Pre-eclampsia is characterized with inadequate cytotrophoblast invasion and remodeling of the spiral arterioles, as well as by an aberrant maternal immune response. This response is characterized by increased Th1 immunity and decreased levels of circulating Th2 cytokines. We previously showed that activated macrophages inhibit trophoblast invasion in vitro. Here, we determined the combined effects of activated macrophages and the Th2 cytokine interleukin (IL)-10 on trophoblast invasiveness.

Methods: Invasiveness of immortalized HTR-8/SVneo human trophoblasts co-cultured with non-activated or lipopolysaccharide-activated human macrophages, or media derived from these macrophages, was determined by an assay that employs reconstituted extracellular matrix (Matrigel) as substrate for invasion. Cell surface expression of the urokinase plasminogen activator receptor (uPAR) was assessed by flow cytometry, and plasminogen activator inhibitor 1 (PAI1) levels were determined by ELISA.

Results: Co-culture with activated macrophages decreased the invasiveness of trophoblast cells. This effect was paralleled by decreased cell surface uPAR expression and increased PAI1 secretion relative to controls. Furthermore, the inhibitory effect of macrophages on uPAR and PAI1 expression was prevented by pre-incubation of the macrophages with IL-10.

Conclusions: These results suggest that decreased Th2 serum cytokine levels associated with pre-eclampsia may contribute to inadequate trophoblast invasion and remodeling of the uterine spiral arterioles. (Supported by the Heart and Stroke Foundation of Ontario).

P17.06
TNFα-MEDIATED INDUCTION OF PAI-1 RESTRICTS INVASION OF HTR-8/SVNEO TROPHOBLAST CELLS
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Objectives: We recently showed that TNFα blocks migration/invasion of primary extravillous trophoblasts suggesting that elevated concentrations of the cytokine could be linked to foiled trophoblast invasion in preeclampsia. Reduced motility of the cells was associated with induction of plasminogen activator inhibitor 1 (PAI-1). Here, we investigated the specific role of the uPA inhibitor and its TNFα-dependent regulation in HTR-8/SVneo trophoblast cells.

Methods: To study the effects of TNFα on migration/invasion and proliferation, wound healing assays, Matrigel invasion assays, counting of accumulative cell numbers and FACS analyses were performed. Apoptosis was measured by immunocytochemistry of the cytokeratin 18 neo-epitope. Expression of MMP9 was studied by gelatin zymography and enzyme activity assays of supernatants of TNFα-treated cells. PAI-1 expression was investigated by RT-PCR, Northern and Western blot analyses. In addition, we studied induction of NFκB using immunocytochemistry and EMSA.

Results: TNFα did not alter proliferation but reduced invasion and migration. The cytokine did not induce apoptosis in the cell line and increased PAI-1 mRNA and protein. Supplementation of PAI-1-blocking antibodies restored invasion of TNFα-incubated cells through Matrigel-coated transwells. Surprisingly, elevation of the pro- and active form of MMP-9 could be detected in supernatants of cytokine-treated cultures suggesting that increased expression of the protease cannot overcome the TNFα-inhibitory effect on cell invasion. Immunocytochemistry revealed TNFα-dependent, nuclear accumulation of the p65 subunit of NFκB. EMSA showed that the cytokine also provoked binding of the inflammatory transcription factor to a NFκB consensus sequence as well as to the NFκB recognition site located in the PAI-1 promoter.

Discussion: The data suggest that TNFα restricts trophoblast invasion mainly by increasing the expression of PAI-1. Induction of the inhibitor may involve TNFα-stimulated activation of NFκB. Increased MMP-9 expression could be part of a compensatory mechanism reconstituting invasiveness of some of the cells. Elevated TNFα concentrations in serum and placental bed of preeclamptic patients could impair trophoblast invasion and might therefore play a critical role in the pathogenesis of preeclampsia.

P17.07
REGULATION OF TROPHOBLAST INVASION.
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Objectives: Invasion of trophoblast cells requires a fine tuning, which is fundamental for correct placentation. This tuning is regulated on intracellular level. Several signal transducers and their suppressors, known from former studies and tumor invasion, are expected to be involved.

Methods: RNA interference (RNAi) was applied to Jeg-3 cells to knock down STAT3, SOCS3 and STAT6. Small interfering RNA (siRNA) and scrambled oligonucleotide concentrations were analyzed by Western blot analyses. In addition, we studied expression and activation of STAT3, SOCS3 and STAT6 by RT-PCR, Northern and Western blot analyses. In addition, we studied induction of NFκB using immunocytochemistry and EMSA.

Results: Knock down of STAT3 reduced proliferation and invasion, whereas SOCS3 knock down increased IL-6 induced Tyr705 phosphorylation of STAT3 simultaneously with proliferation. STAT6 RNAi had no influence on invasion, but IL-4 induced proliferation was inhibited.

Conclusions: Expression and activation of STAT3, SOCS3 and STAT6 regulate proliferative and invasive capacities of Jeg-3 choriocarcinoma cells, which may be regarded as a model for trophoblast cells.

P17.08
ENDOVASCULAR AND INTRAMURAL EXTRAVILLOUS TROPHOBLAST CELLS DISPLAY DIFFERENT IMMUNOSTAINING PATTERNS
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Background: The origin of ‘intramural’ extravillous trophoblast cells (EVT) embedded in fibroid in transformed spiral arteries has been assumed to be from the endovascular rather than from interstitial EVT. In immunohistochemical studies of human placental bed endovascular and intramural EVT are often grouped together as one population.

Methods: Placental bed biopsies were obtained with informed written consent and appropriate ethical approval from women undergoing elective surgical termination of pregnancy (8-10, 12-14 and 16-20 weeks gestation, n=5 each group) at Royal Victoria Infirmary, Newcastle. Biopsies were formalin fixed and paraffin embedded for immunohistochemistry. Sections were immunostained for VEGF-A, -C, -R2, -R1 and NCAM and cyclin E. EVT cells were identified by HLA-G immunostaining. Levels of immunostaining in endovascular, intramural and interstitial EVT populations were scored according to proportion and strength of positive cells on a 12 point scale.

Results: VEGF-A immunostained intramural EVT more strongly than either endovascular or interstitial EVT. In contrast, immunostaining of intramural EVT for VEGF-C, -R2, -R1 and cyclin E was lower than for endovascular and interstitial EVT. NCAM was strongly expressed by endovascular EVT, variably by intramural EVT and interstitial EVT was negative. There were no gestational age effects on the observed results.

Conclusions: The origin of intramural EVT remains unclear. However, the phenotype of intramural EVT differs from both interstitial and endovascular EVT raising the possibility of gene expression alterations induced by myometrial or endothelial cells or fibrinoid and indicating that this population should be considered separately.
**P17.09**

**EFFECTS OF TGF-β, EGF AND FORSKOLIN ON INVASION AND DIFFERENTIATION OF JEG-3 AND BEWO CELLS.**

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**Objectives:** To examine effects of TGF-β, EGF and forskolin (Fk) on viability, migration, invasion, biochemical (hCG) and morphological (syncytialization) differentiation of JEG-3 and BeWo chorocarcinoma cells. Those effects were examined in 20 % O₂ ("normoxia") and 5 % O₂ ("hypoxia").

**Methods:** We developed a novel technique of AlamarBlue reduction to quantify in vitro viability, migration and invasion into fibronectin-coated 12 µ filters in cultures of BeWo and JEG-3 cells treated with 10 ng/ml EGF, 10 ng/ml TGF-β1 or 10 µM hCG secretion was analyzed with ELISA and syncytialization was evaluated by E-cadherin immunocytochemistry.

**Results:** E-cadherin staining showed that only BeWo cells were able to differentiate into multinuclear syncytia. In both JEG-3 and BeWo cells, hCG secretion was stimulated by all three factors (TGF-β, EGF and Fk) and viability was inhibited by TGF-β and EGF. TGF-β and EGF inhibited migration and invasion respectively, in BeWo cells only. Fk inhibited migration of BeWo cells as well Fk also had reversed effects on viability, inhibited in BeWo and stimulated in JEG-3 cells. In hypoxia, effects were limited to Fk, stimulating hCG secretion in both cell types and inhibiting invasion in BeWo only.

**Conclusions:** AlamarBlue is a useful indicator of cell viability, migration and invasion. Biochemical differentiation was associated with reduced cell viability. This reduction in viability disappeared when the cells were incubated under bw oxygen. Inhibition of migration/invasion by TGF-β, EGF and Fk was confined to BeWo cells. The different responses by BeWo and JEG-3 may be related to the inability of JEG-3 to syncytialize or to other intrinsic differences.

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**P17.10**

**TGF-β-DEPENDENT REGULATION OF TROPHOBLAST INVASION DURING PLACENTAL IMPLANTATION.**

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**Objectives:** Implantation of the placenta requires migration of trophoblast cells within the maternal endometrium in an invasion process that is controlled both in time and space though the interplay of various cytokines and hormones. Among these, the transforming growth factor-beta (TGF-β) has been postulated to inhibit invasion. The purpose of our study is to characterize the mechanisms of invasion regulation by TGF-β and the signaling pathways involved.

**Methods:** We have designed an experimental system based on the coculture of human primary endometrial fibroblasts and of trophoblastic villi obtained from early elective abortions (3 to 5 weeks). The trophoblastic villi produce extravillous cytotrophoblast ex vivo and their migration is followed over periods of 24 to 48 hours by time-lapse microscopy.

**Results:** The migration of trophoblast cells within the endometrial fibroblast layer is regulated by TGF-β as shown by treating the trophoblast-endometrium coculture with either recombinant TGFβs (TGFβ1, TGFβ2 or TGFβ3) or TGFβ neutralizing antibodies. Furthermore, the role of the TGFβ-dependent Rho/ROCK signaling pathway is found essential for the inhibition of invasion as shown by using the ROCK chemical inhibitor Y-27632.

**Conclusions:** We have set up an ex vivo model system that allows the study of trophoblastic invasion and of its regulation by TGFβ and identified the Rho/ROCK signaling pathway as essential for this regulation.
**P18.01**

**AGONIST CONSTRICION OF HUMAN PLACENTAL ARTERIES INVOLVES ROK-DEPENDENT MYOFILAMENT SENSITISATION TO CA^{2+}**

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**Objectives** Simultaneous measures of intracellular Ca^{2+} and contraction in human placental arteries indicate that thromboxane receptor stimulation evokes greater constriction, with less Ca^{2+} elevation, than high K^+ depolarising stimulation. This suggests agonist-mediated Ca^{2+} sensitisation of myofilaments. In other vascular beds, this is at least partly due to activation of rho-associated kinase (ROK). Thus, we used a ROK inhibitor, Y27632 (10 μM), to investigate the involvement of this pathway in agonist constriction of human placental arteries.

**Methods** Wire myography was performed on chorionic plate arteries isolated from normal term placentas (with LREC approval). In some experiments, arteries were permeabilised to the bath solution with a-toxin to retain receptor-coupled signalling. Arteries were stimulated with maximum or sub-maximal doses of the thromboxane-mimetic, U46619. At plateau constriction, Y27632 was applied.

**Results** In intact vessels, maximal (1μM) U46619 constrictions were reduced to 76±5% of control by Y27632. Similarily, Y27632 reduced EC_{50} dose of U46619 constrictions to 62±6% of control. In permeabilised arteries sub-maximally constricted with pCa7.6 GTP solution, Y27632 reduced 1μM U46619 constrictions to 62±7% of control.

**Conclusion** A prominent ROK-dependent Ca^{2+}-sensitisation accompanies thromboxane constriction of human placental arteries. Additional, as yet unidentified, pathways are also implicated in this response.

**P18.03**

**PLACENTA GROWTH FACTOR IS NOT ESSENTIAL FOR SPIRAL ARTERY MODIFICATION IN FEMALE MICE**

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**Objectives**: Placenta Growth Factor (PGF) is a vascular endothelial growth factor (VEGF) family member expressed in human trophoblasts and uterine Natural Killer (uNK) cells. Low blood or urinary PGF has been associated with pre-eclampsia. PGF is not well characterized in mouse implant sites and not available in these sites in PGE^{2} mice (Nat Med 2001;7:575).

**Methods**: PGF message was quantified by real time PCR in mesometrium from non pregnant and gestation day (gd) 6-18 C57B1/6 (B6) and alymphoid (RAG-2^{-/-} /γc^{-/-}) mice and in lectin- purified B6 uNK cells. Message was localized by in situ hybridization in gd 6-12 B6 implant sites. Implant sites from PGF^{-/-} mice and their congenic controls were studied morphometrically.

**Results**: PGF was transcribed by B6 and alymphoid mice at equal, constant low rates in virgin and gd 6-12 mesometrium. Transcription elevated between gd 12-18. PGF expression was not detected in uNK cells but was localized to decidual and trophoblast cells. The most distinctive histological feature in implant sites of PGF^{-/-} mice that bore large litters (13-15 pups), was a great elevation in binucleated uNK cells. Spiral artery wall to lumen ratios indicated that arterial modification had occurred by gd10.

**Conclusion**: These data suggest that murine uNK cells are not a major source of PGF and that PGF is unlikely to support murine spiral artery modification. In mice, PGF is more likely to have functional importance late in gestation. Supported by NSERC, OMAFRA and the Canada Research Chairs Program.

**P18.02**

**THE EFFECT OF BETAMETHASONE ON UMBILICAL ARTERY DOPPLER STUDIES IN IUGR PREGNANCIES**

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**Objectives**: Maternal administration of betamethasone in pregnancies complicated by absent end-diastolic flow in the umbilical artery (UA AEDF) is associated with a transient return of end-diastolic flow in the majority of pregnancies. In this largest cohort reported we have defined the frequency of this glucocorticoid effect and asked whether the response, or lack thereof, to steroids is predictive of outcome.

**Methods**: A prospective study of women with a singleton pregnancy complicated by UA AEDF receiving antenatal betamethasone treatment.

**Results**: Outcome data were available for 84 women with UA AEDF for whom Doppler studies were recorded before and after glucocorticoids. Of these, 52 (62%, 95% CI 52-72) had a transient return of flow following betamethasone, 32 (38%, 95% CI 28-48) did not. The median duration of a return in end-diastolic flow was 3 days. There were no differences in mean±SD gestation at diagnosis (196±18 vs 192±18 days) or delivery (205±17 vs 200±16 days) or birthweight (897±727 vs 861±327g) between those with and those without a return of flow but responding women were less likely to have had a pre-existing medical condition (diabetes, SLE etc) than non-responders (21% vs 41%). Overall, there were 35 female infants and 49 male.

**Conclusions**: Betamethasone administration is associated with a transient return of UA EDF in pregnancies with UA AEDF. The response to betamethasone is not of prognostic utility.

**P18.04**

**SOLUBLE HLA-G1 INDUCES ENDOTHELIAL APOPTOSIS: A ROLE IN SPIRAL ARTERY REMODELLING?**

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**Objectives**: Uterine spiral artery remodelling involves trophoblast-induced endothelial apoptosis. Extravillous trophoblasts produce soluble HLA-G1. We have investigated the hypothesis that soluble HLA-G1 is an important regulator of endothelial apoptosis. Endothelial cells (SGHEC-7, a HUVEC-derived cell line) were incubated with conditioned media from cells secreting sHLA-G1, control media or recombinant sHLA-G1 and apoptosis was monitored by time-lapse microscopy and western blot analysis of apoptotic markers.

**Results**: Conditioned media and recombinant soluble HLA-G1 induced apoptotic morphological changes in endothelial cells with the % apoptotic cells after 90h increasing to 40.6% in the presence of sHLA-G1 (100ng/ml, n=8, p=0.001). Apoptosis was confirmed using the broad spectrum caspase inhibitor zVAD-fmk which abrogated sHLA-G1 induced cell death. In addition, western blot analysis showed that sHLA-G1 treated endothelial cells had increased production of the apoptotic marker cleaved PARP. Use of an antibody which blocks FasL interactions partially blocked the sHLA-G1-induced endothelial apoptosis with the effect decreasing to 24.9±4.3, p=0.02.

**Conclusions**: We have shown that sHLA-G1 induces endothelial apoptosis and that this effect may be partly through the Fas/FasL system. Soluble HLA-G1 may therefore have a role in the induction of uterine spiral artery remodelling seen in early pregnancy.
At the end of the first trimester of human pregnancy uterine spiral arteries transform from narrow, high resistance vessels to low resistance vessels. Termed physiological change, this process is essential to allow for the increased blood flow required by the growing fetus. While the mechanisms by which vessels remodel have not been elucidated, histological evidence shows loss of endothelial and smooth muscle cells (SMC) which are replaced by fetal-derived trophoblasts. Defects in remodelling may underlie the development of complications of pregnancy such as pre-eclampsia and intrauterine growth restriction.

Objective: The aim of this study was to determine whether primary trophoblast can cause SMC apoptosis.

Methods and Results: Culture of human aortic SMC with primary first trimester human cytotrophoblast (CTB) caused apoptosis of SMC, as assessed by time lapse microscopy and immunoblotting for cleaved PARP, an apoptotic marker. Furthermore, CTB-induced SMC apoptosis was inhibited by caspase inhibition with zVAD-fmk, indicating apoptosis via activation of caspase 3. A Fas activating antibody induced apoptosis of SMC monolayers in a concentration-dependent manner. Importantly, a Fas ligand blocking antibody significantly inhibited primary CTB-induced SMC apoptosis.

Conclusions: The data demonstrate that trophoblast can initiate SMC apoptosis via activation of the Fas/Fas ligand pathway. We propose that this mechanism is a critical component contributing to vessel remodelling and the associated loss of SMC observed in uterine spiral arteries during pregnancy.

During pregnancy, extravillous trophoblasts invade and remodel spiral arteries to create high flow, low resistance vessels. We have obtained evidence indicating that a soluble factor released by trophoblast initiates a cell death programme in vascular smooth muscle.

Objective: To identify the death signal.

Methods: Human arterial SMC were incubated with primary cytotrophoblast (CTB)-conditioned culture medium, in the presence or absence of the anti-Fas ligand (FasL) antibody NOK-2. Annexin V labelling and flow cytometry were used to measure apoptosis. Human umbilical and spiral arteries were denuded of endothelium and perfused with primary CTB-conditioned culture medium, in the presence or absence of NOK-2. Vessels were frozen and cryosectioned, and SMC apoptosis assessed by TUNEL.

Results: Levels of SMC apoptosis in vitro were increased 5-fold after 1h (P<0.05) and 6-fold after 24h (P<0.05) exposure to CTB-conditioned culture medium. This effect was blocked by NOK-2 but not by a control IgG. Furthermore, NOK-2 pretreated CTB-conditioned medium induced less vascular SMC apoptosis in spiral and umbilical arteries than CTB-conditioned medium pretreated with a control IgG.

Conclusions: Loss of SMC during arterial remodelling in pregnancy involves cell death triggered by soluble FasL released locally by invading CTB.
REACTIVE OXYGEN SPECIES ALTER VASCULAR RESPONSES OF CHORIONIC PLATE SMALL ARTERIES
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Objectives Pre-eclampsia (PE) and intrauterine growth restriction (IUGR) are associated with raised fetoplacental vascular resistance, the cause of which is unknown. Reactive oxygen species (ROS) are implicated in vascular disease and are raised in PE/IUGR. We hypothesise that ROS (superoxide) alter the vascular responses of chorionic plate arteries.

Methods Placentas (n=8) were collected following normal pregnancies. Chorionic plate arteries were prepared for wire myography. Paired vessels were incubated with xanthine (XA: 10^{-4}M) plus xanthine oxidase (XO: 10^{-5}U/ml), to generate superoxide, or with vehicle diluent throughout the experiment. After 10 min, the contractile response of the arteries to U46619 (thromboxane A2 mimetic; 10^{-10}–10^{-6}M) was determined. Endothelium-independent relaxation was assessed in pre-constricted vessels (U46619 EC_{50}) using sodium nitroprusside (SNP, nitric oxide donor, 10^{-9}–10^{-4}M).

Results XA/XO increased passive tension (2.9±0.7 vs controls 0.4±0.2; kPa mean±SE, p<0.05 t-test). XA/XO did not alter the maximum tension induced by U46619 (10.0±1.2 vs 8.4±0.8 kPa control) but shifted the dose-response curve to the left (p<0.01; two-way ANOVA). SNP-induced vasodilatation was enhanced by XA/XO (p<0.05, two-way ANOVA vs time control).

Conclusion ROS generated by XA/XO increase chorionic plate artery basal tone and alter the responses to agonists. We propose that ROS contribute to abnormal fetoplacental vascular resistance in PE/IUGR.

CHANGES IN COMPOSITION OF THE MYOMETRIAL PART OF THE PLACENTAL BED IN ANAEMIA AND IUGR WITH PREECLAMPSIA.
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Objectives: During pregnancy, extravillous trophoblast invades maternal uterine tissues finally reaching spiral arteries. In view of the recent finding of arteriovenous shunts bypassing the placenta (Schaaps et al. AJOG 2005), we analysed the tissue composition of the myometrial part of the placental bed in controls, anaemia and IUGR with pre-eclampsia.

Methods: Full-thickness sections of placental bed from controls, cases with chronic anaemia and with IUGR and preeclampsia were double-stained using anti-smooth muscle actin and anti-CD34 (QBend-10). The fractional areas occupied by smooth muscle, connective tissue, and vessels (lumens + walls) were estimated using randomly-selected microscopic fields of myometrial parts of placental beds.

Results: The fractional area of smooth muscle tissue was significantly higher in controls compared to pathological cases. In IUGR and preeclampsia, there tended to be relatively more connective tissue and vessels compared to control cases. In anaemia, there tended to be relatively less connective tissue but significantly more vessels.

Conclusions: In anaemia, increased vessel tissues compensate for oxygen deprivation. In IUGR with preeclampsia, muscle is replaced by connective tissue and vessels. It needs to be elucidated whether vessels directly approach the placenta (hypothesised for anaemia) or bypass it (hypothesised for IUGR with preeclampsia).