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Opening Keynote Plenary Session (K1, NIH-SI)

[K1]
FETAL AND NEONATAL PULMONARY CIRCULATION IN THE *ALTO ANDINO*

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Pregnancy at high altitude in women and animals cause pulmonary arterial hypertension (PAHT) in the newborns (1). Nevertheless neonatal llamas in the *altiplano* did not have PAHT compared to lowland newborn llamas. We found that carbon monoxide (CO) is an endogenous gas produced by hemoxygenase that plays a key role as vasodilator agent in the llama, with lesser action in the sheep (2). In contrast, basal NO role in the pulmonary circulation in the high altitude llama was negligible, but important in the neonatal sheep pulmonary circulation, that in spite of the high NO tone had PAHT. We postulated that hemin, the oxidized form of heme and hemoxygenase inductor, played a role through CO in the regulation of the pulmonary circulation in high altitude (HA) newborn lambs. The Hemin Group showed basally a decrease in the basal PAP compare to the Control Group. The pulmonary vascular resistance (PVR) in the Hemin Group did not show any change during a superimposed episode of hypoxia, whereas the Control Group showed a significant increase during the hypoxemic period (3). These results may be due to augmented enzymatic activity of sGC and/or to lesser vascular remodeling in the pulmonary artery in the hemin treated neonates. We determined the sGC protein expression and the histology of the small pulmonary arteries. We found that Hemin Group had increased sGC protein expression and lesser smooth muscle layer in the small pulmonary arteries, both findings consistent with the decrease in PAP in the Hemin treated Group. Furthermore, we found augmented CO production and increased protein expression of HO-1, HO-2, BKCa and PKG1 in the lungs of Hemin Group, also consistent with lower PAP than controls (4). In conclusion, hemin could be a good agent to decrease pulmonary arterial hypertension in the neonate.
Supported by FONDECYT 1090355, 1080663-Chile.
(1) Herrera EA et al. Am. J. Physiol. 292:R2234-R2240, 2007.
(2) Herrera EA et al. Cardiovasc Res. 77:197-201, 2008.
(3) Ebensperger G. et al. Fetal and Neonatal Physiological Society Annual Meeting, 2009.
(4) Ebensperger G. et al. VIII World Congress on High Altitude Medicine and Physiology, 2010.

[NIH - SI]
EXPERIMENTAL SELECTION OF HYPOXIA-TOLERANT DROSOPHILA MELANOGASTER: MAJOR ROLE FOR NOTCH ACTIVATION

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Through long-term laboratory selection (over 200 generations), we have generated a Drosophila melanogaster strain that tolerates severe, normally lethal, level of hypoxia. Because of initial experiments suspecting genetic mechanisms underlying this adaptation, we compared the genomes of the hypoxia-selected flies with those of controls using deep re-sequencing. By applying novel computing and analytical methods we identified a number of DNA regions under selection, mostly on the X-chromosome. Several of the hypoxia-selected regions contained genes encoding or regulating the Notch pathway. In addition, expression profiling revealed an activation of the Notch pathway in the hypoxia-selected flies. We confirmed the contribution of Notch activation to hypoxia tolerance using a specific γ-secretase inhibitor, DAPT, which significantly reduced adult survival and lifespan in the hypoxia-selected flies. We also demonstrated that flies with loss-of-function Notch mutations or RNAi-mediated Notch knockdown had a significant reduction in hypoxia tolerance, but those with a gain-of-function had a dramatic opposite effect. Using the UAS-Gal4 system, we also showed that specific over-expression of the Notch intracellular domain in glial cells was critical for conferring hypoxia tolerance in flies. Novel analytical tools, genetic and bioinformatic strategies allowed us to discover that Notch activation plays a major and unsuspected role in this hypoxia tolerance in Drosophila melanogaster.

Plenary Session 1 - Hypoxia (PL1 - PL2)

[PL1]
OXYGEN EFFECTS, TROPHOBLAST BIOLOGY, AND CONTROVERSY: I THINK I AM SUFFOCATING!

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Oxygen is a necessity for life yet is toxic to cells when dysregulated. The oxygen tension in villous tissues until 10-12 weeks' gestation is $\leq$ 15 mm Hg, but the pO 2 at $\geq$ 13 weeks' gestation rises to 40-80 mm Hg and stays in this range throughout the second and third trimesters. Maldevelopment of the spiral arterioles in the first trimester predisposes to placental dysfunction and sub-optimal pregnancy outcomes in the second half of pregnancy. Controversy is intense when investigators propose functional roles for the anatomical maldevelopment of the basal plate and the oxygen concentrations that are normal and abnormal in the placenta in the latter parts of gestation. Placental hypoxia in the second half of pregnancy, ischemia with re-oxygenation injury, mechanical damage due to high flow, or a combination of these phenomena are offered as contributory to placental injury characteristic of placentas from women with pregnancy disorders.
We consider variables that influence the conduct of experiments *in vitro* when the aim is to mimic *in vivo* pathophysiology of the placenta. Most hotly debated is the question of what oxygen percentage is reflective of normal and abnormal oxygen concentrations that occur in vivo? We discuss this controversy and then focus on our studies that show cultured villous trophoblasts express phenotype related differences in their response to insults generally, and hypoxia specifically. We conclude that all *in vitro* models offer some advantages, and some disadvantages, when used to evaluate the effects of insults on trophoblast function. We recommend that investigators who do the actual experiments should breathe 20% oxygen, not other concentrations. Otherwise, they, too, will fall prey and suffocate with the controversy about what oxygen level is ideal and how oxygen affects trophoblast biology and placental function.
(Supported by NIH 29190)
Keywords: trophoblast, oxygen, hypoxia

[PL2]
THE EFFECTS OF OXYGEN ON NORMAL AND ABNORMAL PLACENTAL TISSUE - INSIGHTS FROM METABOLOMICS

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Placental dysfunction is central to many complications of human pregnancy including preeclampsia (PE), fetal growth restriction and stillbirth. The precise nature of placental dysfunction in these conditions in not known, although oxidative and nitrative stress are implicated. Metabolomics can detect and identify endogenous and secreted metabolites in vivo and in vitro. The metabolome describes the complete quantitative collection of small molecular weight compounds which participate in metabolism including amino acids, sugars, organic acids and lipids. Study of the metabolome is advantageous as it represents the downstream product of changes in transcription, translation and provides an accurate snapshot of the biological phenotype.
We hypothesised that a metabolomic strategy could provide a reproducible technique to measure endogenous and secreted metabolites and identify differences in placental cultures from pregnancies complicated by PE and subjected to variations in oxygen tension. We used both gas and liquid chromatography prior to mass spectrometry to separate and detect 1000s of metabolites. Univariate statistical analysis and Principal Components Analysis were used.
We applied an established placental explant culture model to study the "metabolic footprint" (changes in secreted metabolites in conditioned culture medium) and the intra-tissue metabolome. Our early studies confirmed that experimental protocols need to be tightly controlled, including culture medium content. The intra-assay variability was acceptable 6.1-11.6%. We demonstrated changes in the tissue metabolome and metabolic footprint in response to altered oxygen tension. We also demonstrated that explants from normal tissue cultured in 1% oxygen (hypoxia) had similarities to explants from PE cultured at 6% oxygen (normoxia). The metabolite classes included lipid metabolism, glutamate and glutamine, tryptophan metabolism and leukotriene or prostaglandin metabolism. Metabolomics has the potential to identify changes in conditions, such as PE, that are associated with placental pathology. The work complements biomarker studies by providing pathophysiological understanding, via biochemical studies, of complex disease phenotypes.
Keywords: Oxygen, Metabolism, Metabolomics, Placental Explants

Plenary Session 2 - Immunology in the Placenta (PL3 - PL5)

[PL3]
FETAL ANTIGENS: IDENTITY, ORIGINS, AND INFLUENCES ON THE MATERNAL IMMUNE SYSTEM

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Hemochorial placentation represents an unusual physiological situation in which there exists a vast interface between the mother and her genetically disparate fetus. Together with this physical intimacy, the rapid rate of turnover of the chorionic villous trophoblast in the human placenta via shedding of apoptotic nuclei, microparticles and exosomes, results in the release of high amounts of fetal products into the maternal blood stream. Recent observations also reveal that the decidua of pregnancy is heavily populated with lymphatic vessels. Together, these properties of the placenta suggest at least two routes of trafficking from the maternal-fetal interface to secondary lymphoid organs draining the maternal blood and uterus. Our laboratory has adopted a murine model in which a defined fetal antigen can be shown to access the central and peripheral lymphoid organs released from the placenta-blood and/or trophoblast-decidua interface. The result of this interaction appears to be induction of tolerance to fetal antigen-specific CD4+ T cells, as evidenced by swift upregulation of activation and inhibitory cell-surface proteins, as well as induction of fetal antigen-specific regulatory T cells. In contrast, we found surprisingly limited evidence of tolerance induction in CD8+ T cells – a result that was correlated with reduced fetal viability in these animals. Investigations of subcellular localization of defined fetal antigens in human trophoblast cells, fetal macrophages and fetal cord blood leukocytes provides the proof of concept that fetal antigens originate from multiple sources that could access the maternal lymphoid system via trophoblastic deportation, microparticle and exosome release, and microchimerism.
Keywords: T Cells, Maternal-fetal interface, Tolerance, Placenta

[PL4]
HCG, REGULATORY T CELLS, AND PREECLAMPSIA

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Pre-eclampsia (PE) is a devastating pregnancy-associated complication, diagnosed by hypertension, proteinurea, and edema. Dysregulation in hormonal activity, angiogenesis and immunity is considered to contribute to the onset of PE. Our recent results suggest that hCG is a potent angiogenic and immuno-modulatory factor. It is then possible that its dysregulation may contribute to the onset of preeclampsia. The objective of our study was to establish a serum-based causal link between non-functional hCG and PE using *in vivo* and *in vitro* models. hCG levels in serum from normal and PE patients were evaluated. Glycosylation of hCG present in serum from normal and PE patients was determined by a sandwich-ELISA. *In vitro* functional assays included serum-based MAPK signalling and angiogenic cross-talk between the endothelial cells and trophoblasts. Pregnant wild type and IL-10-/- mice were injected (gd 10, i.p) with either normal pregnancy serum (NPS) or PE serum (PES) with or without recombinant hCG. On gd 16/17, blood pressure and pregnancy outcome were recorded. Urinary albumin, creatinine, serum sFlt-1, sEng were measured and renal pathology was monitored. On gd 12/13, splenic and uterine Treg cells (CD4+CD25 +FoxP3+) cells were monitored by FACS. Dysregulation of hCG signalling in PES was demonstrated by defective ERK phosphorylation and disruption in the endovascular cross-talk between trophoblasts and endothelial cells which was reversed with rhCG. We observed higher levels of hCG in PES. Glycosylation pattern showed excessive presence of Sialyl-Lewis a (SLea), Sialyl-Lewis X (SLeX) and Lewis Y (LeY) on PES hCG. *In vivo* treatment with PES resulted in significant reduction in Treg cells at the maternal-fetal interface accompanied by IUGR, hypertension, proteinuria and renal pathology, typical features of preeclampsia. This was reversed by rhCG. Our results will be discussed in the context of establishment of *in vivo* and *in vitro* models of preeclampsia and reversal of pathology by rhCG.
Keywords: hCG, immunity, angiogenesis, proteinuric hypertension

[PL5]

ACTIVATION OF INNATE IMMUNE TYPE CYTOTOTOXIC RESPONSES OF UTERINE-NATURAL KILLER CELL IN THE FETAL-MATERNAL INTERFACE

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Human CD56brightCD16dim and mouse CD3-CD122+ NK1.1-DX5- natural killer (NK) cells are pregnancy specific subset of cytokine producing NK cell that modulate positively the homeostasis of uterine environment for successful pregnancy. On the other hand, this uterine-NK (uNK) cell produce cytotoxic mediators like IFNγ/TNFα pro-inflammatory cytokines and contain perforin/granzymes in the granules for potential innate immune type response. Since its ability to trigger cytotoxic activity affecting the pregnancy is still controversial, our laboratory have evaluated experimentally, the capability of uNK cells response to stress induced in the maternal fetal interface of pregnant mice. The surgical disruption of developing embryo *in loco*, induces increasing of vascular permeability at the mesometrial endometrium as soon as 10 min, where the uNK cells show loss of perforin and proteoglycan contents from the secretory compartment and later (30-60min) the cathepsin D contents from lysosome compartment of granules suggesting releasing of cytolytic mediators. The gene expression profile in this maternal-fetal interface also show increasing of pro-inflammatory IFNγ and TNFα cytokines after 60 min, but not anti-inflammatory IL-6 or Fas/FasL pathway. After 6h of embryo damage, most of the cells at the maternal-fetal interface, including trophoblast cells becomes TUNEL positive. By utltrastructural analysis, the uNK cell granules are heterogeneous and loosing of their contents is not uniform and is time-dependent evidencing the occurrence of uNK functional subsets. Similar effects are seen in the uNK cells of pseudopregnat mice uterus locally injected with amniotic fluid and what component is capable to induce direct or indirectly the quick response of uNK cells is one of challenge to understand the mechanism of cytotoxic activation. Taken all together, uNK cell is capable to trigger its cytotoxic response very fast and seems to work like a sentinel and effector cell responsive to changes of uterine environment homeostasis during pregnancy.

Keywords: uNK cell, cytotoxic, innate immune response, reproductive immunology

Plenary Session 3 - Vascular Imaging and Angiogenesis in the Placenta (PL6 - PL9)

[PL6]

PLACENTA MACROVASCULAR AND MICROVASCULAR ENDOTHELIAL DYSFUNCTION IN DISEASES OF PREGNANCY

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Human endothelial dysfunction is a common feature in diseases of pregnancy such as gestational diabetes (GD), pre-eclampsia (PE) and intrauterine growth restriction (IUGR). Metabolic changes include altered synthesis of nitric oxide (NO), and altered membrane transport of L-arginine and adenosine in primary cultures of human umbilical vein (HUVEC, macrovascular) and placenta microvillous (hPMEC, microvascular) endothelial cells. These alterations are associated with modifications in the expression and activity of endothelial (eNOS) and inducible (iNOS) NO synthases, respectively, an effect maintained up to passage 5 in culture. Expression and activity of the human cationic amino acid transporter 1 (hCAT-1) and equilibrative nucleoside transporters 1 (hENT1) and hENT2, as well as the corresponding *SLC7A1*, *SLC29A1* and *SLC29A2* gene promoter activities, is exhibited by these cell types. Altered gene expression results from increased NO level, PKC, MAPK, hCHOP-C/EBPα and Sp1 transcription factors activation. Reduced ENTs-mediated adenosine transport in GD and PE is associated with stimulation of L-arginine/NO pathway. In addition, hENT2 activity seems to intend to restore the reduced adenosine transport in GD and PE. It is suggested that a common functional characteristic leading to changes in the bioavailability of adenosine is evidenced by human fetal micro and macrovascular endothelium in GD, PE and IUGR.

Supported by CONICYT ACT-73 (PIA), FONDECYT 1070865 & 1080534, Chile.

[PL7]

THE ARTERIAL FETOPLACENTAL AND UTEROPLACENTAL VASCULATURE: IMAGING REVEALS GESTATIONAL AND STRAIN DEPENDENT CHANGES IN MICE

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Objective: The determinants of vascular branching in the placenta are poorly understood but likely depend on genetic, hemodynamic, and environmental factors. Genetically distinct mouse strains may allow for genetic dissection of branching morphogenesis regulation. The aim of the present study was to quantify and compare placental vascular growth in two of the most common mouse strains, C57Bl/6J (B6) and CD-1.
Methods: B6 and CD1 fetoplacental arterial trees from were infused with X-ray contrast agent at embryonic day (E)13.5, 15.5 and 17.5. Uteroplacental arterial trees were perfused at E17.5 ($N = 7$-9 /group), and 3-D micro-CT images were obtained. Uteroplacental datasets were manually segmented to obtain the volumes of specific vascular compartments. Automated vascular segmentation software and flow calculations were used to analyze the fetoplacental trees.
Results: Despite similar late gestational placental weights, fetoplacental arterial vascular volume increased by 71% in CD1 mice and 154% in B6 mice from E13.5 to E17.5 ($p<0.01$). Both strains also increased the depth of their fetoplacental tree suggesting an increase in labyrinthine thickness. Surprisingly the number of small arteriole sized (50-100 μm) vessels did not increase in late gestation in either strain. However B6 increased the number of large vessel segments (>100 μm) by 80% ($p<0.01$) so that by E17.5 B6 had 90% more large vessels than CD1. Arterial vascular resistance decreased by 55% in B6 mice from E13.5-17.5 ($p<0.05$) but was unchanged in CD1 mice. In contrast, the volume of uteroplacental spiral arteries and maternal canals at E17.5 was double in CD-1 mice as compared to B6.
Conclusions: Quantification of the placental vasculature using micro-CT image analysis revealed significant strain-dependent differences in late gestational vascular expansion. These results suggest a significant role for genetics in determining fetoplacental and uteroplacental vascularity.
Funding: Supported by the Heart and Stroke Foundation of Ontario
Keywords: micro-computed tomography, fetoplacental vasculature, uteroplacental vasculature, mouse

[PL8]

ANGIOGENIC AND VASODILATORY PLACENTAL NETWORK

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The angiogenic and invasive properties of the trophoblast are critical to establish the placenta, and to transform the uterine arteries into large bore non-reactive vessels. More recently, priming factors that facilitate invasion by vasodilation have started to be identified.
VEGF is considered the paradigmatic angiogenic factor, but its hyperpermeabilizing and vasodilator effects have been overlooked. VEGF, through its receptors Flt-1 and KDR activates eNOS, via phosphatidilinositol 3-kinase and phospholipase Cγ1 respectively. Bradykinin, a vasodilator, angiogenetic and hyperpermeabilizing peptide, by activation of the B2R receptor through Ca 2+ mediated mechanisms is also a potent stimulus of eNOS, and enhances the effect of VEGF; additionally the B2R transactivates the KDR. Finally, NO also integrates this vasodilator, angiogenic and hyperpermeabilizing network. Different groups have shown in humans immunoreactive expression of VEGF, Flt-1, KDR, B2R and eNOS in villous cyto and syncytiotrophoblasts, fetal capillaries, and in extravillous trophoblasts; moreover the B2R is expressed additionally in intraarterial trophoblasts.
Using the model of the pregnant guinea-pig – the best non-primate species to study trophoblast invasion – we have provided the first demonstration that VEGF, Flt-1, KDR, B2R and eNOS, are expressed in labirynthine syncytiotrophoblast, interlobar trophoblasts, fetal endothelium, subplacenta, invasive, periarterial, intramural and intraarterial trophoblasts, cells that have human morphological and functional correlates.
We postulate that the coincident expression of these functionally interrelated factors in the placenta and in the invasive trophoblast supports an angiogenic and vasodilator network that participates in placental development and in the different stages of the invasive pathway from the anchoring villi/subplacenta to the vascular lumen. In vivo studies need to be done to dissect the individual importance of the VEGF receptors, bradykinin and NO.
Studies in the guinea-pig were supported by grants 1050707-1080228 (Fondecyt, Chilean Agency of Science and Technology, Chile).
Keywords: angiogenesis, vasodilatation, placenta, guinea-pig

[PL9]
FETO-PLACENTAL VASCULARIZATION: A MULTIFACETED APPROACH

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The introduction of Doppler ultrasound technology allowed for the first time the in vivo study of normal and abnormal human feto-placental hemodynamics. Doppler flow velocity waveforms (FVW's) obtained from the umbilical arteries (UA) give informations on downstream blood flow resistance. Pulsatility Index (PI), that quantifies FVW's, decreases throughout normal pregnancy, indicating decreasing blood flow resistance. This finding has been related to previous knowledge about villous tree development. Considering fetal cardiovascular physiology (i.e. increasing perfusion pressure, increasing cross sectional area of UA, the viscous and capacitive characteristics of the vascular bed) and that the villous vessel network develops by dichotomous branching, it could be demonstrated by mathematical models that PI values reflect vascular villous development. PI values are high during the first 15 – 20 weeks of pregnancy, when the villous tree is mainly composed by mesenchimal and intermediate immature villi. They start to decrease around 23 weeks of gestation, when the placental villous tree develops in mature, stem and terminal villi.
It was likewise observed that Fetal Growth Restriction (FGR) is characterized by higher PI values compared to age-matched controls, with the highest values related to the most severe fetal outcomes. The next step was to understand which are the abnormalities of the villous tree underlying the abnormal FVW's. A number of studies using different approaches (animal, in vitro and mathematical models, morphometrical, morphological and immunohistochemical assays) have shown that the worst FVW's patterns are related to a highly abnormal vascular villous tree. Such anomalies are expected to affect placental perfusion, that can be directly monitored using 3D-4D power Doppler sonographic indexes. Indeed these indexes are significantly lower in pregnancies complicated by FGR with abnormal UA FVW's.
The associated study of placental angiogenic and antiangiogenic factors in these cases contributes to the understanding of the pathogenesis of the condition, opening perspectives to innovative therapeutic interventions, like controlling placental hypoxia by local delivery of oxygenated biocompatible nanobubbles.

Plenary Session 4 - SLIMP: Ion Channels in the Placenta (PL10 - PL13)

[PL10]
PLACENTAL MEMBRANE AND IONIC CHANNELS: EXPRESSION, ELECTROPHYSIOLOGY AND LIPID RAFTS (ABSTRACT PLENARY SESSION ON ION CHANNELS IN THE PLACENTA)

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Human placental syncytiotrophoblast (hSTB) is an epithelium responsible for materno-fetal exchange. As in other transport epithelia, ions play multiple roles in human placental syncytiotrophoblast, being transported from mother to fetus and regulating diverse cellular processes. We have been interested in the characteristics of the functional expression of ions channels from membrane fractions of hSTB. Since many functions of the membranes are based on membrane dynamics and structure–function correlation, the knowledge of the characteristics of the ionic channels and its relationship with the domain, subdomain and microdomain of hSTB membranes may play a role mediating different intracellular mechanisms in the placental epithelial barrier that could directly relate to the materno-fetal exchange. Methods: Highly purified membranes were obtained through a double protocol of apical and basal membrane isolation which includes differential centrifugations and basal membrane precipitation with MgCl2. Using differential sucrose density migration we have obtained basal membrane (BM) and described two isolated fractions from the apical membrane: a classical fraction (MVM) and a light fraction (LMVM) where MVM might correspond to the finger-like region and L-MVM could correspond to the basal region of the microvillous. Purified membranes were used in the reconstitution into giant liposomes or their transplantation into Xenopus oocyte membranes followed by electrophysiological recordings. Western blots using ion channel antibodies were performed on apical and basal purified membrane fractions to support our functional findings. We also reported the expression of functional microdomains (lipid rafts) in apical membranes that were studied using detergent resistant membranes (DRMs) and cholesterol sensitive depletion. RESULTS: Our laboratory has worked on the characterization of several chloride and cationic channels in the apical and basal membrane from term human placentas. We also have reported the expression of two functional microdomains (lipid rafts) in both membranes (LMVM and MVM) and the importance of the cytoskeletal participation in their different composition. CONCLUSION: Our results have contributed to the information about ions channels present in the membranes from hSTB and their implications in the physiology of this epithelium in normal and pathological pregnancies. Supported by FONDECYT 1070695 (Chile).
Keywords: ions channels, Human syncytiotrophoblast, membrane, lipid rafts

[PL11]
REGULATION BY CALCIUM OF SYNCYTIOTROPHOBLAST POLYCYSTIN-2 (TRPP2) AND HETERO-COMPLEXES WITH TRPC1

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Introduction: Transient receptor potential (TRP) channels contribute to important sensory functions. Polycystin-2 (TRPP2, PC2) is a large conductance, non-selective cation channel, which permeates Ca2+. PC2 is abundantly expressed in term human syncytiotrophoblast (hST) and is postulated as a principal contributor to Ca2+ delivery from mother to foetus. TRPC1 is a store- and receptor-operated channel, which is also implicated in Ca2+ entry. Both are co-expressed in the primary cilium of renal epithelial cells. Recent studies demonstrated that PC2 and TRPC1 assemble to form functional hetero-complexes with distinct functional properties. The PC2/TRPC1 complexes show a single-channel conductance, ionic permeability and pH dependence, which are distinct from those of either PC2 or TRPC1 alone. Little is known however, about Ca2+ regulation of either TRP channel complex. In this study we review regulatory mechanisms of channel function and assessed the response to Ca2+ of either complex.
Methods: *In vitro* translated PC2 and TRPC1, hST PC2 and purified TRPC1/PC2 hetero-complexes, were assessed for their response to Ca2+. Channels were reconstituted in a lipid bilayer system exposed to a K+ chemical gradient (150/15 mM). The Ca2+ chelating agents BAPTA and EGTA were added to the cytosolic side to a final Ca2+ concentration of 0.3 nM.
Results: The hST PC2 channel activity was inhibited by 70-100% in low cytosolic Ca2+. Proteins extrinsic to the channel mediated this inhibitory effect. Similar experiments conducted with TRPC1, showed Ca2+ sensitivity with a bell shaped Boltzmann's open probability in response to Ca2+. Reducing cytosolic Ca2+ did not change either the I-V curve or the open probability of the PC2/TRPC1 hetero-complex.
Discussion: The interaction between TRPC1 and PC2 in the hetero-complex may confer different biophysical and physiological properties that the observed in the homo-tetramers. Thus, Ca2+ is transported by, and regulates the function of TRP channel activity present in the human placenta.
Keywords: Calcium transport, TRP channels, Human syncytiotrophoblast, Lipid bilayer reconstitution

[PL12]
POTASSIUM CHANNELS AND THE HUMAN PLACENTA

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Potassium (K) channels are fundamentally important for normal cellular function. This is highlighted by diseases directly linked to aberrant K channel function. In the transporting epithelia of renal tubules, inactivating mutations in inwardly rectifying K (KIR) 1.1 channels have been linked to Bartter's syndrome (1). Similarly, in cardiac myocytes numerous "loss of function" mutations in voltage-gated K (KV) 7.1 channels are linked to the long QT syndrome (2). The lack of experimental focus on placental K channels, in particular with regard to regulation of nutrient transport and blood flow, may relate to the fact that as yet no pregnancy complications have been directly attributable to a specific K "channelopathy".
K channel activity is central to epithelial transport processes; therefore it is no surprise that K channels are expressed by syncytiotrophoblast. However our understanding of their role/s in syncytiotrophoblast renewal and / or solute transport is limited.
K channels are also essential for vascular smooth muscle and endothelial cell function. In the lung, K channels participate in the detection of local oxygenation and the concerted response of vascular tissues to hypoxia. KV1.5 channels may be particularly important; restoration of channel expression in a rat hypoxia model restored function (3). Similar roles for K channels have been proposed in placental vascular tissues (4); K channels are expressed in fetoplacental vasculature (4, 5) but their functional significance remains to be elucidated.
Here we summarise current understanding of K channel expression and activity in fetoplacental vasculature in normal and complicated pregnancies.
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Keywords: Potassium Channels, Vasculature, Human

[PL13]
WATER CHANNEL PROTEINS IN THE HUMAN PLACENTA

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In many tissues, water channel proteins known as aquaporins (AQPs) have been implicated in transmembrane water transport. Previous research suggested that water movement across human syncytiotrophoblast (hST) should take place by a lipid diffusion pathway. However, in 2001 we reported that aquaporin-9 (AQP9) and 3 (AQP3) are highly expressed in hST from normal placenta. However, up to now, the physiological function(s) and the regulation of human placental AQPs remain unknown. We also reported that AQP9 protein expression was increased in preeclamptic placentas while AQP3 was decreased. However, we could not relate the higher AQP9 expression with its functionality for the transport of water and mannitol.
Thus, we were focused on establishing the mechanisms that may modulate AQP3 and AQP9 expression and functionality.
In placental explants cultured under hypoxia conditions we demonstrated that changes in oxygen tension may be responsible for the dysregulation of both proteins. In addition, insulin treatment decreased AQP9 but had no effect on AQP3 expression.
On the other hand, we studied hST membranes lipid composition and observed that the apical membrane fluidity was reduced in preeclamptic placentas. Furthermore, recently we found that CFTR expression was almost undetectable in preeclamptic placentas and failed to regulate AQPs activity suggesting that CFTR may be involved in the regulation of trans-cellular water flux in human placentas.
In conclusion, our finding suggested that in preeclamptic placentas insulin was not effective to down-regulate AQP9 expression, although AQP9 gen has a negative insulin-response-element, and the intermittent hypoxia contribute synergistically to its up-regulation. In contrast, AQP3 decrease may be only caused by changes in oxygen tension. Our results also proposed that the unfavorable lipid environment and the reduced expression of CFTR may be affecting AQPs functionality. However, whether the alterations of AQPs could play a role in the development of preeclampsia is still unknown.
Keywords: aquaporins, syncytiotrophoblast, human placenta, preeclampsia

Plenary Session 5 - New Regulatory Mechanisms (PL14 - PL16)

[PL14]
GENETIC AND EPIGENETIC VARIATION IN HUMAN PLACENTAS ASSOCIATED WITH NORMAL OR ADVERSE PREGNANCY OUTCOMES

WP Robinson*, Luana Avila, Ryan Yuen, Dan Diego Alvarez, Maria Pena-herrera, Peter von Dadelzsen et al, [1]University of British Columbia, Canada, [2]Child & Family Research Inst., Canada

Background: Abnormal placental growth and function may be influenced by genetic errors (e.g. trisomy) as well as epigenetic changes, such as those involving altered DNA methylation. However, before an association between DNA methylation and placental pathology can be investigated, normal intra-placental variation and the effects of sample location, local cell composition, gestational age, as well as mode of delivery and sample processing needs to be understood.
Methods: To further our understanding of normal epigenetic variability detailed sampling was obtained from 14 normal term placenta. Sites exhibiting variable methylation (KISS1, PTPN6, CASP8, *APC*, *AR*, and LINE1) were quantified by pyrosequencing. Expression of genes specific to a subset of placental cells (*CDH1*, *CDH11*, *ID2*, *PLAC1* and *KISS1*) were evaluated by real-time PCR. In addition, 2-3samples from each from over 130 normal and IUGR/preeclampsia associated placentae were obtained for evaluation of genetic (trisomy) and methylation changes (targeted and by array-based approaches).
Results: Processing time (0-24 hours) had a dramatic effect on mRNA level for most genes; while methylation tended to remain relatively constant over this same time period. Considerable intra-placental variability was observed that did not correlated with site location or depth. The patterns of site-to-site correlation in expression and methylation from unrelated genes suggest that some variability reflects chance cell-composition differences between samples. Only a subset (up to 10% in the IUGR group) of placentas show evidence of chromosomal trisomy, however, methylation changes can also be identified—adding new knowledge to the changes associated with poor placentation.
Conclusion: Sites that are differentially methylated in different placental cell types (e.g. KISS1 or PRPN6) can be used to infer cell composition associated with abnormal placentation, while methylation differences that are not influenced by cell type may be more informative for understanding the cellular/physiological changes between normal and abnormal placentae.
Keywords: Genetics, DNA methylation, Epigenetics

[PL15]
FUNCTIONAL PHYLOGENOMIC DATA ELUCIDATE CONSERVED AND DERIVED ASPECTS OF PLACENTA BIOLOGY

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The placenta is a complex organ, and the regulatory mechanisms that result in its development are diverse. Moreover, it is the first organ to be differentiated during development, and its anatomy varies extensively across the clade of eutherian mammals. Phylogenetic studies have demonstrated that the human hemochorial placenta is the ancestral type among placental mammals. Comparative genomic analyses provide evidence for ancient adaptive evolution (i.e. $dN/dS>1$) during the emergence of the eutherian placenta in genes that are highly expressed in placentas of extant mammals. Independent confirmation that these genes are crucial in placentation comes from knockout mice models that produce abnormal placental phenotypes and/or result in embryonic or perinatal lethality. Despite the great degree of conserved adaptations across many eutherian placenta expressed genes, there is also evidence for lineage specific adaptations. In addition to possessing genes with elevated rates of evolution, humans have expanded gene families with placenta specific expression including galectins, gonadotropins and growth hormones. New data from comparative placenta transcriptome studies highlight both evolutionarily conserved and derived patterns of gene expression in placentas from diverse mammalian species with a wide range of anatomical variation. Taken together, these studies contribute to a comprehensive view of the evolution of the placenta as well as identifying novel candidate genes for unravelling the regulation of placenta development, function, and structure.
Keywords: transcriptome, evolution, gene gain and loss, phylogeny

[PL16]
PLACENTA-SPECIFIC MICRORNAS (MIRNAS) DERIVED FROM THE CHROMOSOME 19 MIRNA CLUSTER IN NORMAL PREGNANCY AND PREECLAMPSIA

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MicroRNAs (miRNAs) are single-stranded non-coding RNAs of approximately 22 nucleotides in length, and accumulating evidence indicates that they are involved in a variety of patho-physiological processes. Furthermore, miRNAs have been investigated for the development of clinical diagnostic and prognostic tools. We performed small RNA library sequencing using human placental tissues, which led to the identification of placenta-specific miRNAs (e.g., *MIR517A*) linked to the chromosome 19 miRNA cluster (C19MC). The miRNA cluster genes were differentially expressed in placental development. Subsequent analyses by real-time PCR and *in situ* hybridization revealed that villous trophoblasts expressed placenta-specific miRNAs. We also analyzed small RNA libraries from the blood plasma and showed that the placenta-specific miRNAs were abundant in the plasma of pregnant women. We also demonstrated the rapid clearance of the placenta-specific miRNAs from the plasma after delivery, indicating that placenta-specific miRNAs enter into maternal circulation. By using the trophoblast cell line BeWo in culture, we demonstrated that miRNAs were extracellularly released via exosomes. Taken together, our findings suggest that miRNAs are exported from the human placental syncytiotrophoblast into maternal circulation. Next, we determined differential expression of miRNAs in placentas from normal and preeclampsia (PE) pregnancy quantitatively using a real-time PCR-based miRNA array system. We identified 44 miRNAs upregulated significantly in placentas from PE pregnancy, 10 of which were placenta-specific miRNAs in C19MC. Further, the *in silico* target genes of some PE-related miRNAs were closely related to placenta functions.
Collectively, our study suggests that some miRNAs including placenta-specific miRNAs possibly regulates the expression of genes involved in placenta functions and also that some miRNAs are dysregulated in PE placentas. Since PE increases maternal and infant mortality and morbidity, early detection of PE is one of the most important issues in Perinatology. These miRNAs could serve as promising biomarkers for the diagnosis of PE.

Plenary Session 6 - Lipids and the Placenta (PL17 - PL19)

[PL17]
LIPID METABOLISM IN THE DIABETIC PLACENTA

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In maternal diabetes, the placenta is exposed to alterations in maternal lipid metabolism, and is involved in the alterations in the quantity and quality of the lipids that reach the fetal compartment. The excess of lipids is present in the mother, the placenta and the fetus. Fetal lipid accretion is related to the increased placental transport of glucose and lipids, and to alterations in several placental lipid metabolic pathways. As a result of the pro-inflammatory environment there is an increase in lipid peroxidation in intrauterine tissues that leads to a loss of essential fatty acids (EFAs), which are highly susceptible to peroxidation. EFAs have important functions as signaling molecules in feto-placental development and metabolism. They are ligands of the nuclear receptors PPARs, and substrates for the synthesis of bioactive lipids such as prostacyclin, 15deoxyΔ12,14prostaglandinJ2 and leukotriene B4, respective ligands of PPARδ, PPARγ and PPARα.
Results: By studying experimental models of diabetes and pregnancy, we found that activation of the three PPAR isotypes regulates the synthesis and catabolism of placental and fetal lipids. Their activation also induces anti-inflammatory pathways in the placenta and the fetus. Altered concentrations of PPARs and their endogenous ligands are found in placentas and fetuses from diabetic rats. Dietary activation of PPARs in maternal diabetes regulates lipid metabolic pathways and reduces matrix metalloproteinases overactivity in the placenta, while it prevents lipid accretion and reduces lipid peroxidation in the fetuses.
In conclusion, in maternal diabetes, the lipid overaccumulation and impaired formation of bioactive lipids that activate PPARs challenge the metabolism and function of the placenta as well as the development and metabolism of the fetuses. Modulation of the quality and quantity of dietary lipids may help to regulate feto-placental lipid metabolism, pro-inflammation and impaired development in diabetic gestations.
Keywords: Diabetes, pregnancy, lipid, placenta

[PL18]
THE UPTAKE AND TRANSPORT OF MATERNAL LIPIDS TO THE FETUS

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The fetus has a high demand for lipids. Triglycerides and/or fatty acids are used for energy and as membrane substrates. Cholesterol is used for membrane substrates, as precursors for various signaling moieties, ie LXR, and as mediators of membrane function, ie lipid rafts are cholesterol-rich. Lipids can be derived from the various lipoproteins circulating in maternal plasma. LDL and HDL are excellent sources of cholesterol. VLDL and chylomicrons (CM) are sources of both cholesterol and triglycerides and circulating levels can be readily manipulated. This is important information assuming the amount of lipid presented to the fetus can impact upon fetal growth rates. Optimal growth is important since overgrowth as well as undergrowth can lead to age-related diseases. Growth can be manipulated directly as a source of energy and membranes for the fetus itself and can be indirectly involved by affecting placental metabolism, ie placental signalling as a result of altered membrane composition leading to a change in uptake and transport of other growth-promoting nutrients.
Lipoproteins are taken up by lipoprotein receptors that are expressed on the apical side of trophoblasts. Trophoblasts express a number of lipoprotein receptors that allow for uptake of all lipoproteins. Proteins are also expressed that can mediate uptake of free fatty acids from the circulation. Triglycerides and cholesteryl esters are hydrolyzed to fatty acids and cholesterol which are utilized by cells or transported across the cells to the basolateral side where the lipid moities are effluxed or secreted as free lipids, as simple complexes with proteins, or as lipoproteins.

[PL19]
THE MOLECULAR FUNCTIONS OF PPARGAMMA IN THE PLACENTA

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The nuclear receptor Peroxisome Proliferators-Activated Receptor gamma (PPARg) is essential for placental development. To gain better understanding of PPARg function in the placenta, we screened for PPARg target genes by integrating expression profiles of *Pparg*-null and *Rxra*-null placentas, *Pparg* hemizygous placentas, trophoblast stem cells (TSC) treated with a PPARg agonist, and *Pparg*-null TSC. Significant overlaps between profiles pinpointed plausible PPARg target genes. Microarray analyses of placentas deficient for the transcriptional coactivator AIB3 revealed that it has a ubiquitous role in the expression of putative PPARg targets. Diverse spatial and temporal expression patterns of candidate PPARg targets demonstrated that PPARg regulates genes at multiple stages of trophoblast differentiation and in diverse combinations of trophoblast lineages. A novel cohort of targets that encode interlinked metabolic enzymes offers fresh insights into essential roles of PPARg in placental energy metabolism. Our data provide new leads into the placental functions of PPARg and advance the basic understanding of placental development and metabolism in general.
Keywords: PPARgamma, placenta, trophoblast, microarrays

New Investigator Oral Session 1 (N1 - N6)

[N1]
ANTENATAL GLUCOCORTICOID TREATMENT DOWN-REGULATES SYSTEM A TRANSPORT IN THE MURINE PLACENTA

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Introduction: Synthetic glucocorticoids (sGCs), which are administered to women threatened with preterm labour, differentially regulate the system A amino acid transporter *in vitro*, but there is no comparable data *in vivo*. Since ~30% of women who receive sGCs carry to term, our objective was to examine the short and long-term consequences of antenatal sGCs on placental system A transport using a murine model. We hypothesized that sGC treatment would differentially regulate placental system A transport based on timing from sGC exposure.
Methods: System A transplacental transport (measured using specific substrate 14C-methylamino-isobuytyric acid) was characterized in pregnant C57BL/6 mice in mid-late gestation on embryonic day (E) 12.5, E15.5 and E18.5 (n=6; term~E19.5). Secondly, pregnant dams were treated with either saline or dexamethasone (DEX; 0.1mg/kg) on E13.5 and E14.5 to assess short-term (E15.5) and long-term (E17.5, E18.5) consequences of sGC treatment on system A activity (n=5-8). Changes in passive permeability (14C-mannitol; n=3) were examined. We also determined whether these effects were sex-specific.
Results: System A transport increased from E12.5 to E18.5 ($p<0.01$). DEX treatment had no short-term effect at E15.5 or E17.5, but resulted in a significant decrease in system A transport prior to term at E18.5 ($p<0.05$), in both sexes. DEX did not affect passive permeability of the placenta or fetal weight. Female fetuses appear more susceptible to reduced placental growth, as DEX reduced female placental weight at E18.5 ($p<0.05$).
Discussion: System A transport dramatically increased over the second half of gestation, consistent with increased fetal growth. There are no short-term effects of mid-gestation sGC treatment, however a substantial reduction in system A mediated transport occurred when gestation persists to term. As such, prenatal use of sGC therapy may lead to a reduction in availability of neutral amino acids and alter fetal development.
Funding: Canadian Institutes for Health Research.
Keywords: System A, Synthetic Glucocorticoids, Amino acid transport

[N2]
HIGH DENSITY LIPOPROTEIN (HDL) REGULATES PLACENTAL VASCULAR TONE INDEPENDENT OF ENDOTHELIAL NITRIC OXIDE SYNTHASE (ENOS)

Manuela Augsten[1], Gerald Wölkart[2], Kurt Schmidt[2], Bernd Mayer[2], Gernot Desoye[1], Christian Wadsack*[1] et al, [1]Department of Obstetrics and Gynaecology, Medical University of Graz, Austria, [2]Department of Pharmacology and Toxicology, Karl-Franzens University of Graz, Austria

Introduction: Blood flow in the human feto-placental circulation is regulated by a variety of vasoactive molecules. NO, enzymatically produced by eNOS, is a potent endothelium-derived vasodilator. eNOS activation can be promoted by HDL through its binding to the scavenger receptor class B type 1 (SR-BI). Recently, we demonstrated SR-BI and eNOS expression on placental endothelial cells and HDL binding to SR-BI. Here we hypothesized that HDL could be a potent eNOS/NO mediated vasodilator in the feto-placental vascular system.
Methods: Immunoblotting was performed to detect eNOS phosphorylation after placental endothelial cells were treated with fetal HDL. eNOS activity was measured by the conversion of L-argingine to L-citrulline. Vasoactive *ex vivo* capacity of HDL was investigated by wire myography. The vessels were precontracted with the thromboxan mimetic U46619, HDL was supplied and the vasoactivity was measured. Different inhibitors of eNOS (L-LAME), cyclooxygenases (COX) (Diclofenac, Indomethacin) and of SR-BI receptor (BLT-1) were added to identify activated resistance mechanisms.
Results: Neither phosphorylation nor activation of the eNOS [HDL (1.50±0.4%) vs control (1.65±0.8%); n=4] were induced by HDL. However, HDL showed a significant endothelial dependent dilatation of placental arteries (-75±6%) and veins (-77±4%) after precontraction in wire myography. This vasoactivity could not be blocked neither by eNOS nor by COX inhibitors, whereas BLT-1 totally inhibited HDL-mediated vasodilatation in placental vessels.
Conclusion: HDL is a potent SR-BI-mediated vasodilator in the feto-placental circulation. This is independent of eNOS activation and of the cyclooxygenase-prostacyclin cascade. We speculate that the HDL-mediated vasodilatation involves the endothelium-derived hyperpolarizing factor (EDHF), which would represent a novel regulator of feto-placental vasotonus, but this needs further investigation. (Austrian Jubilee Fund 12601 to GD and 13533 to CW)
Keywords: High denisty lipoprotein (HDL), vasodilatation, Scavenger receptor class B typ-1, endothelium derived hyperpolarizing factor (EDHF)

[N3]

PLACENTAL VASCULATURE REMODELING BY ADENOVIRAL-MEDIATED PLACENTAL GENE THERAPY OF INSULIN GROWTH FACTOR (AD-HIGF-1) IN A MOUSE MODEL OF PLACENTAL INSUFFICIENCY (PI)

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Introduction: Recent work in our lab showed that Ad- hIGF-1 restores fetal weight in rat, rabbit and mouse models of PI. The mechanism of action of Ad-hIGF-1 is unknown. We hypothesize that Ad-hIGF-1 restores fetal weight in a murine PI mice model through remodeling of the placental vasculature.

Methods: Time mated pregnant C57 Black /6J mice were divided into 4 groups :1-Sham operated control 2- PI induced by mesenteric uterine artery ligation (MUAL)3-MUAL with Ad-Lacz $1x10^8$ pfu 4-MUAL with Ad-IGF-I $1x10^8$ pfu. At gestational day 18, through laparotomy MUAL was performed and treatment was administered. Cesarean delivery was done on day 20. Fetal and placental weights recorded. Morphometric analysis of labyrinth depth and vascular density was performed following immuno-histochemical staining with endothelial specific MECA 32. Expression of Vascular Endothelial Growth Factor-A (VEGF-A), Angiopoietin-1 & 2 (Ang-1 & 2) and soluble FMS-related Tyrosine Kinase (sFLT) was assessed by qPCR and IHC.

Results: Placental labyrinth depth in MUAL group(679.7 ± 57.9 um) ($n=3$) was significantly reduced by 23% ($p<0.05$) and 26%in comparison with sham-operated(887.3 ± 65.3 um, $n=5$) and MUAL with Ad-IGF-1(913 ± 50.1 um, $n=2$) respectively. Similarly, a significant reduction in fetal vessel counts was recorded by 28 % (37 ± 1.5 vs. 50 ± 1.9, $p<0.05$) and 31%(37 ± 1.5 vs. 54 ± 1.6) in the ligated group as compared to the sham-operated and MUAL with AD-IGF-1 respectively. No changes were seen in the RNA expression levels of any of the angiogenic factors among the 4 groups. IHC showed increased Ang-2 expression in the MUAL group as compared to others. In contrast, both sFLT and VEGF-A expression were increased in the MUAL with Ad-HIGF-1 group.

Conclusion: Placental vascular remodeling may be one of the mechanisms by which placental gene transfer of IGF-1 restore fetal weight in a surgical mouse model of PI.

Keywords: Placental insufficiency, gene therapy, angiogensis

[N4]

THE EFFECT OF LOW SHEAR STRESS ON TROPHOBLAST-INDUCED ENDOTHELIAL CELL APOPTOSIS INVOLVED IN SPIRAL ARTERY REMODELLING

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Background: In the first trimester of pregnancy trophoblast migrate along uterine spiral arteries (SA) and replace endothelial cells (EC) lining these vessels by mechanisms that may involve EC apoptosis. Inadequacies in SA remodelling have been associated with pre-eclampsia and IUGR. Until 10-12 weeks of gestation trophoblast plug SA, preventing maternal blood flow into the intervillous space, resulting in slow, high resistance flow in these vessels. This work aimed to determine the effect of shear stress on trophoblast-induced EC apoptosis.

Methods: Cells were cultured using the BioFlux200 system. SGHPL-4, Jar, primary extravillous trophoblast (EVTs) and HUVECs were cultured for 12-30hrs under 0.02-7dyne/cm shear stress. Apoptosis was quantified by time-lapse microscopy.

Results: 1) Shear stresses from 0.02-3dyne/cm did not induce SGHPL-4, Jar or HUVEC apoptosis. **2)** TNFα/Actinomycin-D induced significantly less apoptosis in trophoblast cultured in 3dyne/cm than in 0.5dyne/cm cultures ($p<0.05$). This protective effect was seen to a small extent in HUVECs cultured at 3dyne/cm, but was not significant. **3)** Jars and EVTs cultured on HUVEC monolayers at 0.5 or 3dyne/cm significantly induced apoptosis in directly adjacent HUVECs, in comparison to HUVECs >2 cells away or HUVEC only controls ($p<0.05$), with a progressive decrease in the amount of apoptosis induced from 0.5 to 3 to 5dyne/cm cultures ($p<0.05$). However, Jars failed to significantly induce HUVEC apoptosis in 5 or 7dyne/cm cultures. Blocking antibodies to Fas-ligand inhibited the ability of Jars to induce apoptosis in adjacent HUVECs.

Conclusions: Trophoblast demonstrate a survival advantage over HUVEC at low levels of shear stress (3dyne/cm). Jars and EVTs are able to induce HUVEC apoptosis in this model by Fas/Fas-ligand interactions in a similar manner to trophoblast *in vivo*, and this process is inhibited by increasing shear stress. Therefore, low shear stresses in plugged first trimester SA may aid trophoblast-induced EC apoptosis involved in SA remodelling.

Keywords: Shear Stress, Endothelial cell apoptosis, Trophoblast, Spiral artery remodelling

[N5]

RHOA/ROCK SIGNALLING PATHWAY IS NOT IMPLICATED IN ENOS INACTIVATION BY HYPOXIA IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Regulation of blow flow to the placenta depends on structural changes and vasoactive factors, mainly to nitric oxide (NO). NO derives from the metabolism of L-arginine to NO and L-citrulline by the NO synthase (NOS). Reduced endothelial NOS (eNOS) activity has been implicated in vascular dysfunction, and a competition over the L-arginine availability has been described between eNOS and arginases (ARG). Due to its role in vascular dysfunction, the RhoA/ROCK signaling pathway could be implicated in the regulation of eNOS expression and/or activity, through its phosphorylation on serine^{1177} (i.e., activated eNOS, p^{1177}~eNOS) or threonine^{495} (i.e., inactivated eNOS, p^{495}~eNOS) and/or arginase II (ARGII). We studied the role of the RhoA/ROCK pathway on eNOS and ARG expression in human umbilical endothelium (HUVEC) under hypoxia.

Methods: HUVEC primary cultures were exposed to normoxia (5% O_2) or hypoxia (2% O_2) for 0-24 hours. Cells were exposed to *S*-(2-boronoethyl)-L-cysteine (BEC, arginase inhibitor, 100 μM) or fasudil (ROCK inhibitor, 10 μM). RhoA, phosphorylated ERM (p~ERM, index of activated RhoA/ROCK signalling pathway), ARGII, total eNOS, p^{Ser1177}~eNOS and p^{Thr495}~eNOS were determined by western blot.

Results: Higher p^{Thr495}~eNOS paralleled by reduced p^{Ser1177}~eNOS, with no significant changes in total eNOS was observed in hypoxia (24 hours), an effect unaltered by fasudil or BEC. In hypoxia, total eNOS protein abundance did not change with fasudil. No changes were observed in RhoA under hypoxia or in the presence of BEC. Hypoxia induced ARGII expression, an effect unaltered by fasudil. RhoA and pERM were neither modified in hypoxia nor with fasudil.

Conclusions: ARGII over-expression, together with reduced p^{Ser1177}~eNOS and increased p^{Thr495}~eNOS could explain the endothelial dysfunction exhibited by HUVEC exposed to hypoxia, a phenomenon that seems independent on the RhoA/ROCK signalling pathway.

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Keywords: eNOS, Arginase, Hypoxia, RhoA/ROCK pathways

[N6]

CHANGES IN DNA METHYLATION PATTERNS THROUGHOUT HUMAN PREGNANCY: IMPLICATIONS FOR EVOLUTION, FUNCTION AND BIOMARKER DISCOVERY

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DNA methylation is the most commonly studied epigenetic modification, and is involved in the control of gene expression and maintenance of chromosome stability. DNA methylation marks are potentially reversible, and can be altered by environmental factors, such as diet, drug/alcohol abuse, and stress. Recent studies searching for biomarkers of fetal and placental disease have identified DNA methylation differences between placenta and maternal blood; however most of these studies have used term placenta methylation as a proxy of first trimester methylation status. This is potentially flawed, because a recent preliminary study, identified differences in placental DNA methylation between $<$34 and $>$34 weeks.[1] We hypothesise that the profile of DNA methylation within the placenta varies over time in association with the developmental stage of pregnancy. Further we speculate that environmental changes throughout gestation influence this process and are associated with the adaption of the developing pregnancy to the in utero environment. The aim of the current study was to analyse changes in DNA methylation on a genome-scale level between three gestational ages, 8 weeks, 12 weeks and term.

Genome-scale DNA methylation analysis was performed using the Illumina Infinium HumanMethylation27 platform, which analyses 27,578 CpG sites covering 14,595 genes.

Our analysis revealed a large number of CpG sites that change significantly throughout placental development (650 probes showed absolute differences of 20% between first trimester and term). We found very few differences between 8 and 12 weeks, suggesting a relative robustness in DNA methylation during this period. We also found evidence of greater variation between placentas from different individuals at term, potentially as a response to environmental influences throughout gestation. Our study has revealed a suite of first trimester-specific placental DNA methylation markers with potential utility for development of novel diagnostic tests for placenta-associated diseases of pregnancy.

[1] Yuen et al., EJHG, 2010

Keywords: Epigenetics, DNA methylation, Gestation, first trimester

New Investigator Oral Session 2 (N7 - N12)

[N7]

DECIDUAL HOXA10 REGULATES TROPHOBLAST MIGRATION *IN VITRO*

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The initiation of pregnancy requires coordinated differentiation of the maternal endometrial stroma in to the decidua and the embryonic trophoblast tissue. Several factors secreted by decidual cells and trophoblast cells make an ideal micro-environment for the initial attachment of blastocyst to the receptive endometrium. Based on several *in vitro* experiments it has been shown that the decidua regulates trophoblast migration/invasion and this regulation is necessary to dictate the degree of placentation. However, the genetic players that regulate this differentiation have not been identified. Previous studies have identified the Homeobox gene HOXA10 as an important factor involved in decidualization. Mice knockout for HOXA10, or human decidual cells knocked down for HOXA10 have defective decidualization underscoring the importance of this factor. Since decidua regulates trophoblast migration and as HOXA10 is required for decidual response, we hypothesized that loss of HOXA10 in the decidual cells must affect trophoblast migration.

To investigate in to this hypothesis, we knocked down HOXA10 expression in the decidual cells by siRNA and used the supernatants in an *in vitro* assay to determine trophoblast invasion. The trophoblastic cell lines JEG3, ACH-3P and HTR-8SV/neo were employed for the study. Invasion was assessed by Matrigel invasion assay, changes in gene expression of invasion related genes viz Integrin subunits alpha 5,6, v, Matrix metalloprotease (MMP) 2, 3 and 9 and Tissue Inhibitor of MMPs (TIMPs) 1, 2 and 3 was assessed by realtime PCR and phosphorylation of STAT3 in trophoblast cell lines was studied by western blotting.

The results revealed that as compared to control medium, in absence of decidual HOXA10, the invasion of all the three cell lines increased by two fold. At the molecular level, this increase in invasion was associated altered expression of alpha 5 and alpha v, MMP2, 3 & 9 and TIMP 1,2,3, and activation of STAT3.

These observations for the first time demonstrate that HOXA10 in the decidua regulates trophoblast invasion. At the molecular level, this ability is derived by the virtue of HOXA10 to regulate decidual secretome which influences integrins, MMP and TIMP transcription in the trophoblastcells.

Keywords: Trophoblast Invasion, HOXA10, Decidua, Endometrium

[N8]

SYNCYTIAL NUCLEAR AGGREGATES ARE NOT UNIVERSALLY APOPTOTIC IN NATURE AND ARE CLOSELY ASSOCIATED WITH CYTOKERATIN, β-ACTIN AND β-TUBULIN

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Introduction: Syncytial nuclear aggregates (SNA) are clusters of nuclei found in the syncytiotrophoblast of the human placenta. The origin and fate of SNAs is unknown and contrasting theories exist. SNAs may have a role in disease as their number is increased in common pregnancy pathologies. We hypothesise that SNAs are formed by cytoskeletal protein interactions with nuclei, and that SNAs are not exclusively apoptotic.

Methods: Fresh placental villous tissue was fixed and wax-embedded (n=7). Apoptosis was assessed on single sections by staining for M30 and TUNEL. α-tubulin, β-tubulin, β-actin, γ-actin, cytokeratin-7, dynein intermediate-chain-1 and kinesin 5B were localised using immunofluorescence. An archive of electron micrographs was reviewed to investigate nuclear morphology and surrounding cytoskeletal proteins.

Results: At term, 20% to 26.5% of SNAs contained apoptotic material as tested by TUNEL and M30 respectively (Figure 1). Immunofluorescence showed cytokeratin-7 in syncytiotrophoblast associated with SNAs. β-tubulin and β-actin were found throughout the trophoblast and underlying some SNAs. α-tubulin and γ-actin were observed throughout the trophoblast but were not associated with SNAs. Dynein intermediate chain 1 was found sparsely at localised regions in the trophoblast but was not associated with SNAs. Transmission electron microscopy confirmed that intermediate filament arrays often surround SNAs. It also showed various morphologically distinct stages of apoptosis in nuclei within the same SNA.

Discussion: Only a minority of SNAs contain apoptotic nuclei, suggesting they may serve different functions, rather than just the extrusion of senescent nuclei as has previously been suggested. Cytoskeletal proteins, and especially cytokeratin, were found within and surrounding SNAs and may have a role in forming or maintaining these nuclear clusters. Further research is required to determine how cytoskeletal proteins are involved in SNA formation, and how apoptosis is activated in specific SNA nuclei.

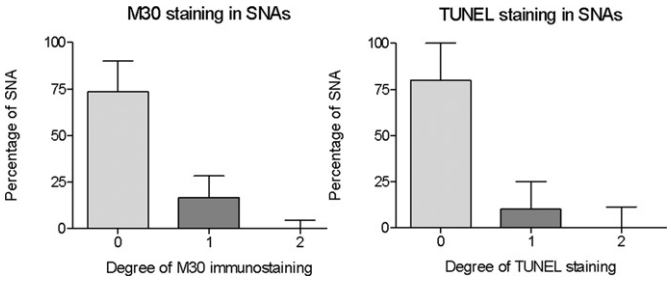

Figure 1. Graph showing proportion of SNAs with nuclei showing signs of apoptotic cell death shown by M30 immunostaining and TUNEL respectively. Grade 0 = no staining, Grade 1 = weak staining in one nucleus, Grade 2 = strong staining in one or more nuclei.

Keywords: syncytiotrophoblast, syncytial nuclear aggregates, apoptosis, cytoskeleton

[N9]

CERAMIDE, ACID SPHINGOMYELINASE AND CERAMIDASE EXERT DIFFERENTIAL EFFECTS ON TROPHOBLAST DIFFERENTIATION AND FUSION

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Introduction: Although trophoblast syncytialisation has been actively investigated for decades, various contradicting theories describing the fundamental nature of trophoblast differentiation and fusion exist. A realisation that the biochemical and morphological features of trophoblast differentiation can be dissociated has surfaced in recent findings. We have previously demonstrated a novel role of Ceramide, and sphingolipid synthesis/metabolic enzymes in trophoblast differentiation. To clarify the role of sphingolipid metabolism in the syncytialisation process, we have further dissected the distinct roles of Ceramide in differentiation and fusion, and the signalling pathways involved in this process.
Methods: Cultured cytotrophoblasts, isolated from term human placentas were allowed to differentiate over 7 days in culture. Trophoblast differentiation was assessed by measuring hCG secretion, placental alkaline phosphatase (PLAP) activity and E-cadherin expression and immunostaining.
Results: Exposure of trophoblasts for 72 hours to short chain Ceramide, acid sphingomyelinase (aSMase) and a ceramidase inhibitor (B13) enhanced hCG secretion, indicating a role for Ceramide in differentiation but not fusion. No changes were detected in PLAP activity. Syncytialised trophoblasts expressed high levels of ceramidase, suggesting that this enzyme may be involved in maintaining the syncytial phenotype. Consistent with this view, B13 treatment reduced fusion as shown by enhanced E-cadherin expression. Inhibition of the Ceramide-responsive pathways JNK-II and PP2A did not abolish the effects of Ceramide, and in fact increased hCG production while having divergent effects on fusion (PLAP and E-cadherin).
Discussion: This study highlights the complexity of the pathways that regulate trophoblast differentiation and fusion (syncytialisation). Different agents (e.g. Ceramide) can regulate differentiation independently of effects on fusion, while regulators of fusion can have no or even opposite effects on differentiation. Pregnancy complications such as preeclampsia, where a moderate increase in syncytialisation is observed with no effect on differentiation, may be the end result of perturbations in one or more of these pathways.
Keywords: Differentiation, Fusion, Ceramide, trophoblast

[N10]

INVOLVEMENT OF THE TRANSCRIPTION FACTOR KLF6 IN HUMAN TROPHOBLAST DIFFERENTIATION

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Krüppel-like factor 6 (KLF6) is a ubiquitous zinc finger transcription factor involved in differentiation, cell cycle control and proliferation in several cell systems. In human and mice placenta, KLF6 is highly expressed and *klf6*-/- knockout mice exhibit impaired placental development. An increase in KLF6 expression during morphological and biochemical differentiation of cytotrophoblasts (CTB) and Jeg3 cells, was previously demonstrated. Here in, we postulate that KLF6 is an important regulator of genes implicated in trophoblast differentiation. Initially, we investigated whether KLF6 regulates the promoter activity of pregnancy-specific glycoprotein (PSG) genes, which are early markers of trophoblast differentiation. Transfection assays in trophoblast cells demonstrate that KLF6 transactivates PSG3 and PSG5 gene promoter constructs in a dose-dependent manner, an activation enhanced when cells are differentiated. Moreover, the KLF6 carboxy-terminal zinc finger DNA-binding domain is required for transactivation, and gel shift assays indicate that KLF6 specifically interacts with the PSG promoter region. Furthermore, qRT-PCR assays reveal that KLF6 overexpression induces endogenous mRNA levels of not only PSG3 but also human chorionic gonadotrophin β-subunit and the glial cell missing 1 (GCM1) trophoblast specific transcription factor, both strongly involved in trophoblast differentiation. Interestingly, the PSG promoter is activated by GCM1 and by KLF6 suggesting that cooperation between both transcription factors is, at least in part, responsible for the markedly increase in PSG transcript levels observed in CTB differentiation. To conclude, these results provide further evidence for a role of KLF6 as a transcriptional regulator of genes in trophoblastic cells and suggest that it might be involved in villous CTB cell differentiation., Supported by CONICET, FONCyT, MinCyT of Córdoba and SECyT-UNC
Keywords: transcriptional regulation, trophoblast cells, differentiation

[N11]
CYTOMEGALOVIRUS IS HARBOURED BY THE PLACENTA THROUGH REVERSIBLE BINDING TO HEPARAN SULPHATE PROTEOGLYCANS

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Introduction: Human Cytomegalovirus (HCMV) is the most common cause of congenital infection in newborns. One mechanism for viral transmission is across the syncytiotrophoblast (ST) layer of the placenta. The ST is extensively microvilliated, presents a large surface area to maternal blood and has high levels of heparan sulphate proteoglycans (HSPGs) to which HCMV initially binds. We hypothesized that HCMV is protected by reversibly binding to ST on HSPGs, specifically Syndecan-1 (Syn-1). This leads to accumulation of HCMV in the placenta and increases the risk of fetal transmission by virtue of its localization.
Methods: In a clinical based pilot study, viral load was quantified by q-PCR in placentas, maternal blood and urine. Cultured ST were challenged with HCMV, or HCMV treated with Heparin, at a multiplicity of infection (MOI) = 10 for 4 hours, extensively washed and then further incubated. Supernatants and cell lysates were then assayed for infectious virus. The expression of Syn-1 was also assessed in cultured ST in the presence or absence of HCMV.
Results: Over 50 patient samples have been assessed to date (maternal blood, urine and placental tissue); 4 out of 51 samples have tested positive for HCMV in placental tissue only. In vitro studies showed that reversible binding was reduced when HCMV was pre-treated with heparin. Cell-surface Syn-1 expression decreased as HCMV MOI increased when compared to unchallenged controls.
Discussion: Our results indicate that HCMV is harboured preferentially in the placenta and may be protected from inactivation through reversible binding to HSPGs. The perseverance of HCMV allows high viral levels to accumulate in and around the ST thereby increasing the potential for transmission to the fetus. Conversely, the cell-surface reduction in Syn-1 expression as the amount of virus inoculum increases suggests protective cellular mechanisms reduce potential viral receptor expression in the presence of infectious virus.
Keywords: Human cytomegalovirus, Binding, Fetal, Maternal

[N12]
EXPRESSION OF CHEMOKINE (C-C MOTIF) LIGAND 25 AND ITS RECEPTOR *CCR9* DURING EMBRYO IMPLANTATION IN MOUSE

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Background: Interactions between the blastocyst/trophoblast and uterine tissues during embryo implantation are partly mediated by cytokines and chemokines. CC' and CXC' terminal chemokines are known to be involved with blastocyst polarity with the uterus, leukocyte recruitment and capture/coordination of leukocyte migration. In particular, the chemokine *Ccl25* is highly expressed in the thymus, in periosteum progenitor cells and mesenchymal stem cells (inducing gene activation, cell polarity, reorganization of membranes and cytoskeleton and migration) and also in the mouse post implantation trophoblast.
Objectives: The aim of this study is to investigate whether *Ccl25* and its receptor *Ccr9* are expressed during embryo implantation in mice.
Methodology: *Ccl25* and *Ccr9* transcript abundance was measured by quantitative Real-Time PCR and normalized to YWHAZ expression in blastocysts flushed from uterine horns at gestation days (gd) 3.5, 4.5 and 5.5 (n=30).
Preliminary results: Expression of both chemokine and receptor was not detected in the pre-implantation blastocyst on gd3.5. Expression of *Ccl25* mRNA increased after the onset of implantation on gd4.5 and 5.5, being maximal on gd4.5 followed by a corresponding increase in *Ccr9* on gd4.5 ($p<0.05$, Mann-Whitney test).
Discussion: The expression of *Ccl25* and its receptor during the adhesion, attachment and initial process of invasion of the blastocyst into the uterine epithelium suggests an autocrine role for this chemokine during mouse implantation and adds a new putative element to our understanding of trophoblast-endometrium interactions.
Financial support: FAPESP, CNPq and CAPES
Keywords: Embryo Implantation, Blastocyst, Chemokine, Real-Time PCR

Early Career Researcher Plenary (ECR1 - ECR2)

[ECR1]
A STRATEGY FOR INVESTIGATING HEMOCHORIAL PLACENTATION

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Hemochorial placentation is a strategy involving modification of the maternal-foetal interface for the purpose of facilitating nutrient and waste exchange and ensuring development of healthy offspring. Building a hemochorial placenta is a complicated process involving the expansion and lineage-specific differentiation of trophoblast stem cell populations. Developmental fates emerge under the direction of an intrinsic genetic program and the modulatory effects of the maternal environment. Modern biomedical research has benefitted from the use of accessible model organisms (especially rodents) to study fundamental physiological and pathological processes. The implementation of such a research strategy has not been the norm for gaining insights into hemochorial placentation. The purpose of this presentation is to extol the merits of the rat as a model system for studying hemochorial placentation. There are striking conserved elements in rat and human hemochorial placentation. Among the shared features is a robust intrauterine trophoblast invasion, which contributes to uterine spiral arteriole remodelling. There is also a wealth of experimental tools that can facilitate dissection of rat placentation phenotypes. These strategies can be complemented with techniques for manipulating the rat genome and the generation of mutant animal models with gene-specific perturbations. The availability of in vitro rat trophoblast stem cell models and methods for trophoblast-specific gene manipulation make the rat an attractive model organism. In summary, the rat possesses the requisite structural features and breadth of research strategies to provide important mechanistic insights regarding the regulation of hemochorial placentation. (Supported by NIH HD20676)

[ECR2]
DIFFERENTIATION POTENTIAL AND PARACRINE ACTIONS OF AMNIOTIC MEMBRANE-DERIVED CELLS: IN VITRO AND IN VIVO STUDIES

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In seeking novel stem cell sources for application in regenerative medicine, the amniotic membrane of human term placenta has attracted increasing interest in recent years due to its early embryological origin and essential role in fetomaternal tolerance, suggesting that it could harbour cells with differentiation capacity and immunomodulatory features that would make them applicable for cell therapy. The amniotic membrane *in toto* has long been applied for treatment of wounds, burns and various ocular surface disorders, conferring beneficial effects including wound healing, enhanced epithelialization, increased neovascularization, suppression of inflammation and fibrosis. Recent studies have investigated the properties of the two main cell populations which can be isolated from amniotic membrane, namely, human amniotic epithelial cells (hAEC) and human amniotic mesenchymal stromal cells (hAMSC). In particular, hAEC express several markers of embryonic stem cells, and differentiate *in vitro* toward cell types of all three germ layers. Meanwhile, hAMSC, which display phenotypic and functional characteristics reminiscent of mesenchymal stromal cells of other sources including bone marrow and adipose tissue, have been shown to differentiate toward mesodermal lineages, indication of ectodermal and endodermal differentiation has also been reported. Interestingly, these cells also display immunomodulatory effects, both through suppression of T cell proliferation, as well as blocking of monocyte maturation into dendritic cells. *In vivo*, we have demonstrated that fetal membrane-derived cells have anti-fibrotic and anti-inflammatory effects in a mouse model of bleomycin-induced pulmonary fibrosis, while in rat models of cardiac injury and biliary fibrosis, we observed that application of amniotic membrane patches reduced post-ischemic injury, and inhibited fibrosis progression, respectively. While the exact mechanisms (ie. differentiation vs. paracrine actions) underlying the observed beneficial effects of amniotic membrane or its derived cells remain to be defined, the promising data obtained to date constitute strong evidence supporting their future application in regenerative medicine.

Trophoblast Research Award (TR1)

[TR1]
UTERINE-SPECIFIC NODAL DELETION DISRUPTS PLACENTATION AND CONTRIBUTES TO PRE-TERM BIRTH IN MICE

C. B. Park*, D. Dufort, McGill University, Canada

Introduction: Pre-term birth (PTB) is the leading cause of perinatal mortality, accounting for over 75% of perinatal death. Despite recent progress, PTB has continued to rise over the years and remains an important clinical dilemma worldwide. Failure to decrease the rates of PTB is attributed, in part, to our lack of understanding of the causes and mechanisms that underlie pre-term delivery. In order to aid in the ongoing pursuit of elucidating these mechanisms, our laboratory has been characterizing the expression of the TGF-β superfamily member, Nodal, in the uterus and investigating the potential role this factor may play in facilitating the birth of healthy offspring.
Methods: Utilizing the loxP-Cre recombinase system, we have generated a conditional knockout of Nodal in the female reproductive tract of adult mice (Progesterone Receptor-Cre), bypassing embryonic lethality.
Results: Interestingly, the Nodal deficient mice exhibit various reproductive abnormalities, including reduced rates of establishing pregnancy, intrauterine growth restriction (IUGR) and pre-term delivery late into development (d17.5). The placenta of the Nodal conditional knockout mothers exhibits significant disruption of the maternal basal plate by d12.5 and, coincidentally, resembles the morphology of a late-stage placenta. Furthermore, apoptosis is dysregulated at the maternal-fetal interface. We hypothesize that deleting uterine Nodal affects placentation and disrupts the endocrinological framework that underlies labour thereby inducing premature delivery in our mouse model.
Discussion: We report here, a detailed phenotypic characterization of a uterine Nodal knockout strain, implicating the Nodal signalling pathway in facilitating healthy pregnancy. Our observations indicate that Nodal ligand from a maternal source may play a crucial role in decidualization and proper placenta development and its absence leads to IUGR and PTB. Understanding the mechanisms that underlie IUGR and pre-term delivery are paramount in the ultimate goal of eliminating complications during pregnancy leading to pre-eclampsia, PTB and embryonic loss.
Keywords: Nodal Signalling Pathway, Pre-Term Birth, Intrauterine Growth Restriction

SLIMP Award Lecture (SLIMP1)

[SLIMP1]
EXPRESSION OF LEPTIN, A KEY PLACENTAL HORMONE

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The process of embryo implantation and trophoblast invasion is currently considered as the most limiting factor for the establishment of pregnancy. Leptin has been proposed to play a relevant role in the regulation of the embryo implantation as well as its maintenance. Leptin is a 16000 MW protein product of the obese gene, originally considered as an adipocyte-derived signaling molecule for the central control of metabolism. However, leptin has been suggested to be involved in other functions during pregnancy, particularly in placenta, where it was found to be expressed. In our work we aimed to study the signal transduction pathways activated by leptin in placenta and to elucidate the mechanisms that mediate the antiapoptotic effect of leptin. Moreover, we attempted to study the regulation of leptin expression in placenta. BeWo and JEG-3 choriocarcinoma cell line, as well as trophoblastic cells from human placenta explants were used. Western blot analyses were carried out to detect leptin expression as well as the phosphorylated form of proteins involved in mayor signaling pathways. Apoptosis was assayed by flow cytometry and by western blot of caspase-3 fragmentation. Transient transfection assays with a plasmid construction containing different leptin promoter regions and the reporter gene luciferase, and expression vectors for some intermediates of signaling pathways were used to determine the transcriptional regulation of leptin. *q*RT-PCR was performed to determine leptin mRNA expression. We have found that leptin stimulates JAK-STAT, MAPK and PI3K pathways in placental cells and in human placental explants. The effect of leptin on JEG-3 survival was completely reversed by blocking p42/44 MAPK activation employing the MEK inhibitor PD98059. We have also found that hCG added to BeWo and JEG-3 cells showed a stimulatory effect on leptin expression. Moreover, hCG treatment enhanced leptin promoter activity and mRNA expression .Similar results were obtained with placental explants evidencing physiological relevance. We found that dbcAMP counteracted hCG effect on leptin expression. Moreover, hCG effect on leptin is mediated by the MAPK pathway. We showed that cAMP stimulates leptin expression in placental cells. More interestingly, we demonstrated that the cAMP effect on leptin expression probably involves both the PKA classic signaling pathway and the MAPK signal transduction pathway. A cross talk between these pathways would be responsible for the observed effects.
In summary, our results further support the importance of leptin in the biology of reproduction

Than Award Lecture (THAN1)

[THAN1]
TRANSFORMATION OF THE SPIRAL ARTERIES IN HUMAN PREGNANCY: KEY EVENTS IN THE REMODELLING TIMELINE

L Harris*, The University of Manchester, United Kingdom

During human pregnancy, the uterine spiral arteries are progressively remodelled to form dilated conduits lacking maternal vasomotor control. This phenomenon ensures that a constant supply of blood is delivered to the materno-fetal interface at an optimal velocity for nutrient exchange. Impaired vascular transformation is associated with poor pregnancy outcome and an increased risk of pre-eclampsia, fetal growth restriction and second trimester miscarriage. Conversion of a tonic maternal arteriole composed of multiple layers of vascular smooth muscle, elastin and numerous other extracellular matrix components, into a highly dilated yet durable vessel, requires tight regulatory control and the coordinated actions of multiple cell types. Initial disruption of the vascular wall, characterised by foci of endothelial cell loss, and separation and misalignment of vascular smooth muscle cells (VSMC), is coincident with an influx of uterine natural killer (uNK) cells and macrophages. uNK cells are a source of angiogenic growth factors and matrix degrading proteases, thus they possess the capacity to initiate changes in VSMC phenotype and instigate extracellular matrix catabolism. However, complete vascular cell loss, mediated in part by apoptosis and dedifferentiation, is only achieved following colonisation of the arteries by extravillous trophoblast (EVT). EVT produce a variety of chemokines, apoptotic cytokines and matrix degrading proteases, enabling them to influence the fate of other cells within the placental bed and complete the remodelling process. The complex interplay of cell-cell and cell-matrix interactions required for effective vascular transformation will be discussed.

Poster session 1 (P1.1 - P1.83)

[P1.1]
DEPORTATION OF SYNCYTIAL SPROUTS IN NORMAL TERM PLACENTAS

GJ Burton*, University of Cambridge, United Kingdom

Introduction. Trophoblast is deported from the placenta into the maternal blood throughout pregnancy. In early pregnancy this involves the detachment of syncytial sprouts containing euchromatic nuclei. At term, it has been suggested that deportation involves the extrusion of syncytial knots containing nuclei with heavily condensed chromatin, interpreted by some as apoptotic. Syncytial sprouts are still present at term, and it was recently suggested that these may continue to be deported [1]. This conclusion was rightly criticised because of the danger of sectioning artefacts [2].
Materials. Paraffin-embedded blocks were selected at random from 5 normal term placentas fixed by immersion in formalin immediately after delivery before the tissue samples were excised, entrapping the contents of the intervillous space. 40 serial 7 μm sections were cut for each placenta, scanned and the images examined.
Results. Many sprouts with euchromatic nuclei were identified arising from the villous surface in each placenta. Examples of apparently detached sprouts were also observed in individual sections. The majority proved to be attached to the villi when followed through serial sections. However, in each placenta examples were found where sprouts were genuinely detached, and had no connection to a villus.
Conclusion. This study provides conclusive proof that syncytial sprouts, containing euchromatic nuclei, are deported into the maternal blood in late pregnancy. The incidence of these sprouts is increased in pre-eclampsia [3], and so they may contribute to the overall increase in trophoblast deportation observed in these pregnancies.
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[2] Huppertz B. IFPA Award in Placentology Lecture: Biology of the placental syncytiotrophoblast–myths and facts. Placenta 31 Suppl:S75-81.
[3] Alvarez H, Benedetti WL, De Leonis VK. Syncytial proliferation in normal and toxemic pregnancies. Obstet Gynecol 1967;29:637-43.
Keywords: Syncytial sprouts, Trophoblast, Deportation

[P1.2]
A NEW POSSIBLE FUNCTION FOR PLACENTAL PERICYTES

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Introduction: The pericyte is a multifunctional cell closely associated with endothelial cells and may play a role in angiogenesis and vessel stabilisation. In this study, archival material from an investigation into ultrastructural changes in placental development and pathology has been examined to identify previously undescribed structures associated with the pericyte of the human placental terminal villus.
Methods: Placentae were collected between 1973 and 1976 from St Mary's Hospital, Manchester; they were obtained immediately after delivery and processed for electron microscopy. Electron micrographs from 100 specimens (normal term: 14, postmature: 21, diabetes: 14, intrauterine growth restriction: 11, rhesus incompatibility: 12, preeclampsia: 28) were examined for this study.
Results: Structures in the form of outgrowths from the main body of the pericyte were found. These generally had a narrow neck rich in cytoplasmic filaments and terminated in swollen tips which appeared to bleb off the pericyte and form electron lucent stromal vesicles. These vesicles were distributed through the connective tissue stroma of the chorionic villus, sometimes making contact with the trophoblast basal lamina and stromal cell processes. Such features were occasionally found in placentae from normal term pregnancies but were increasingly identified where capillaries showed abnormalities such as contraction and thickening of the endothelial cells and a failure to form sinusoids, as in pregnancies complicated by diabetes, postmaturity, rhesus incompatibility and preeclampsia. Changes associated with apoptosis were not generally associated with the cells bearing outgrowths.
Conclusion: It is suggested that such outgrowths and associated blebs or stromal vesicles may contribute to fluid homeostasis where normal mechanisms are impaired by thickened or damaged endothelial cells.

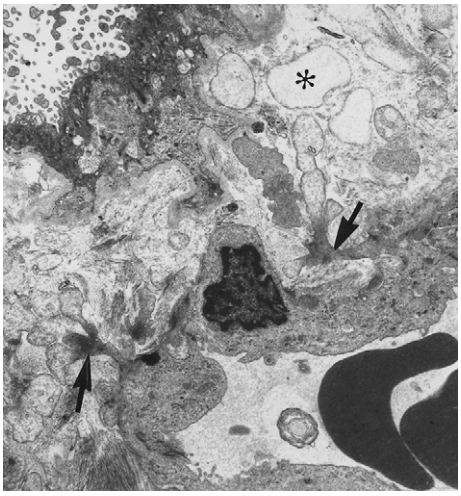

Figure 1. Pericytes showing outgrowths with narrow necks (arrows) terminating in swollen tips. A large stromal vesicle (*) can be seen.

Keywords: Placenta, pericyte, ultrastructure, capillary

[P1.3]
THE CHARACTERIZATION OF FIBROCYTES: A NOVEL FIBROBLASTIC CELL OF THE HUMAN PLACENTA

M.R. Riddell[*1], B. Lowen[2], S.T. Davidge[1,3], L.J. Guilbert[2], [1]University of Alberta, Department of Physiology, Canada, [2]University of Alberta, Department of Medical Microbiology and Immunology, Canada, [3]University of Alberta, Department of Obstetrics and Gynecology Medical Microbiology and Immunology, Canada

Introduction: The placenta is the site of oxygen, nutrient, and waste exchange between the mother and fetus, and as such is primarily a vascular organ. Microenvironmental conditions are known to be important in the control of angiogenesis thus the placental stroma as well as the trophoblastic shell likely regulate the expansion of the villous vasculature. This study identifies, for the first time in the placenta, a population of fibroblastic cells positive for monocyte/macrophage lineage markers previously identified in adult circulation as fibrocytes and examines their role in placental angiogenesis.
Methods: Fibroblastic cells were isolated from human first trimester, early third trimester, and term placentas and expanded in culture. The cells were stained with a panel of fibroblastic and macrophage markers and were assessed for the ability to phagocytose fluorescent beads. The effect of fibrocytes on the formation of vascular tubes in Matrigel was assessed.
Results: A population of placental fibroblastic cells positive for the macrophage markers CD14 and CSF-1R, negative for myofibroblast marker alpha-smooth muscle actin and capable of phagocytosing fluorescent beads were defined as fibrocytes. The cells could be isolated by CD45 (a hematopoietic marker) flow cytometry (along with Hofbauer cells) but became CD45 negative in two weeks of culture although CD14 and CSF-1R expression were maintained. Vascular tube formation in Matrigel of human umbilical vein endothelial cells (HUVECS) was disrupted in a 48 hr co-culture with CD45 negative fibrocytes. Staining of early third trimester and term placental villous sections revealed a large pool of spindle shaped cells dispersed throughout the villi positive for CSF-1R and CD14 with a smaller number being positive for CD45.
Conclusions: Due to their abundance and localization in the villi and their ability to disrupt tubule formation in Matrigel we propose that fibrocytes may have important roles in the regulation of placental angiogenesis.
Keywords: Angiogenesis, Fibrocyte, Fibroblast

[P1.4]
TWIN PREGNANCY: A MODEL OF PLACENTAL ADAPTATION

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Introduction: The U shaped curve of perinatal mortality rate, showing an increase after 41 weeks of gestational age, is shifted to the left in twin pregnancies, where the increase occurs at 37-38 weeks This might be due to the fact that placental changes occur earlier compared to those of single pregnancies. The aim of this study was to compare villous patterns of twin and single placentae at 34-37 weeks by morphometric analisys.
Methods: We examined the placentas from two dichorionic diamniotic uneventful twin pregnacies with normal fetal growth and two age-matched single pregnancies with normal fetal growth. Twin and single placentae were fixed in neutral buffered 4% formaldehyde solution and weighed. Three vertical full-thickness slices (one central, one intermediate and one peripheral) including chorionic and basal plates were excised. Infarctuated areas were excluded. Three villous sections for each placenta were examined to determine structural morphometrical composition (percentage of fibrinoid, intervillous space, and villous tissue) and relative amount of villous type (stem, mature intermediate, and terminal villi).
Results: Twin placentas are characterized by a higher percentage of terminal villi (42.77%) compared to age-matched single placentae (33.89%).. No differences were observed in intermediate villi pattern between twin (30.4%) and single (36.6%) placentae.
Discussion: Our results suggest that maternal haemodinamic changes occurring during multiple pregnancies can't supply to the requirements of two fetuses starting at 35-36 weeks thus inducing an increase in terminal villi in twin dichorionic placentae as previously described in single pregnancies with feto-placental hypoxia.
Keywords: Placenta, Twin pregnancy, Morphometry, Villous morphology

[P1.5]
PATTERN OF THIRD TRIMESTER PLACENTAL POSITION IN AN AFRICAN POPULATION

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Background: Placenta is an apposition or fusion of the foetal membranes to the uterine mucosa for physiological exchange. This fusion occurs at the embryonic pole where the foetal chorionic tissue fuses with the maternal decidual tissues. Once the fusion has occurred the placenta thereafter becomes a functional unit at about 10weeks of gestation. As a result of continuous growth of the uterus, the placenta continually migrates but attains a definite position in the third trimester. Placental location is important in patients that are to undergo caesarean section or amniocentesis. Clinicians believe that the placenta is more likely to implant near an existing uterine scar in a subsequent pregnancy. Placenta previa is also common in patients with previous caesarean section, multiple gestation, anaemia or previous abortion.
Materials and Methods: Using ultrasound at the University College Hospital Ibadan, three hundred and ninety one pregnant women in their third trimester were prospectively evaluated after obtaining an informed consent. Pregnant women whose placental site could not be easily identified were excluded. Scanning was performed using a transabdominal approach with 3.5 MHz sector probe.
Results: The ages of patients range between 17 and 43 years with a mean of 30 years. Ten different placental positions were identified. primigravidas represented only 29.7% .An anterior placental position had the highest frequency with 37.1%. Fundal position represented 7.7% while both lateral-right and left locations accounted for 7.9%. Low lying placenta or placenta previa was found in 1.8% of patients. Placenta position showed no significant variation with parity, number of foetuses or previous caesarean section.
Conclusion: Third trimester placental position can be wide-ranging and affected by pre-existing maternal conditions. For quality antenatal care, ultrasound examination may be recommended in the third trimester for assessment of placental location, as it has become readily available in developing countries like Nigeria.
Keywords: placenta, ultrasound, pregnancy, position

[P1.6]

A SYSTEMATIC AND STANDARDIZED CLASSIFICATION OF PLACENTAL PATHOLOGY A NORWEGIAN ENTERPRISE

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Introduction: A systematic, objective and standardized classification of placental pathology, useful to clinicians, has never been agreed upon. This lack of a unified clinical-pathologic registration of placental structural abnormalities hampers wider comparisons, clinical-pathologic research and the understanding of the clinical relevance of the findings.
Methods: A group of eight Norwegian, experienced pathologists with special interest in placental pathology has formed a national group, meeting regularly, discussing current knowledge and new basic insight in placental development and reaction to various type of injury. We additionally discuss these concepts in a wider network with Nordic and European placental pathologists. Through systematized case discussions and discussion of current relevant literature, we have agreed upon a common, standardized, classification scheme. All of us are currently using this classification scheme and are collecting information and feedback on the clinical usefulness of such a classification.
Results: We have agreed upon a simple, clinically easy- to- understand, standardized line of a main diagnosis, as stated below. In the standardized main diagnosis, mature or immature is additionally added.
1. Normal placenta
2. Placenta with chorioamnionitis
3. Placenta with villitis (usually VUE)
4. Placenta with materno-placental circulatory disorder
5. Placenta with feto-placental circulatory disorder
6. Placenta with maturation disturbance
7. Placenta with findings suggestive of gene aberration
8. Placenta with placentation defect
9. Placenta with other pathology

All diagnoses include a comment with additional specification of the findings and discussion of the clinical relevance etc.
Discussion: The feedback from clinical colleagues is so far very positive. The classification is easy to use by pathologists, and relate to defined criteria from well documented research. The criteria and practical use of the classification will be discussed.
Keywords: Placental pathology, Classification scheme, clinical-pathologic research

[P1.7]

DIFFUSIONAL SCREENING OF CAPILLARIES WITHIN VILLI OF THE HUMAN PLACENTA

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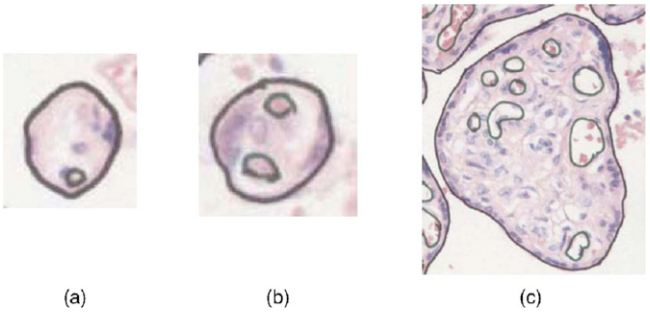
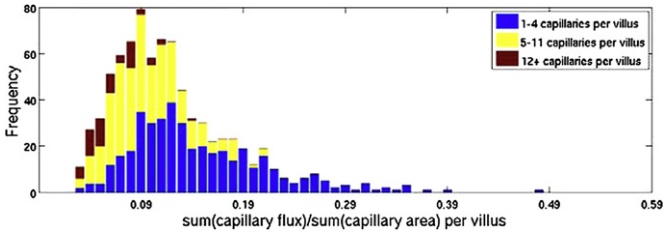
Goal/Background: Respiratory transfer across the placenta to the fetus occurs by (i) maternal blood bathing the chorionic villi in oxygen, (ii) oxygen permeating the villus surface and diffusing into the fetal capillaries, and (iii) oxygen transport to the fetus by fetal blood. The diffusive current in (ii) is influenced by factors such as the number and spatial arrangement of the capillaries within the villi. This can lead to diffusional screening of inner by outer capillaries, which reduces oxygen transport to the fetus by the screened capillaries.
Materials/Methods: Step (ii) is modelled as stationary oxygen diffusion within the villi. The oxygen concentration at the villus boundary is taken as a constant and the flux into the capillaries is described by a Robin boundary condition. The boundaries of the villi and capillaries are obtained from digitized images that are representative of placentas with no abnormalities and those with pre-existing and pregnancy-induced hypertension (PIH).
Results: Panels (a), (b), and (c) show villi with one, two, and nine capillaries. The error made in calculating the total capillary flux as the sum of independent fluxes for each capillary (with the same circular area) is 0.6% for (a), 11% for (b), and 64% for (c). The increasing error is due to diffusional screening, i.e. the competition between adjacent capillaries for diffusing oxygen. Panel (d) shows how the number of capillaries within individual villi affects the flux per capillary area through diffusional screening. Clinical implications of these results can be seen in the table, which shows that placentas with pre-existing hypertension have larger villi, more capillaries, but a lower average flux per capillary area per villus than the other placentas.
Conclusions: Diffusional screening has been shown to be a strong function of capillary number within villi and its correlation with pre-existing hypertension has been suggested.





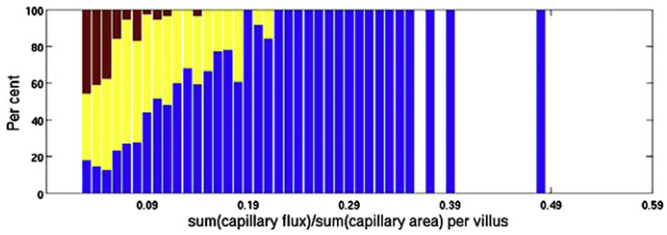


Table 1

	All data[1]	No complications[2]	PIH[3]	Hypertension[4]
Capillaries per villus	4.75 ± 0.18	4.56 ± 0.20	4.71 ± 0.28	9.3 ± 2.5
Capillary area (px)	759 ± 14	756 ± 19	705 ± 22	1100 ± 80
Flux per capillary	55.9 ± 0.6	57.4 ± 0.8	54.1 ± 1.0	51.0 ± 2.6
Capillary flux per villus / Capillary area	0.120 ± 0.02	0.125 ± 0.003	0.115 ± 0.003	0.083 ± 0.009

[1] 812 villi, 3856 capillaries
[2] 512 villi, 2335 capillaries
[3] 276 villi, 1299 capillaries
[4] 24 villi, 222 capillaries

Keywords: villous capillary, oxygen flux, diffusion screening, placental function

[P1.8]

FUNCTIONAL ANALYSIS OF CAPILLARIES WITHIN VILLI OF THE HUMAN PLACENTA: AREA, FLUX, AND BIRTH WEIGHT, PLACENTAL WEIGHT AND GESTATIONAL AGE

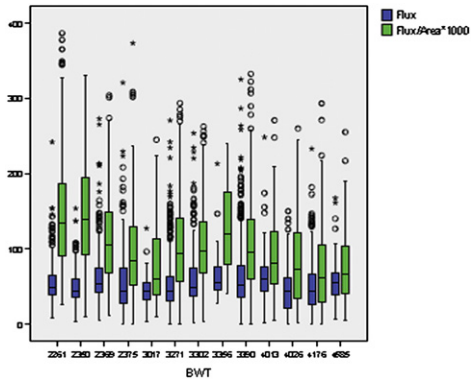
JS Gill*[1], D Grebenkov[2], CM Salafia[3], DP Misra[4], D Vvedensky[1], [1]Imperial College, United States, [2]Ecole Polytechnique, France, [3]Placental Analytics LLC, United States, [4]Wayne State University, United States

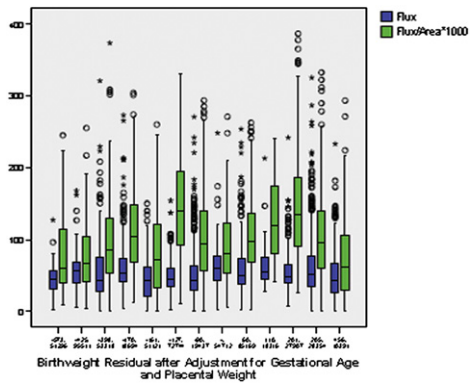
Goal/Background: We have, through image analysis and solution of diffusion equations, obtained oxygen flux data for individual villi. Oxygen flux is crucial for fetal viability, and can be considered proportional to nutrient exchange. Our goal is to determine if our measures account for birth weight variance that is not explained by gestational age or placental weight. This would provide additional insights into aspects of placental function key to fetal growth and health

Materials/Methods: 13 placentas were selected from a consecutive birth cohort that has been extensively studied for chorionic surface shape, cord insertion and chorionic vasculature. From these, a minimum of 7 photomicrographs were taken from routine stained placental samples. Images were obtained from the central zone of non-marginal samples and were selected for those in which blood retention made the capillaries visible. The villous perimeters and each capillary were manually traced by a single observer (CMS). The stationary oxygen concentration within each villus is the solution of Laplace's equation, with a fixed value at the villous surface and a Robin boundary condition at the capillary boundary.

Results: A total of 3855 capillaries were measured; the number varied from 57 to 863 capillaries per case. Birth weights ranged from 2261-4585g (3086+612 g), gestational ages from 258-290 days (277+7 days), and placental weights from 240-740 g (413+97g). Mean capillary areas, perimeter, oxygen flux and the flux/area ratio were 759+888, 87+53, 55.9 +36.1 and 0.11+0.063, respectively. Ordering the cases by increasing birth weight, flux and flux area tended to decrease (Figure 1). However, after adjustment for gestational age and placental weight, flux and flux/area tended to increase with the residual birth weight (Figure 2).

Conclusions: Individual capillary oxygen flux and flux per area account for birth weight independent of gestational age and placental weight.



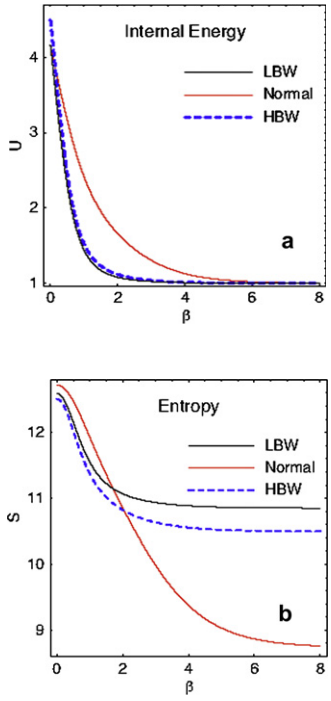


Keywords: Morphology, villous capillary, oxygen flux, birth weight

[P1.9]
STATISTICAL TOPOLOGY OF PLACENTAL VASCULATURE

R-K Seong[1], CM Salafia*[2], D Vvedensky[1], [1]Imperial College, United Kingdom, [2]Placental Analytics LLC, United States

Goal/Background: The vascular system of the placenta emanates from two arteries and one vein that extend from the fetus to the placenta through the umbilical cord. The veins and arteries branch from their insertion in the placenta, first within the chorion in a tree-like manner and then, upon leaving the chorion, diving into the placenta with further branching. The goal of this project is to develop a quantitative measure of the branching of the placenta that distinguishes between normal and abnormal placentas.
Materials/Methods: We characterize the placental vascular network by its edges and vertices. Each edge connects an *n*th generation vertex to and *(n+1)*st generation vertex, with the origin designated as the first generation vertex. The network is then represented by triangles that connect an *n*th generation vertex to two *(n+1)*st generation vertices. A partition function is constructed for this triangulated network that weighs the triangular elements according to their generation and area from which area characteristics associated with different generations are determined.
Results: Panel (a) shows the areas of a high birth-weight (HBW), a low birth-weight (LBW) and a normal placenta with increasing generations (decreasing β). Panel (b) shows the corresponding distribution of the areas among the generations. The entropy indicates that in the early generations (large β), there is a much small disparity of triangulated areas in the normal placenta, even though the total areas are comparable. The triangulated increases with generations more rapidly for the nor-mal placenta, but with a distribution that approaches the other two placentas. The final states for all three types of placenta are similar.
Conclusions: Our analysis provides a way of distinguishing normal from abnormal placentas solely from the topology of their vasculature. Further work will determine the sensitivity of our methodology to other types of abnormalities.



Keywords: Chorionic plate, chorionic vasculature, vascular structure, branching angoiogenesis

[P1.10]
INFLUENCE OF PROTEIN S & PROTEIN C ON PLACENTA VILLOUS STRUCTURE A STEREOLOGICAL STUDY

A. O'Malley[1], D.S. Gibbons[1], J. Donnelly[1], S. Cooley[1], M. Geary[1], J.E. Gillan*[1,2] et al, [1]Rotunda Hospital, Ireland, [2]Trinity College, Ireland, [3]Our Lady's Hospital for Sick Children, Ireland

Introduction: Protein S (PS) and Protein C (PC) deficiency are claimed to cause impairment of maternal blood flow to the placenta. This study evaluated the effect of these thrombophilias on placental terminal villous structure using stereological measurement.
Methods: Clinical cohorts were obtained from low risk primigravid mothers of 36-41 weeks gestation.. These cohorts comprised 5 cases each with (i) PS levels <20%; (ii) PS levels 20-25%; (iii) PC levels <70% of normal values. 8 placentas from mothers with preeclampsia associated with IUGR (PET-IUGR) and 8 normal cases provided positive and negative controls respectively. The volume of each placental disc was measured followed by uniform random sampling of 10 full thickness biopsies. 5 fields were examined from coded haematoxylin and eosin stained sections. Stereological assessment comprised star volume and surface area measurements of terminal villi (TV) and of their capillaries. Two-dimension enumeration of syncytial knots was also performed in each case.
Results: There was a statistically significant reduction in the volume fraction of capillaries (PS<20 *p=0.043*; PS 20-25 *p=0.005*; PC *p=0.002*) and of terminal villi (PS<20 *p=0.05*; PS 20-25 *p=0.13*; PC *p=0.005*) in the thrombophilia groups when compared with control values. The levels were commensurate with those noted in the PET-IUGR cohort (capillaries: *p=0.002*; TV: *p=0.03*). In addition the surface area of capillaries were reduced in the thrombophilia groups (PS<20 *p=0.01*; PS 20-25 *p=0.005*; PC *p=0.0002*) when compared with control values. This was similar to that found in PET-IUGR (*p=0.002*). The surface area of terminal villi was reduced in all 3 cohorts similar to PET-IUGR, but was only statistically significant (*p=0.017*) with protein C. Syncytial knots were not increased when compared with controls.
Conclusions: These findings demonstrate that PC and PS affect placental villi in a way which is similar to PET-IUGR. In addition, the alterations evident in the intermediate PS cohort highlights a requirement for heightened clinical concern in this group.
Keywords: Stereology, Thrombophilia, Protein S, Protein C

[P1.11]

SPATIAL ARRANGEMENT OF PERIPHERAL PLACENTAL VILLI AND VILLOUS CAPILLARIES IN TYPE 1 DIABETES

M. Jirkovská*, J. Kaláb, M. Jadrnícek, V. Niedobová, M. Moravcová, V. Krejcí, Charles University in Prague, First Faculty of Medicine, Czech Republic

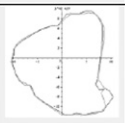
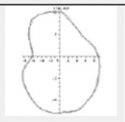
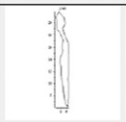
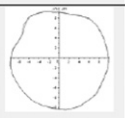
Objectives: Maternal diabetes manifests itself by the great variability of placental villous vascularity. As shown previously, the capillary bed of terminal villi is more branched in placentas from pregnancies complicated by gestational diabetes. This contribution deals with the spatial organization of villous capillaries in placentas from Type 1 diabetes (DM1).
Methods: Specimens collected by the systematic random sampling from 14 normal and 16 DM1 placentas were fixed in formalin with admixture of eosin, and embedded in paraffin. In sections cut at 120 μm, the topology of capillaries in terminal villi was analyzed by the confocal laser scanning microscope, and the frequency of variable types of capillary bed was analyzed. Based on the stacks of optical sections, villous capillaries and mutual relationships of villi in the intervillous space were visualized.
Results: Two simplest types of villous capillary bed, i. e. U-like loop and Y like branching were found in both studied groups. Some villi had capillary segments interconnected with one or more "redundant" connections" (RC), i.e. capillaries that can be removed without disconnecting the capillary bed. The proportion of capillary beds without RCs was higher in normal placentas (80%) than in DM1 placentas (67%) respectively whereas the mean number of RCs per villus was higher in DM1 group (0.41) than in control group (0.23). Diabetic placentas displayed two types of abnormal villi: 1) large villi with few capillaries in edematous stroma, and 2) large villi displaying chorangiosis. 3D reconstruction showed a markedly waved capillaries of highly variable diameter in the latter type. The arrangement of capillaries in such villi influenced their size and shape, which resulted in changed shape and dimensions of adjoining parts of the intervillous space.
Discussion: In order to compensate the maternal metabolic disorder, the DM1 placenta manifests more intense angiogenesis resulting in both the more dense villous capillary bed and abnormalities of its spatial arrangement. The consequent changes of the villous shape and size may locally influence the haemodynamics in the intervillous space.
Keywords: capillary bed, Type 1 diabetes, spatial arrangement, angogenesis

[P1.12]

ABNORMALITY OF THE PLACENTAL VASCULATURE AFFECTS PLACENTAL THICKNESS

O Shlakhter[1], M Yampolsky[1], CM Salafia*[2], DH Mandel[2], [1]University of Toronto, Canada, [2]Placental Analytics LLC, United States

Background. Our empirical modeling suggests that deformation of placental vascular growth is associated with an abnormal placental surface shapeand lowered placental functional efficiency. We hypothesize that placentas with deformed vascular trees have irregular vascular branching and both increased variabilityof placental thickness and lower mean thickness.
Materials and Methods. Fourier analysis quantified placental shape variability, including non-central umbilical cord insertion, and regularly irregular shapes. To quantify the thickness, from a cross-section of the placenta along an oriented diameter is measured mean placental thickness and thickness variation. The functional efficiency is quantified as the scaling exponent β=log (Placental Weight)/log(Birth Weight). The normal value of the scaling exponent is $\beta \approx 0.75$, reflecting the fractality of the vasculature. Higher β corresponds to lower functional efficiency.
Results. Non-centrality of the placement of the umbilical cord is strongly significantly correlated with thickness of the placenta, consistent with our earlier findings (Spearman's ρ=0.128, p=0.002). Placentas with deformed shape are strongly significantly associated with lower overall thickness and higher variability of thickness. ρ lies between -0.173 and -0.254 ($p<0.001$) for correlations of Fourier coefficients quantifying shape deformations with normalized mean thickness. Finally, both lower mean thickness and high variability of thickness are strongly positively correlated with β (reduced placental efficiency).
Conclusions. Our findings confirm the predictions of our empirical modeling. A regularly deformed placental surface is associated with non-uniform placental thickness, and lower average thickness. We speculate that lower thickness is caused by sparse vascular coverage due to an abnormal placental vascular growth. As a confirmation of this, placentas with lower average thickness are less functionally efficient.

Shape	Traced placental perimeter	Traced central slice	Fourier coefficients	Mean thickness	Normalized mean thickness
(a) tri-lobate placenta: large C_3, small mean thickness			C_1=1.57 C_2=0.80 C_3=1.42 C_4=0.7 C_5=0.25	1.134	0.071
(b) bi-lobate placenta: large C_2, small mean thickness			C_1=1.78 C_2=2.18 C_3=1.07 C_4=0.39 C_5=0.38	1.005	0.072
(c) normally shaped placenta			C_1=0.64 C_2=0.49 C_3=0.34 C_4=0.46 C_5=0.28	2.243	0.134

Keywords: Villous arborization, umbilical cord insertion, chorionic disk shape, chorionic vasculature

[P1.13]

ADVANCED PARENTAL AGES AND PLACENTAL THICKNESS ASSOCIATED WITH IQ AT AGE 7 YEARS

CM Salafia*[1,2], DP Misra[3], AK Charles[4], RK Miller[5], [1]Placental Analytics LLC, United States, [2]Institute for Basic Research, United States, [3]Wayne State University, United States, [4]Princess Margaret Hospital, Australia, [5]University of Rochester, United States

Background: We hypothesized that placental disk thickness reflects branching growth of viscera including neuronal dendritic trees, and thus be associated with variation in childhood IQ.
Methods: IQ at age 7 was assessed using the Wechsler Intelligence Scale. Paternal and maternal ages were centered to minimize collinearity. Parental ages, placental weight (grams), and placental thickness (mm) were used as the independent variables in regression analyses of age 7 year IQ in 12,926 singleton liveborns delivered 34-42 weeks with complete placental data. Analyses were stratified on gender and race with covariates of socioeconomic status, parity, and gestational age.
Results: All models were adjusted for age at IQ testing. After adjustment for SES, parity, and gestational age, parental ages and placental measurements independently predicted IQ at age 7 years but results varied by gender-race subgroups. Older paternal age was associated with a statistically significant reduction in IQ in all subgroups except Black boys. Older maternal age was associated with an increase in IQ in all four subgroups with effects about two times larger in girls than in boys, regardless of race. Placental thickness was positively associated with higher IQ for White boys and White girls but not Black boys or Black girls. Tests for interactions of gender, race, and paternal age detected statistically significant interactions between gender and paternal age, race and paternal age, and gender and race together with paternal age (3way) on IQ. There is also a significant interaction between race and thickness on IQ.
Conclusion: Placental thickness is correlated with age 7 yr IQ but the findings vary by race and gender. Further exploration of the possible interaction of these factors on the arborization reflected by placental thickness that correlates with age 7 yr IQ is indicated.

	IQ Effect (β, 95% confidence interval)				
	Whites		**Blacks**		
	Boys	Girls	Boys	Girls	
Paternal age (centered)	-0.133*	-0.148**	-0.067	-0.155**	Placental thickness matters in whites as well as black boys but not black girls.
Maternal age (centered)	+0.182*	+0.399***	+0.234**	+0.396***	
SES	+6.26***	+6.56***	+3.15***	+4.16***	
Nulliparity	-1.91***	-2.45***	-2.88***	+0.575	
Gestational age	+0.442***	+0.478**	+0.07	+0.281*	
Placental weight	-0.004	+0.005	+0.006	+0.01*	
Thickness	+0.299***	+0.258***	+0.114	+0.033	

All adjusted for chronological age at time of testing,
Footnote p-values for each cell *p<0.05 **p<0.01 ***p<0.001

Keywords: parental age, IQ, placental thickness, placental pathology

[P1.14]

MORPHOLOGICAL ANALYSIS OF PLACENTAS OF SHEEP PRODUCED BY INTRACYTOPLASMIC SPERM INJECTION (ICSI)

I La Rosa*[1], F Pereyra Bonnet[1,2], R Fernandez-Martín[1], A Gibbons[2], D. F. Salamone[1], [1]Laboratory of Animal Biotechnology, Agriculture Faculty, University of Buenos Aires, Argentina, [2]Bariloche Experimental Station, National Institute of Agriculture Technology of Argentina (INTA), Argentina

New reproductive technologies in farm animals, like cloning, are usually associated with placental abnormalities after transferences to recipients; i.e. Large Offspring Syndrome, polyhydramnios, hydroallantois, larger placentomas, reduced vascularization, extended gestations, dystocia, etc. So, it is of great interest to analyze placenta gross morphology as a measure of how the maternal- fetal physiology is affected by the ICSI. The length of in vitro embryo culture is also an important factor affecting the placentation. We have performed ICSI followed by chemical activation in ovine. Embryos were transferred at day 2 of in vitro culture to contralateral oviducts (treatment 1, T1) or at day 7 to ipsilateral utherine horn (Treatment 2, T2) of synchronized merino ewes. A total of 228 T1 and 46 T2 resulted in two pregnancies (one of each treatment). Parturitions occurred at term by normal delivery. Born lambs were healthy. ICSI placentas showed no signals of tissue degeneration or excess of fluid or blood. Total weight, cotyledon number and weight and cotyledons/total weight were registered. Placenta 1 weighted 396 g (30% cotyledonary) and contained 83 cotyledons with a mean weight of 1.43 g (± 0.8). Placenta 2 weighted 365.7 g (21% cotyledonary) and contained 72 cotyledons with a mean weight of 1.06g (± 0.77). In placenta 2, we observed a lower percentage of cotiledonary tissue and a lower number of cotyledons than in placenta 1. In T2, Individual weights of cotyledons were statistically lower compared to T1 (t-test). These differences could be attributed to longer in vitro culture before embryo transfer in T2. Our results indicate that there are not major placental abnormalities in ICSI produced sheep. To our knowledge, previous studies about ICSI ovine placentas have never been reported.
Keywords: Ovine, ICSI, in vitro produced embryos, Cotyledons

[P1.15]

VASCULAR CAST OF PLACENTA IN A MONOCHORIONIC PREGNANCY WITH SELECTIVE IUGR FETUS

S Visentin*, V Macchi, A Porzionato, R De Caro, E Cosmi, University of Padua, Italy

Objective: Selective IUGR in monochorionic pregnancy is defined as the presence of a intrauterine growth restriction in a single fetus and the incidence is 5-15%. The most important cause is the irregular subdivision of the placenta and can coexist with a twin to twin transfusion syndrome. The aim of this study was to describe the application of vascular cast technique to the single placenta of a monochorionic twin pregnancy to observe the characteristics of the site of umbilical cord insertion and the possible one blood circulation system shared.
Method: A twin gestation was conceived naturally by a 29-year-old healthy woman. A monochorionic biamniotic pregnancy was diagnosed at 10 weeks' gestation by ultrasound scan and was followed every week for up to 23 weeks of gestation when there was a diagnosis of selective IUGR and marginal umbilical cord insertion. Vascular corrosion cast was performed through injection of metachrilate resins in the placenta vessels
Result: The twins were delivered by caesarean section at 29 weeks of gestation due to Doppler abnormalities of the smallest fetus. The cast model of the monochorionic biamniotic placenta showed that the insertion of the umbilical cords into the placenta was central for normal twin and marginal for the selective IUGR twin. Moreover, the marginal cord insertion of the IUGR twin rised to a monopoidal pattern in which the vessels were distributed asymmetrically along both sides of the placenta's circumference.
Conclusion: Fetal growth in monochorionic twins is proportional to the placental territory and arteriovenous anastomoses. Placenta vascular anatomy demonstrated a shared blood circulatory system.
Keywords: monochorionic pregnancy, selective IUGR, plastination, cord insertion

[P1.16]

MORPHOLOGICAL ANALYSIS OF RAT PLACENTA IN MATERNAL UNDERNUTRITION CONDITIONS

S San Martin*[1,2], B Ponzio[3], Z Fortes[3], T Zorn[3], A Moller[1], D Martinez[3] et al, [1]Universidad de Valparaiso, Chile, [2]CREAS, Chile, [3]University of Sao Paulo, Brazil, [4]Federal University of Sao Paulo, Brazil

Introduction: During the fetal development, nutrient and hormonal milieu is strongly influenced by the placenta. Experiments in several species have shown that maternal nutrition can profoundly influence placental growth. Studies of the effects of maternal malnutrition on placental morphology have revealed some striking changes, related to decrease of villous mass in the human and a significant decrease in glycogen concentration in the rat. However, it is not known whether maternal malnutrition model promotes morphological changes in the placenta during their development.
Objectives: To determine whether the maternal undernutrition promote differences in the development and morphology of the rats placentas.
Material and Methods: The experimental (undernutrition) group was fed 50% of the ad libitum intake, determined by the amount of food consumed by the control group from day 1 of pregnancy until parturition. Placentas from control and restricted groups were collected on days 14, 17 and 20 postcoitum (dp). We used histological technique to identify the placental regions and images was visualized and captured with a CX81 (Olympus) microscope and digital camera.
Results: The placentas from undernutrition mother have disarranged in the labyrinth region with a decrease area and minor presences of fetal blood vessels. Different to placentas form control animals, placentas from undernutrition mother show premature presences of glycogen rich cells in the junctional zone with a minor develop of spongiotrophoblast cells. Also, there are minor presences of giant trophoblast cells was observed.
Conclusion: The maternal undernutrition during the pregnancy is related with several morphological changes in the rats placentas. The altered morphology in the different regions of this organ could be related with a minor nutrients exchanges and growth factor synthesis, all biological process related with a normal function of the placenta.
Acknowledgment: Dipuv 07/2008; CI 05/2006 (Universidad de Valparaíso, Chile) and CONICYT (ACT-73), Chile.
Keywords: Undernutrition, Placenta, Rat

[P1.17]

CHARACTERISTICS OF THE PLACENTA AT TERM IN SMALL FOR GESTATIONAL AGE NEWBORNS (SGA)

R Prieto*, M Rojas, Universidad de Chile, Chile

The aims of this study were (1) to identify the morphometric parameters differences as the area of the villi, the area of the vessel, the number of vessels of small for gestational age (SGA) in connection with placentas of newborns adequate for gestational age (AGA), (2) to identify the differences in the distribution of the placental lactogene and IGF-1 receptor on free-chorionic villi, between SGA and AGA placenta.

Methods. We used 25 placenta at term (37-42 weeks), 12 AGA, and 13 SGA newborns. The samples were obtained from the Maternity Hospital Hernán Henriquez Aravena of Temuco, Chile, and were fixed in 10% formalin. The histological techniques were H-E Alcian blue, Masson's Trichromic. For immunoperoxidase technique primary antibodies used were anti placental lactogen (polyclonal, dilution 1:200, NCL-PLP, Novocastra) to label cytoplasmic granules from the syncytial trophoblast and anti IGF-1 (dilution 1:200, NCL-GHR, Novocastra). The following parameters were evaluated: cross section area, number of blood vessels and blood vessel area.

Results. The areas of the villi, the area of the vessel and the number of vessels of SGA showed significant differences between the control group AGA and the SGA group. The immunostaining for the IGF-1 receptor was evidenced in some intermediate and stem chorionic villi. Placental lactogen immunostaining was observed as granules in the cytoplasm of the syncytial trophoblast in both groups.

Conclusion. The intention of the score or placental factor proposed in this study is to contribute to the assessment of the placental function, strongly related with neonatal clinical care.

[P1.18]

PLACENTATION IN *LAGOSTOMUS MAXIMUS MAXIMUS* "PLAINS VISCACHA" (RODENTIA, CHINCHILLIDAE)

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Plains viscacha is a species with an ample distribution in Argentine. The gestational period is 145 -166 days, with two offspring. As member of the suborder Hystricognathi, showing a high potential as experimental model. However, only physiological data related to placentation are available so far, whereas the structural and developmental characteristics are unknown. We herein provide details based on 6 placentae of free-living animals and including stages from early pregnancy to term. Samples were obtained from Estación de Cría de Animales Silvestres, Buenos Aires, the necropsy was performed at Faculty of Veterinary Sciences, La Plata University, fixed in 4 % paraformaldehyde. The material was processed for light microscopy in Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, sectioned (5μm) and stained with haematoxylin and eosin. Macroscopically the placenta had a discoidal shape, attacked to the uterus by a peduncle, similar to other South American hystricognath rodents. The chorioallantoic placenta showed a typical lobulated structure with central maternal vessels and labyrinth, surrounded by the trophospongium. The labyrinth was dually vascularized from maternal and fetal capillaries, with trophoblast in between. The distinct subplacenta was richly vascularized, organized in lamellae situated on fetal mesenchyme and associated with layers of syncytial and cellular trophoblast. It was attached to decidua which possessed giant cells with evident chromatin. The parietal and visceral yolk sac were present. The inverted visceral yolk sac is villous with abundant blood islands. In conclusion the viscacha placenta is similar to other rodents from Suborder Hystricomorpha already studied. Once again, the current survey indicated that major qualitative aspects of placentation were independent of scale dimensions, even in larger species such as the viscacha or capybara. The current findings support the value of the guinea pig as a most promising animal model for human pregnancies, even being a small animal.

Keywords: Lagostomus maximus, placentation, subplacenta, yolk sac

[P1.19]
DISTRIBUTION AND FORM OF ENDOTHELIOCHORIAL PLACENTAS

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Endotheliochorial placentas occur in orders from all four major clades. Species with this type of placenta include the smallest (short tailed shrew) and largest (elephant) land mammals. The endotheliochorial placenta as a definitive form has an interhemal area consisting of maternal endothelium, interstitial lamina, trophoblast, individual or conjoint basal laminas, and fetal endothelium. We commonly think of such placentas as having hypertrophied maternal endothelium with abundant rough endoplasmic reticulum (rER), and as having hemophagous regions. Considering the group as a whole, the trophoblast may be syncytial or cellular, fenestrated or nonfenestrated, and there may or may not be hemophagous regions. Variations also appear in the extent of hypertrophy of the maternal endothelium and in the abundance of rER in these cells. This combination of traits and a few other features produce many morphological combinations. In addition to endotheliochorial as a definitive condition, a transitory endotheliochorial condition may appear in the course of forming a hemochorial placenta. In sciuromorph rodents and vespertilionid bats the maternal endothelium is displaced by trophoblast, transforming an endotheliodichorial placenta into a hemodichorial or monochorial placenta. In some emballonurid bats the early endotheliochorial placenta is two-layered, but the definitive placenta lacks an outer syncytial trophoblast layer. In mollosid bats a well developed endotheliochorial placenta is present for a short time even after a definitive hemochorial placenta has developed in a different region. It is concluded that the endotheliochorial placenta is more widespread and diversified than originally thought, with the variant with cellular trophoblast in particular appearing in several recently studied species.
Keywords: endotheliochorial, trophoblast, hemophagous regions, comparative

[P1.20]
CHARACTERISTICS OF THE TROPHOSPONGIUM AND GIANT CELL REGION IN THE PLACENTA OF *NECROMYS LASIURUS* (RODENTIA, CRICETIDAE, SIGMODONTINAE)

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After fertilization the trophoblast giant cells (TGC) are among the first definitive cell type to differentiate. The associated region, i.e. the trophospongium and giant cell region showed a great variety of cells. Herein we provide details of this region, based on 5 placentae of *Necromys lasiusus* obtained from a breeding group at the UFERSA, Mossoró, Brazil. Tissues were fixed in 4% paraformaldehyde or 2.5% glutaraldehyde and subjected to standard histological processing, using H&E and PAS, immunohistochemistry for cytokeratin and PCNA 1:300, DBA-lectin (1mg/ml; Sigma). Samples were processing by TEM. Trophospongium region mainly consisted of spongiotrophoblast and large numbers of TGC. Both syncytial and cellular trophoblast was found. Partly, the trophoblast formed thin syncytial layers associated with the maternal blood spaces. Moreover, TGC were dispersed within the trophospongium, closely associated with the maternal blood system. They had large nuclei, evident chromatin, and showed positive reaction for cytokeratin. Immunostaining for PCNA likewise revealed high proliferation activity of TGC. Neither the spongiotrophoblast nor the TGC associated with the trophospongium showed positive reactions to PAS. This distinguished them from another type of cell, the so-called glycogen cells, the only type in this region that showed positive reaction to PAS. PAS positive cells were present inside the trophospongium as well as on its outer border, entering the decidual region. Some of these cells were positive for DBA-lectin on decidua, suggesting being NK cells, which are involved to remodeling of implantation sites. Therefore, we observed a continuous band of TGC, situated between the decidua and trophospongium. This band was connected to an area of accumulated TGC at placental border. Giant cells were mostly bi or multinucleated in nature. The current findings confirm that this region consists of different subtypes of trophoblast cells and support the importance of this region for a normal development. Financial support: Fapesp.
Keywords: Sigmodontinae, placentation, trophospongium, immunostaining

[P1.21]
APOPTOSIS PERCENTAGE AND CALCIUM CONCENTRATION IN BOVINE PLACENTOME

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The mammalian placenta consists by an overlap of both fetal and maternal tissues. The maintenance of tissue homeostasis in multicellular organisms is ensured by different biological regulatory mechanisms, which include cell proliferation and programmed cell death or apoptosis. Apoptotic bodies formed during the cattle gestation are largely phagocytosed by macrophages. This process occurs during pregnancy and, lastly, decreasing the overlap of fetal tissue on maternal tissue during late gestation for release the fetal membranes after parturition. Proliferation decrease as the apoptosis increase, it is more evident in the maternal epithelium [1]. The changes in the intracellular calcium concentration are related to cell death. The response to the increase of intracellular calcium promotes the externalization of phosphatidilserina resulting in the case of mitochondria in the liberation of the intermembrane protein namely cytochrome c, so it is responsible for a formation of a caspase activation complex namely apoptosome [2, 3]. We analyzed the calcium concentration using X-ray fluorescence and the percentage of apoptotic cells using flow cytometry in both non manipulated and cloned bovine placentome at different ages (90, 135, and 225) of gestation. Cloned samples showed an increase in the calcium concentration [Ca] as far as in the percentage of apoptotic cells in relation to non manipulated samples. Cloned samples with 90 days of gestation showed 637.29 ($\mu g\ g^{-1}$) of [Ca] and 50.85% of apoptosis, whereas non manipulated showed 262.13 and 12.0%, respectively. To 135 days the [Ca] was 445.38 ($\mu g\ g^{-1}$) and the apoptosis 39.45% for cloned samples, and 257.96 ($\mu g\ g^{-1}$) and 10.7% for non manipulated samples. To analyzed samples from 225 days were 329.98 ($\mu g\ g^{-1}$) of [Ca] and 39.4% of apoptosis, whereas non manipulated showed 285.42 and 10.7%, respectively. The current findings confirm the relation between the calcium with the percentage of apoptotic cells during the gestation.

Keywords: apoptosis, bovine placentome, calcium, flow cytometry

[P1.22]
OCT-4 EXPRESSION IN EQUINE EMBRYOS

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Researches involving stem cells showed the protein OCT-4 is the most used as a marker of pluripotential it was first described in embryonic cells. The aim of this study was to identify the pluripotency of embryonic cells from horse embryos through immunostaining for OCT-4. It was used 6 equine embryos, with gestational ages between 21 and 28 days, fixed in paraformaldeyde 4%, dehydrated and embedded in paraffin. Sequential slides of 5μm were carried out, followed by the processing for immunohistochemistry. There were realized 3 dilutions of antibody: 1:300, 1:400, and 1:500. Positive cells were identified in all embryos, and the better dilution was 1:400. Embryos with 21 days of gestation showed positive marker in the heart ventricle, liver, intestine, kidney (glomerulus and tubule), blood cells and adrenal gland. Embryos with 25 days of gestation showed positive marker in the heart, liver, intestine, lung, kidney, gonads and somites. The embryo with 26 days of gestation showed positive cells in the liver, lung, spinal cord, kidney, somite and a strongly positive marking in the heart. The embryo with 28 days, on the other hand, showed strongly positive marking in liver and positive marker in kidney and choroid plexus. Although it is described in literature that OCT-4 expression is restricted to germ cells in rodents, pigs and cattle, our results showed the marking of OCT-4 in equine embryos. We can also say that the staining was positive for the liver, kidney and heart, mainly, elucidating that these stem cells were scattered in the tissues, and demonstrating that some cells in these organs have pluripotency to germ lines.

Keywords: OCT4, Immunohistochemistry, Embryo, Equine

[P1.23]
ORIGIN AND FATE OF INVERTED YOLK-SAC PLACENTA REVISED IN GUINEA-PIG (*CAVIA PORCELLUS*) EMBRYOGENESIS

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Inverted yolk-sac placenta (IYSP) predicts direct exposure of extra-embryonic endodermal cells to the maternal tissues which does not make sense to the current paradigm of reproductive immunology. In the aim to better understand the origin and organization of IYSP, we revised the embryogenesis and placentation of guinea pig *(Cavia porcellus)*. Pregnant animals on gd 6th to 35th were perfusion fixed for histological processing and paraffin serial sections were evaluated by hematoxylin-eosin stain, a panel of anti-cytokeratin immunocytochemistry and lectin cytochemistry. The anti-cytokeratin CK7 (M7018- Dako Co, USA) and *Erytrina cristagali* lectin selectively reacted with trophoblast cell and used to distinguish these cells from blastocyst trophectoderm to all subsets found in the fully developed placenta. The reconstruction of serial sections showed two distinct cell mass in the gd 7th blastocyst cylinder. Different from other embryos, the embryblast inner cell mass (ICM) growth positioned in blastocyst at antimesometrial side, separated from trophectoderm at mesometrial side forming trophoblast cell mass will originate the ectoplacental cone. The early blastocele cavity was covered by CK7+ trophoblast single cell layer and the embryoblast of ICM facing the blastocele cavity will originate epiblast columnar cell sheet (gd 8-9th), while latterly those facing the trophoctoderm differentiate to flattened hypoblast cell sheet (gd 9-10th). The hypoblast derived endoderm cells migrate laterally through blastocele cavity to gradually form parietal endoderm of the choriovitelline unit (gd 10-11th) covering yolk-sac cavity. By serial sections of developing embryo, the organogenesis of amnion cavity, allantoid and chorioamnioallantoid placenta were further reconstituted for better understand of guinea pig embryogenesis. Taken all together, the yolk sac placenta remains as intact extra-embryonic membrane through the pregnancy without breaking of trophoblast sheet or exposing extra-embryonic endoderm. The hypothesis of complete or partially IYSP seems to be a miss understanding of guinea pig embryogenesis.
Grants: CAPES and FAPESP.
Keywords: Inverted yolkc-sac placenta, Guinea pig (Cavia porcellus), Blastocyst

[P1.24]
FETOPLACENTAL GROWTH DYNAMICS IN THE VERVET MONKEY (*CHLOROCEBUS AETHIOPS*)

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Fetal growth is largely mediated by placental growth and development. As fetal growth accelerates later in pregnancy, there is a shift toward greater placental efficiency. Much of the literature on placental efficiency across gestation comes from murine, ovine, and porcine models that are not fully applicable to primate fetal development and pregnancy, which are uniquely shaped by a higher degree of maternal investment, placental invasion, and fetal brain growth. A rare time series of 50 vervet monkey (*Chlorocebus aethiops*) fetuses and placentas from the St. Kitts Biomedical Research Foundation was examined to characterize the shift in placental efficiency across the latter half of gestation, days 83-159 of a species-typical 167-day gestation. Both fetal mass and placental mass increased significantly with gestational age (Pearson's correlations: $r=0.85$, $p<0.001$; $r=0.64$, $p<0.01$, respectively), but this growth was not symmetrical. Placental efficiency during period 2 (d. 131-159) was 43% greater than that of period 1 (d. 83-130) (T-test: $t=-3.60$, $p<0.001$), indicating that there is a significant shift in the functional properties of the placenta as gestation progresses in the vervet monkey. The plateau in placental growth in the vervet monkey occurs around day 130. The microscopic morphology of the maternal-fetal interface of the placenta will be considered as a key mechanism in the nature and temporality of this functional shift.
Keywords: placental efficiency, vervet monkey, fetal growth, morphology

[P1.25]
YOLK SAC DEVELOPMENT OF SHEEP EMBRYOS

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The yolk sac is the only embryonic attachment present in all species of vertebrate embryos. In eutherian mammals it is initially large, but subsides when the definitive placenta develops. This study aimed to characterize gross and microscopically the yolk sac from sheep embryos at 15 and 25 days of gestation. Five yolk sacs were fixed in Methacarn for microscopic analysis. The embryo at 15 days showed yolk sac located in the ventral region, whitish and form a "V". The results showed blood islands throughout the membrane region and delineation of three layers (mesoderm, endoderm and mesenchyme). The yolk sac of the embryo at 21 days showed orange color, vascularization and blood islands with large amounts of primitive blood cells, however regions of apoptosis. The embryo at 22 days presented the yolk sac had dark orange color, smaller size and blood islands of larger size with a large number of cells within them. In the embryo of 23 days, the yolk sac had reddened and reeled to the umbilical cord, overlapping the amnion. Microscopically showed large amount of blood islands of greater size in the entire length of the membrane and demarcation of the three cell layers. The embryo of 25 days showed yolk sac in the regression phase, with reddish, moving away from the ventral region of the embryo. Microscopic findings showed a blood grouping of islands, occupying large area of the membrane and apoptosis. The yolk sac was modified according to the embryonic period in color, appearance, size and location. Microscopically the yolk sac blood islands are grouped as a result of advancing gestational period and hematopoietic established, in addition to evidence apoptosis.
Keywords: sheep embryo, yolk sac, development, morphology

[P1.26]

BOVINE PLACENTA LIPID PROFILING BY DIRECT INJECTION MASS SPECTROMETRY

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Mass spectrometry (MS) is a leading analytical technique for lipidomic studies. It allows the detection of intact molecules from complex lipid extracts, also providing chemical structural information by using a molecule fragmentation approach (MS/MS). Usually, liquid chromatrography coupled to MS is used for lipid species separation. Herein we proposed the use of electrospray ionization (ESI) MS for the direct profiling (i.e., without previous separation) of placental lipid extracts. Chorion allantoic samples were collected from bovine placentae on Day 225 of gestation (n=3) and placentomes at term (n=2) one as control and other from somatic cell nuclear transfer bovine. Samples were submitted to Bligh & Dyer lipid extraction protocol. A Waters Micromass Q-TOF mass spectrometer equipped with an ESI source was used for the experiments. Lipid extracts were resuspended in methanol/chloroform (7:3) with 0.1% formic acid and sodium and injected using a syringe pump at a rate of 10 μL/min. Lipid species detected in the positive ion mode at the mass range of 500-1200 *m/z* were mostly sodiated ions represented by sphingomyelins (as [SM 16:0) + Na+], phosphocholines (mainly [PC (34:2) + Na^+], [PC (34:1) + Na^+], [PC (36:3) + Na^+], [PC (36:2) + Na^+], and [PC (36:1) + Na^+], and sodiated triacyglicerols composed of 50-52 carbon chain-long unsaturated acyl groups with 1 to 3 double bonds [as TAG(52:1) + Na^+, for example]. It is broadly known that placental function and morphology may be altered in some concepti derived from *in vitro* fertilization (IVF) and nuclear transfer (NT) procedures. Further experiments using placental samples on the negative ion mode will likely increase the detection of additional lipid species. In conclusion, the use of direct injection of placental lipid extracts by ESI-MS allows a rapid and useful detection of a range of lipid classes that may be involved in placental structure and metabolism. This strategy will contribute to the understanding of the pathophysiology underlying altered placental function and morphology in bovine placentae from IVF- and NT-derived bovine concepti. Financial support: FAPESP, CAPES and CNPq.

Keywords: lipidomics, clone catle placenta, bovine, comparative placentation

[P1.27]

IMPRINTED GENE NETWORK IMPLICATION IN THE DEVELOPMENT OF EXTRA EMRYONIC TISSUES AND PLACENTA IN COW

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The newly described1 **I**mprinted **G**ene **N**etwork (IGN), is clearly implicated in the control of differentiation and growth of the foeto-placental unit2. In mice, it has been established that *In Vitro* Fertilization3,4 (IVF) impacts the expression regulation of the IGN. In order to validate the universal biological significance of this IGN, we analysed it in bovine species. Moreover, we compared different reproductive technologies, taking advantage to the opportunity to use reproductive cloning (Somatic Cell Nuclear Transfer, SCNT) in this species.

Our present objective is to analyze the IGN in cow to understand i) how the coordinated pattern of gene expression could take place during early developmental stages in extra embryonic tissues and placenta ii) which the cellular localization of the expression of these genes and iii) which molecular processes are involved in the transcriptional control. The analyses were performed in different extra embryonic tissues and in placenta, at different gestational stages and following different reproductive technologies.

Twenty genes are implicated in the IGN, such as *H19, Igf2, Igf2r, Phlda2, p57kip2, Dcn, DLK1, Gtl2, Grb10, Mest, Peg3*. Firstly, we verified the imprinted status of these genes. Using crossbreeding between two French bovine species (Holstein X Charolais) to generate Single Nucleotide Polymorphism, we determined the heterozygosis in genomic DNA and researched the loss of heterozygosis in cDNA generated from RNA of extra-embryonic tissues. Secondly, we quantified the gene expression by qRT-PCR and determined the specific-cell lineage localization by In situ hybridization. Finally, we analysed the methylation status of the differential methylated regions (DMRs; closely related to imprinted status) and of the promoter regions by bisulfite conversion of genomic DNA and pyrosequencing.

The results highlighted a spatio- temporal regulation of the expression of the IGN suggesting a major role of these genes in the development of the extra-embryonic tissues and placenta in cow., This study was supported by a grant from the EU (Cutting Edge genomics for Sustainable Animal Breeding; SABRE contrat-CT-2006-01625). The authors wish to thanks the technicians of the experimental farm (UCEA; INRA) at Bressonvilliers for animal management.

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Keywords: Imprinting, DNA methylation, gene expression, reproductive technologies

[P1.28]

GENE EXPRESSION IN THE PLACENTA OF A PRECOCIAL SPECIES - THE SPINY MOUSE

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Introduction: The long gestation of the spiny mouse (39 days) relative to other rodents renders this species useful to study precocial fetal and neonatal development, as pregnancy ends after major organogenesis is complete. Less is known about placental development; specifically, what might be necessary to support fetal growth and development to term. It is known that the placental labyrinth is present from approximately day (d) 20 of gestation. Therefore we quantified the expression of genes known to be involved in labyrinth development. We also examined expression of genes which facilitate nutrient transfer in the placenta.
Methods: *Glial cell missing 1* (*Gcm1*), *Mitogen activated protein kinase kinase 1* (*Map2k1*), *Glucose transporter 1* (*Glut1*), *Insulin-like Growth Factor 1* (*Igf1*), *Vascular endothelial growth factor A (Vegfa),* and *Vascular endothelial growth factor receptor 2 (Vegfr2)* were examined across gestation using qPCR.
Results: Increases in *Map2k1* and *Gcm1* were observed at the time (d20) when the labyrinth could be first clearly distinguished. Increases in *Glut1* in the Junctional Zone, and *Igf1* (in the labyrinth) were greatest when the labyrinth reached its maximum size relative to the placenta as a whole on d25. *Vegfa* in the labyrinth increased steadily until term; expression of its receptor (*Vegfr2)* in the labyrinth was relatively constant from d20 to term.
Discussion: The increase in *Gcm1* and *Map2k1* coincide with the structural development of the labyrinth, consistent with transgenic mouse studies showing these genes are essential for labyrinth development. The increase in *Glut1* and *Igf1*, observed after the labyrinth reaches its relative maximum size suggests increasing capacity of labyrinth tissue to allocate nutrients necessary to support the exponential increase of fetal growth that occurs after this time. The expression patterns of *Vegf* and its receptor suggests that vascular development is an ongoing process in the spiny mouse placenta throughout gestation
Keywords: Spiny Mouse, Labyrinth Development, Map2k1, Vegf

[P1.29]

IMMUNOLOCALIZATION OF THE CAVEOLINS IN THE MATERNAL-FETAL INTERACTION IN TERM CLONED CATTLE PLACENTA

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This work was conducted in order to analyse the contribution of the caveolins -1, -2 and -3 proteins in the placental transport by immunohistochemistry since that proteins are implicated in internalization of substances into the cells and in many signaling functions. In our work we utilized cloned fetuses produced by nuclear transfer of somatic cells. Histology and immunohistochemistry (anti- caveolin -1, -2 e -3) were performed on placentomes and uteri from 5 cloned (term gestation) and 5 controls in the same gestation period. The tissues were snap-frozen or formalin fixed. The caveolins -1 were localized in the fetal and maternal villi, but stronger in the endometrial stroma. Caveolins -2: positive stain in the trophoblast and specifically in a trophoblast giant binucleate cell (BNC). Caveolins -3: positive in the trophoblast epithelium and in BNC, endometrial stroma and in the muscular layer of the placental vessels. This suggests that caveolins -1, -2 and -3 could participate in the placental transport, mainly in internalization of substances into maternal and fetus cells of the maternal-fetal interaction and influence the occurrence of abnormalities in the in cloned cattle placenta.
Funding: FAPESP and PROPE UNESP
Keywords: placental transport, caveolins, cloned cattle placenta, Imunolocalization

[P1.30]

GFP EXPRESSION IN TROPHOBLAST CELLS OF TRANSGENIC CLONED BOVINE PLACENTA

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Introduction: The migratory ability of trophoblast giant binucleated cells (TGC) towards to maternal tissues has been previously described in many species including the bovine. These cells migrate and fuse with maternal epithelial cells to deliver fetal proteins to maternal side. TGCs are reported to express specific proteins, such as, placental lactogens and pregnancy associated glycoproteins. In cloned bovine placenta an increase of TGCs number accompanied to many other placental abnormalities was reported. Herein, we used the transgenic GFP cloned embryo model to evaluate the migration of fetal cells in the bovine placenta.
Methods: Samples of placentome and intercaruncular endometrium from pregnant and nonpregnant uterine horns were obtained from GFP transgenic cloned embryo pregnancies at 60 (n=3) and 90 (n=3) days. Samples were preserved in 4% buffered PFA and paraffin-embedded for immunohistochemistry (IHC) analysis or snap-frozen in liquid nitrogen for western blotting (WB), Real time PCR and fluorescence microscopy.
Results: Through this model we expected to observe the migration of TGCs to maternal tissues. However no positive cells for GFP were observed in maternal side. The IHC analysis showed a lack GFP expression in the TGCs whereas the expression of the GFP was observed throughout the trophoblastic cells in all analyzed samples. In WB analysis, as expected GFP was present in placentome and placental membranes. We also observed GFP expression in the intercaruncular endometrial samples from both pregnant and non-pregnant uterine horns.
Conclusion: The absence of expression of GFP in TGCs suggests that a GFP gene silencing in response to the highly specific protein synthetic profile. Presence of GFP in intercaruncular region and non-pregnant uterine horn tissues may be generated by the high GFP solubility or by other mechanisms allowing permeability of the uterus to fetal protein products during pregnancy in the cow.
Funded by FAPESP.
Keywords: Green Fluorescent Protein, Trophoblast Giant Cell, Transgenic Cloned Embryo

[P1.31]

EXPRESSION OF TIGHT JUNCTIONAL PROTEINS IS NOT NECESSARILY ASSOCIATED WITH PHYSIOLOGICAL BARRIER FUNCTION IN CULTURED BOVINE PLACENTAL CELLS

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Introduction: Tight junctions (TJ) are a parameter for cellular polarity due to their ability to regulate the paracellular flow of molecules and to separate molecules into apical and basolateral domains. In the synepitheliochorial bovine placenta the feto-maternal barrier is composed of maternal caruncular epithelial cells and trophoblast cells interdigitating with each other via a microvillous brush border. Any substance passing from one compartment to the other has to move through these two layers. To study fetal-maternal interaction and transportation through the feto-maternal barrier *in vitro*, the aim of this study was to characterize junctional complexes, as well as polarity and barrier function in bovine caruncular epithelial cells (BCEC-1) and trophoblast cells (F3).
Methods: The presence of TJ in cultured bovine placenta cells was evaluated by immunohistochemistry of TJ proteins such as ZO-2, occludin and claudin. TJ complexes were visualized by transmission electron microscopy (TEM). Furthermore, BCEC-1 and F3 cells were examined morphologically by scanning electron microscopy (SEM). The barrier function of TJ was assessed by measuring the transepithelial electrical resistance (TEER) in cells growing on inserts.
Results: ZO-2, occludin and claudin-11 were detected in BCEC-1 and F3 cells by immunofluorescence. Junctional complexes occurred frequently in BCEC-1 but were observed less numerous in F3 by TEM. SEM showed that the apical cell membrane of BCEC-1 and F3 cells was covered densely with microvilli. F3 cells did not form compact monolayers as seen in BCEC-1. Only light microscopically confluent BCEC-1 developed considerable TEER values, while F3 cells developed no detectable resistance.
Conclusions: Our results have shown that cultured bovine placental cells are polarized and exhibit junctional complexes. However, only maternal BCEC-1 cells develop a functional physiological barrier. From these *in vitro* studies, we speculate that different properties of permeability are present in the maternal and fetal compartment of the bovine placenta.
Keywords: bovine, trophoblast, caruncular epithelium, tight junctions

[P1.32]

SYSTEMIC MATERNAL-FETAL TOLERANCE IN COWS

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The conceptus is a semi-alogeneic graft so the maternal immune system is constantly challenged by it. Therefore, for its successful implantation and development, several immune-regulatory mechanisms, local and systemic, are activated to tolerate the conceptus antigens. They include the regulatory roles of CD4+CD25+Foxp3 T cells, indoleamine 2,3 dioxygenase enzyme- IDO, uterine macrophages, T cell Ig and mucin domain (Tim)-3 among others. Nevertheless, most of this data originates from rodents and human observation. Differently from these species, bovines have a synepitheliochorial type placenta, practically non-invasive, that drastically reduces the conceptus antigens exposure to the maternal immune system. Little is known regarding the tolerance mechanisms in this species and how do they behave in this context. Therefore, this work aimed to verify if there is any systemic immune-tolerance in pregnant cows by accessing some innate and adaptive immune responses features, as the capacity of proliferation of the peripheral blood mononuclear cells (PBMC) and their phagocytic capacity, respectively.
Peripheral blood of six non-pregnant and twelve pregnant cows was used. The PBMC was separated by Ficoll-Paque, added 10 mM of CFSE, treated with Concanavalin A, incubated and analyzed by flow cytometry. The phagocytic capacity was conducted using a commercial kit PhrodoTM and analyzed by flow cytometry. The results demonstrate that the proliferation of the PBMC of non-pregnant cows stimulated with Concanavalin A was significantly higher ($p < 0.001$) than those of pregnant cows disregarding the gestational period. The phagocytic capacity of pregnant cows was reduced when compared to non-pregnant animals ($p < 0.05$). The reduced proliferation index of peripheral lymphocytes as well as the phagocytic capacity of macrophages from PBMC of pregnant cows compared to non-pregnant ones strongly suggests a systemic regulation of the maternal immune response.
Keywords: Immune-tolerance, Bovine, Lymphocyte Proliferation, Phagocytic Capacity

[P1.33]

WHAT IS GOING ON WITH NON-PREGNANT HORN IN PLAINS VISCACHA?

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Plains viscacha *Lagostomus maximus maximus* (Rodentia, Hystricognathi, Chinchillidae) belongs to Argentine wild faune. Various studies so far deal with their very distinct characteristics of reproductive parameters and endocrine organs. In particular, female viscachas produced around 300-800 ova at each cycle, but only 10% were fertilized, resulting in 2 offspring with advanced developmental status (Mossman & Duke, 1973). In its natural environmental plains viscacha had a monoestrous cycle, but in captivity they could be polyoestrous. However, there are still only few data on the reproductive system itself. We therefore investigated structural parameters of the early developmental processes. Four female reproductive apparatus were obtained from Estación de Cría de Animales Silvestres, Buenos Aires, Argentine; the necropsy was performed at the Faculty of Veterinary Science, La Plata University, and fixed in 4% paraformaldehyde. Samples were processed for light microscopy in the Faculty of Veterinary Medicine and Animal Science, University of Sao Paulo, Brazil. Sections of 5μm were stained with haematoxylin and eosin. Female reproductive system was composed by two ovaries, two uterine tubes, a bicornuate uterus, and one vagina. After the mean cycle, early embryologic stages were present in both uterine horns. Multiple implantations were observed in the uterine horns, in a variable number and shape, but not exceeding 6 for each horn. These implantations showed a clustered appearance while proceeding pregnancy. Implantations near to uterine tube were associated with a dark color of the uterine body as well as amorphous appearance and irregular shape. Larger implantations showed light color with a circular cavity. When studied late stages of pregnancy were only found embryos near to the uterine body, while the others had disappeared. Data suggest the position of the implantating eggs in uterine horns is crucial for its developmental success and the uterine tissue has the power to regulate implantation processes.

Keywords: Lagostomus maximus, female reproductive apparatus, uterus, morphology

[P1.34]

MATERNAL TREATMENTS WITH FOLIC ACID AND SAFFLOWER OIL PREVENT MATRIX METALLOPROTEINASES OVERACTIVITY IN THE EMBRYO AND DECIDUA FROM DIABETIC RATS

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Introduction: Maternal diabetes induces embryo malformations, related to the intrauterine pro-inflammatory environment. Maternal treatments with folic acid and safflower oil (enriched in linoleic acid, an endogenous agonist of peroxisome proliferator activated receptors (PPARs)) can prevent congenital malformations, although the mechanisms involved are unclear. Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in developmental processes that can be overactivated in a pro-inflammatory environment.

Aim: To analyze the effects of maternal treatments with folic acid (FOL) and/or safflower oil (SAF) on oxidative stress and MMPs activities in the embryo and decidua from control and diabetic rats on day 10.5 of pregnancy.

Methods: Diabetes was induced in rats by streptozotocin administration (50 mg/kg) prior to mating. Treatments with FOL (0.5% s.c.) and SAF (6%, dietary supplementantion) were given from days 0.5 to 10.5 of gestation. Isoprostanes and TBARs were measured as indexes of oxidative stress. MMP-2 and MMP-9 activities were measured by zymography.

Results: Embryos from diabetic rats showed increased levels of isoprostanes ($p<0.01$) when compared to controls, whereas FOL and FOL+SAF treatments prevented these anomalies ($p<0.001$). TBARs were increased in decidua from diabetic rats ($p<0.05$) when compared to controls and were highly reduced by SAF, FOL and FOL+SAF ($p<0.001$). In embryos from diabetic rats MMP-2 and MMP-9 activities were enhanced ($p<0.05$), whereas both SAF ($p<0.05$) and FOL+SAF ($p<0.05$) prevented MMPs overactivation. The decidua from diabetic rats showed enhanced activity of MMP-2 and MMP-9 ($p<0.001$), SAF diminished MMP9 overactivity ($p<0.05$) and FOL+SAF reduced both MMP-2 and MMP-9 ($p<0.01$) overactivities. The elevated malformation rate in diabetic rats ($p<0.001$) was reduced with maternal treatments with FOL, SAF and FOL+SAF ($p<0.05$).

Conclusions: In embryos and decidua from diabetic rats, FOL and SAF have the ability to reduce the malformation rate at least in part through the regulation of oxidative stress and MMPs overactivity.

Keywords: matrix metalloproteinases, inflammation, folic acid, PPARs

[P1.35]

IMPACT OF MATERNAL MILD HYPERGLYCEMIA ON RAT OFFSPRING DEVELOPMENT AND BEHAVIOR

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Introduction: Exposure to the diabetic intrauterine milieu has long been recognized to have important consequences for the fetus and the newborn [1,2,3,4]. The present study aimed to evaluate the impact of maternal mild hyperglycemia of Wistar rats on female and male offspring development and behavior in different moments of life.

Methods: At birth, female rats were assigned either to Control (subcutaneous (sc) citrate buffer;n=20) or STZ group (streptozotocin (STZ)-100 mg/kg-sc.;n=61). Rats were mated (90 days) and on pregnancy day (PD) 15 the insulin tolerance test (ITT) was carried out and on PD17 the glucose tolerance test (GTT). Glycemia was measured on PD 0, 7, 14 and 21. Around PD22 the rats delivered naturally and the newborns were classified according to their birth weight (First generation–F1). At birth, F1 newborns were divided into 4 groups: male offspring of control mothers (MControl;n=93); female offspring of control mothers (FControl;n=74); male offspring of diabetic mothers (MSTZ;n=76), and female offspring of diabetic mothers (FSTZ;n=103). Physical development was daily evaluated. Body weight, length and anogenital distance were measured. Behavioral activity was evaluated in an open field.

Results: STZ rats presented increased glycemia on PD0 and impaired glucose tolerance when compared to Control rats. Offspring from STZ dams presented increased body weight on postnatal day (PND) 0. Physical landmarkers of development such as pinna unfolding and eye opening were forwarded in the STZ rats offspring while hair growth was delayed. Open field behavior showed decreased immobility in offspring of diabetic dams in PND10 for females and PND75 for males.

Discussion: Streptozotocin administration in the neonatal period caused mild hyperglycemia during pregnancy. Offspring of diabetic dams presented alterations in physical development landmarkers and open filed parameters, in agreement with other studies [5,6,7], thus concluding that even a mild hyperglycemia during pregnancy is enough to impair offspring development and behavior.

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Keywords: Mild Hyperglycemia, Behavior, Offspring, Development

[P1.36]

MATERNAL-FETAL REPERCUSSIONS OF TREATMENT WITH *AZADIRACHTA INDICA* (NEEM) IN DIABETIC AND NON-DIABETIC PREGNANT RATS: PRELIMINARY RESULTS

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Introduction: Women with poorly controlled *Diabetes mellitus* have a much higher incidence of pregnancy complications1. In Brazil, *Azadirachta indica* (Neem) is one plant frequently used as anti-diabetic remedies2. The aim was to investigate the effects of oral treatment with Neem oil on maternal reproductive outcome; fetal and placental development of the diabetic and non-diabetic pregnant rats.

Methods: Diabetes was induced by streptozotocin (100 mg/kg, sc)3 in *Wistar* rats (mild diabetes group - MD) on the day of birth. As adults, rats were mated and, when positive diagnosis of pregnancy was identified, were distributed into 4 experimental groups: G1) Non-diabetic treated with vehicle; G2) Non-diabetic treated with Neem oil (1.2 mL/day) during whole pregnancy; G3) MD treated with vehicle; G4) MD treated with Neem oil in the pregnancy. Glycemia was measured at days 0 and 20 of pregnancy. At term pregnancy, each rat was anesthetized and killed for reproductive performance analysis, fetal and placental weights. Maternal and fetal data were analyzed by ANOVA followed by the Student Newman–Keuls test.

Results: G3 rats presented higher glycemia on day 0 of pregnancy and reduced fetal weight (4.14±1.08g, $p<0.05$) compared to other groups (G1=5.17±0.86g; G2=5.10±0.43; G4=5.23±0.49g). It was verified significantly increased liver weight in the G2 (18.30±2.36g) and G4 (18.20±1.76g) groups compared to their control groups (G1=13.13±0.01; G3=15.30±1.50g). The placental weight showed no alteration in the different groups.

Discussion: Neem oil caused alteration lipid metabolism leading increased liver weight, which suggests a liver steatosis. However, there were no alterations on the maternal reproductive outcome but there was an improvement in fetal weight from diabetic rats after treatment. Thus, using the data obtained at the present moment, we concluded this product contributed to avoid intrauterine growth restriction caused by diabetes.

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Keywords: diabetes, fetus, *Azadirachta indica* (Neem), pregnancy, rats

[P1.37]

PERIVASCULAR CELLS OF FETOPLACENTAL VESSELS IN PREGNANCIES COMPLICATED BY TYPE I DIABETES MELLITUS

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Introduction: Angiogenesis takes place in the placenta until delivery as demonstrated by expression of markers for neovasculature in the placenta at term. Perivascular cells such as smooth muscle cells and pericytes participate in the vascular wall formation in neovessels. Here we studied the extent of pericyte coverage in microvessels of normal pregnancies and pregnancies complicated by type I diabetes mellitus (DMI). In addition, we characterized the phenotype of pericytes in normal and DMI pregnancies.
Material and Methods: The placentas from normal pregnancies (n=8) and placentas from mothers with DMI (n=18) were obtained at the time of delivery. The specimens were collected using unbiased systematic random sampling, fixed with formaldehyde and embedded in paraffin. Immunohistochemical detection was performed using standard procedures.
Results: Pericytes of capillaries in terminal and intermediate villi were immunoreactive for smooth muscle actin (SMA), but they were negative for intermediate filament desmin. In addition, perivascular cells expressed PDGFR-beta, although the expression was variable. We thus used SMA as a marker for quantitation of pericyte coverage in placental microvessels. The proportion of capillaries covered with SMA+ pericytes (microvessel pericyte coverage index) was 84±13% in normal vs. 79,5±13% in DMI pregnancies. Extent of pericyte coverage around the vessel circumference was 38±11% in normal vs. 33±10% in DMI pregnancies.
Discussion: Immunohistochemical phenotyping of perivascular cells in human fetoplacental vessels showed that pericytes surrounding capillaries in terminal and intermediate villi are SMA+/desmin- perivascular cells. This further demonstrates great variability of these cells in different organs. The phenotype of pericytes in normal pregnancies and in pregnancies complicated with DMI was virtually identical. A statistically significant difference in the extent of pericyte coverage around the vessel circumference between DMI and normal pregnancies was not found.
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Keywords: angiogenesis, pericytes, capillaries, diabetes mellitus

[P1.38]

VASOCONSTRICTION INDUCED BY SEROTONIN IS REDUCED BY ADENOSINE IN HUMAN UMBILICAL VEINS FROM GESTATIONAL DIABETES

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Adenosine is a vasoactive nucleoside that contributes to the physiological vasomotor function of the human umbilical vein. Adenosine-triggered signalling mechanism have been shown to be altered in umbilical vein endothelial cells (HUVEC) from gestational diabetic pregnancies, an effect proposed to be determinant for the abnormal vasoreactivity observed in response to vasoconstrictors or vasodilators in gestational diabetes. We evaluated adenosine role on 5-hydroxytriptamine (5HT)-induced vasoconstriction in isolated human umbilical vein (HUV) rings from normal (HUV-N) and gestational diabetic (HUV-GD) pregancies.
Methods: HUV intact or endothelium-denuded rings were mounted in a wire myograph to determine vasoactive response to 5HT (0.1 nM - 10 μM). HUV rings were preincubated (1 min) in absence or presence of adenosine (0.1 μM-1 mM) and ZM241385 (10 μM, A2A adenosine receptors antagonist). Responses were expressed as a percentage of KCl-induced contraction (%Kmax).
Results: 5HT-induced contraction in intact HUV-GD exhibited higher ($P<0.05$, two way ANOVA test) half-maximal effect (EC50 33 ± 4 nM 5HT) compared with HUV-N (11 ± 1 nM 5HT), an effect that was independent of endothelium. Adenosine mimicked gestational diabetes effect in the response to 5HT (EC50 39 ± 5 nM 5HT at 10 μM adenosine) in HUV-N, but did not alter the vascular response in HUV-GD. Adenosine effects were blocked by ZM241385 and independent of endothelium. In addition, ZM241385 in absence of exogenous adenosine blocked the effect of gestational diabetes on 5HT-induced vasoconstriction.
Conclusion: Gestational diabetes is associated with altered umbilical vein reactivity to vasoconstrictors most likely due to activation of A2A adenosine receptors by adenosine. The vasocontrsitcion induced by 5HT seems not to involve the vascular endothelium in this phenomenon.
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Keywords: Gestational Diabetes, Endothelium, Vasocontriction, Adenosine

[P1.39]

FETAL-DERIVED ENDOTHELIAL PROGENITOR CELLS MAY CONTRIBUTE TO PLACENTAL VASCULATURE AND THE PATHOGENESIS OF IUGR

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Introduction: Circulating Endothelial Progenitor Cells (EPCs) are recruited from the bone marrow and contribute to vessel formation. One subpopulation, Endothelial Colony Forming cells (ECFCs), form endothelium. A second subpopulation, Circulating Angiogenic Cells (CACs), stimulate ECFCs. We aimed to investigate (1) whether EPCs migrate to the placenta from the human fetus and contribute to its vasculature; and (2) whether in intrauterine growth restriction (IUGR) the amounts of EPCs sequestered by the placenta differ or their proliferative capacity is changed.

Methods: (a) CAC- and ECFC- blood counts (as percentage of mononuclear cells) were determined by five-colour flow cytometry in the umbilical arteries and veins of 23 normal and 9 growth restricted newborns (Individualised Birth Weight Ratio<5) at 36-41 gestational weeks. ECFCs were defined as $7AAD^-/CD31^{bright}/CD45^-/KDR^+/CD34^+$ and CACs as $7AAD^-/CD31^+/CD45/CD133^+/CD34^{+1}$.

(b) ECFCs were isolated from fetal peripheral mononuclear cells by a culture technique, which selectively propagates these cells[2]. The phenotype of outgrowth cells was carefully confirmed by a range of established surface markers and functional assays.[3]

Results: (1)CAC- and ECFC-levels were significantly higher in the umbilical artery than vein (CAC:$5.5x10^{-1}$[$2.7\ x10^{-1}$-$8.2\ x10^{-1}$] vs. $4.0x10^{-1}$[$1.8\ x10^{-1}$-$7.1x10^{-1}$];$p<0.05$,n=13, ECFC:$3.0x10^{-3}$[$7.2x10^{-4}$-$7.0x10^{-3}$] vs. $5.0x10^{-4}$[0.0-$2.4x10^{-3}$], n=17; Median[Interquartile Range];$p<0.05$). (2) Flow cytometry showed that more EPCs are sequestered by the placenta in normal pregnancy than in IUGR (CAC:$8.4x10^{-2}$[$4.6x10^{-2}$-$1.3x10^{-1}$];n=12 vs. $9.7x10^{-4}$[$-6.0x10^{-2}$-$5.5x10^{-3}$];n=9, ECFC:$2.3x10^{-3}$[$4.6x10^{-4}$-$4.2x10^{-3}$]; n=17 vs. 0.0[0.0-$1.7x10^{-5}$];n=9;$p<0.05$). In culture, normal ECFCs formed more colonies (8.1 [4.8-12.2];n=12 vs.1.0[0.4-3.5]; n=8;$p<0.05$) and had longer population doubling times (1.5[1.0-3.1];n=9 vs. 3.5[3.1-3.6]n=7;p,0.05) than did those from IUGR. Differences in colony forming times were not significant (12[9-16.5];n=13 vs.8[12.5-21.5]n=8;$p>0.05$).

Discussion: These data suggest that EPCs migrate from the fetal circulation to the placenta, where they may contribute to vessel formation. IUGR is associated with decreased EPC migration and abnormal behaviour in culture. These anomalies in the numbers or function of EPCs may contribute to the pathophysiology of placental development in IUGR.

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Keywords: Fetal Endothelial Progenitor Cell, Placental Angiogenesis, Intrauterine Growth Restriction

[P1.40]

ENHANCED PROLIFERATION OF JEG3 CELLS IN RESPONSE TO BENZO[A] PYRENE: IMPLICATIONS FOR INTRAUTERINE GROWTH RESTRICTION

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Objective - Maternal cigarette smoking is associated with intrauterine growth restriction (IUGR) and placental abnormalities by incompletely understood mechanisms. Benzo[a]pyrene (B[a]P), a major component of cigarette smoke, is embryotoxic. Our objective is to investigate the growth and cytotoxic responses of JEG3, a human trophoblast-like choriocarcinoma cell line, to B[a]P.

Methods - $5x10^4$ JEG3 cells were exposed to 0.0, 0.1, 1.0, 10.0 and 40.0μM B[a]P for 5 days in 5 separate experiments. The effect of B[a]P on cell growth was evaluated using MTT cell proliferation assay, and acridine orange and TUNEL assays for apoptosis. To investigate the cellular pathways modulated by B[a]P, we determined the activities of total and activated ERK mitogen activated protein kinase (ERK-MAPK), cyclin D1 a cell cycle regulating protein and epidermal growth factor receptor (EGFR) by Western blot analysis. We then performed micro-array analysis of cRNA from control and treated JEG3 cells to ascertain if exposure to B[a]P is associated with alterations in expression of genes relevant to cell proliferation.

Results – Exposure to 0.1μM, B[a]P caused a modest but significant increase in proliferation of JEG3 cells, an increased activity of cyclin D1, enhanced activation of ERK-MAPK and increased phosphorylation of EGFR. Exposure to 0.1μM B[a]P also caused a modest increase in expression of genes that regulate cell proliferation including the cyclins and MAPKs. Exposure to 10.0 or 40.0μM B[a]P was associated with a 20-25% increase in apoptosis.

Conclusion - Exposure of JEG3 cells to low concentrations of B[a]P is associated with enhanced cell proliferation through increased activity of cell growth associated proteins. This may partly explain the trophoblast hyperplasia often observed in placental villi of smoking mothers. Such proliferation may interfere with the syncytial nature of the trophoblast epithelium, increase the thickness of the villous epithelium and interfere with transplacental transport, which may contribute to IUGR.

Keywords: Benzo[a]pyrene, JEG3, Proliferation, Apoptosis

[P1.41]

ALTERED PLACENTAL EXPRESSION OF PAPPA2 DOES NOT AFFECT BIRTH WEIGHT IN MICE

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Introduction: Pregnancy-associated plasma protein A2 (PAPPA2) is an insulin-like growth factor binding protein (IGFBP) protease expressed in the placenta and upregulated in pregnancies complicated by pre-eclampsia. The mechanism linking PAPPA2 expression and pre-eclampsia, and the consequences of altered PAPPA2 expression, remain unknown. We previously identified PAPPA2 as a candidate gene for a quantitative trait locus (QTL) affecting growth in mice and in the present study examined whether this QTL affects placental PAPPA2 expression and placental or embryonic growth.
Methods: Using a line of mice that are genetically homogenous apart from a 1 megabase QTL region containing the PAPPA2 gene, we bred mice homozygous for alternate QTL genotypes and collected and weighed placentae and embryos at E12.5. We used quantitative RT-PCR to measure the mRNA levels of PAPPA2, as well as mRNA levels of IGFBP-5 (PAPPA2's substrate), and PAPPA (a closely related IGFBP protease) to examine potential feedback and compensation effects. Western blotting was used to quantify PAPPA2 protein. Birth weight was measured in pregnancies allowed to proceed to parturition.
Results: PAPPA2 mRNA and protein expression levels in the placenta differed by a factor of 2.5 between genotypes, but we did not find a significant difference between genotypes in embryonic PAPPA2 mRNA levels. Placental IGFBP-5 and PAPPA expression levels were not altered in response to PAPPA2 levels. The observed difference in placental PAPPA2 expression had no significant effect on placental or embryonic mass at mid-gestation, birth weight or litter size.
Discussion: Despite a significant difference between genotypes in placental PAPPA2 expression similar in magnitude to the difference between pre-eclamptic and normal placentae previously reported, we observed no difference in embryonic, placental or birth weight. Our results suggest that elevated PAPPA2 levels are a consequence, rather than a cause, of pregnancy complications.
Keywords: PAPPA2, pregnancy associated plasma protein, pappalysin, insulin-like growth factor binding protein

[P1.42]

PLACENTAL GROWTH RESTRICTION INDUCED BY DEXAMETHASONE (DEX) TREATMENT OF PREGNANT MICE IS NOT TRANSMITTED TRANSGENERATIONALLY BY THEIR MALE OFFSPRING

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Introduction: Placental size is a major determinant of fetal growth, which, in turn, affects postnatal phenotype, whether it affects placental phenotype in the next generation remains unknown. Here, we examine the consequences for F2 placentas of reducing F1 placental and fetal size by giving F0 dams DEX in mid- and late-pregnancy.
Methods: Pregnant C57BL6/J mice were given DEX (200ng/g daily, s.c.) from days (D) 11-15 (MID, maximal placental growth) or D14-18 (LATE, maximal fetal growth, term=D20.5). Controls were untreated. Postnatal fractional growth rate (FGR) was monitored after culling litters to 4. At 4 months, F1 males were mated with virgin females to produce an F2 generation. Placental and fetal weights were measured at D19 (n=7-19 litters) in both generations after euthanasia. Five month-old F1 males received a glucose tolerance test (GTT, 1g/kg i.p.) after 15h fasting. Differences between treatments were determined by univariate GLM with litter size as a covariate or one-way ANOVA and considered significant when $P<0.05$.
Results (Table)
F1 placental and fetal weights were significantly less in dex treated than control groups. Relative to controls, MID DEX F1 pups grew slower prior to weaning but faster afterwards, with intermediate values in the LATE DEX group. Offspring glucose tolerance differed with F0 treatment, with lower basal and maximum increments in blood glucose in LATE DEX males. There were no significant differences in F2 placental or fetal weights with F0 treatment at either D16 or D19.
Discussion: These results demonstrate that there is no paternal transgenerational inheritance of F1 feto-placental growth restriction induced by F0 DEX treatment. Furthermore, the F1 male phenotypic outcome of DEX-induced intrauterine growth restriction is more pronounced when treatment is given during the period of maximal placental growth.
Table: Mean ±SEM fetal and placental weights (n=7-19 litters) and F1 male FGR (n=5-25) in control, MID DEX and LATE DEX groups. a,b denote significant difference.

		Control	EARLY DEX	LATE DEX
F1 conceptus weight (mg)	D19 placenta	88 ± 2^{a}	79 ± 2^{b}	79 ± 2^{b}
	D19 fetus	1165 ± 16^{a}	1110 ± 16^{b}	1111 ± 18^{b}
F1 FGR (g/g/week)	Suckling 0-3weeks	2.08 ± 0.07^{a}	1.77 ± 0.05^{b}	1.96 ± 0.05^{ab}
	Weaning 3-6 weeks	3.80 ± 0.17^{a}	4.66 ± 0.16^{b}	4.50 ± 0.29^{ab}
F2 conceptus weight (mg)	D19 placenta	82 ± 1^{a}	83 ± 1^{a}	82 ± 1^{a}
	D19 fetus	1213 ± 17^{a}	1215 ± 21^{a}	1256 ± 18^{a}

Keywords: glucocorticoids, mouse, transgenerational programming, IUGR

[P1.43]

DIFFERENTIAL EXPRESSION OF PLACENTAL ENDOTHELIAL CELL-SPECIFIC HOMEOBOX GENE *HEX* IS ASSOCIATED WITH ABNORMAL ANGIOGENESIS IN FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA

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Abnormal angiogenesis, particularly in the villous microvasculature, is frequently associated with fetal growth restriction (FGR) and pre-eclampsia (PE) (1). In the placenta, angiogenesis is regulated by homeobox gene transcription factors. *HEX* is a negative regulator of endothelial cell function in cardiovascular development (2). Here, we hypothesised that *HEX* expression is altered in idiopathic FGR and in PE. Placentae were collected from idiopathic FGR or PE and from gestation age-matched controls (GMC). The level of *HEX* mRNA was determined using real-time PCR as described previously (3). Relative quantitation of *HEX* mRNA normalized to *GAPDH* revealed *HEX* expression was increased in FGR compared with GMC [1.38 ±0.27, FGR (n=25) vs. 1.08±0.21, control (n=25), t-test, $p<0.05$] and decreased in PE compared with GMC [0.84 ±0.04, PE (n=25) vs. 1.08±0.21, control (n=25), t-test, $p<0.05$]. Functional role of *HEX* was determined using human placental microvascular endothelial cells (HPEC) and three independent siRNAs (si) designated as si1, si 2 and si 3. The loss of *HEX* mRNA was confirmed by real-time PCR [1.0 ± 0.57, control vs 0.3 ± 0.16, si1; 0.4 ± 0.22, si2; and 0.3 ± 0.17, si3, (n=3), t-test, $p<0.05$]. Following *HEX* inactivation, the rate of migration of HPEC showed a significantly lower number of migrated cells in si treated HPEC compared with controls [6 ± 2, (si1); 6 ± 3, (si2); 7 ± 0.5, (si3) vs 52 ± 5 control (n=3), t-test, $p<0.01$]. Proliferation of HPEC demonstrated a significantly decreased rate of proliferation in si treated HPEC compared with controls [3016 ± 327, (si1); 3159 ± 290, (si2); 2500 ± 130, (si3), vs 7522 ± 402, control, (n=3), t-test, $p<0.001$]. Our study suggests that the altered expression of *HEX* may contribute to the abnormal non-branching and branching angiogenesis observed in FGR and PE, respectively.

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Keywords: placental angiogenesis, gene expression, fetal growth restriction, pre-eclampsia

[P1.44]

DOWN-REGULATION OF STARD7 BY RNA INTERFERENCE INCREASES β-HCG PRODUCTION AND SECRETION IN JEG-3 CELLS

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StarD7 is a member of the START-domain protein family whose function remains poorly defined. We have recently reported that StarD7 is partially relocated from the cytoplasm to the plasma membrane during *in vitro* cytotrophoblast differentiation into syncytiotrophoblast. Furthermore, we have shown that ß-catenin activates human StarD7 expression through Wnt/ß-catenin signaling. Herein, to explore its function in JEG-3 cells StarD7 expression was down-regulated by siRNA. Silencing of StarD7 expression, confirmed by qRT-PCR and western blot, led to a marked decrease of ß-catenin, Cnx43, iNOS, MBD2, ABCG2 and TGFβRII mRNA levels, all of them associated with Wnt signalling. In contrast, expression of syncytial formation markers, such as β-hCG protein production and secretion, as well as β-hCG mRNA levels, was increased by StarD7 siRNA. In addition, fluorescent microscopy performed by immunostaining for desmoplakin suggested that there was a reduction of intercellular desmosomes between adjacent JEG-3 cells after knocking-down StarD7 expression.

Whether this response is unique to this cell line is being currently investigating using other models such as BeWo and primary trophoblast cells. These findings indicate that the inhibition of StarD7 transcript level alters the expression of several critical genes suggesting that it may play a role in placental development.

Supported by CONICET, FONCy, MinCyT of Córdoba and SECyT-UNC
Keywords: StarD7, hCG production, JEG-3 cells, siRNA

[P1.45]

CONVERGENCE OF β-CATENIN SIGNALING AND SF-1 TRANSCRIPTION FACTOR ON STARD7 GENE PROMOTER

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StarD7 belongs to the START-domain proteins that are involved in intracellular transport and lipid metabolism. StarD7 was first identified over-expressed in JEG-3 cells compared to normal and benign trophoblastic samples. We previously reported that StarD7 expression was independently regulated by β-catenin signaling and SF-1 factor at the transcriptional level. Herein, a possible convergence between both activating factors was investigated. To evaluate this hypothesis, JEG-3 cells were co-transfected with the SF-1 or/and the constitutively active β-catenin S33Y expression plasmids, together with the -938/+157 StarD7 promoter construct, which contains the SF-1 and TCF consensus binding sites recognized by each protein. In addition, co-transfection assays were performed using these protein expression plasmids and mutated promoter versions in the SF-1 and TCF consensus sites (-938/+157mut SF1-1 and -938/+157mut SF1-1/mut TCF).

The results of this work show that SF-1 and β-catenin synergically activate StarD7 promoter. Moreover, these findings indicate that the TCF site localized at -614/-608 bp plays an important role in this activation. Furthermore, deletion of the 235-238 amino acids in the SF-1 transcription factor, involved in the β-catenin physical interaction, abolished this transcriptional activation; demonstrating that the interaction between the two proteins is necessary for an efficient StarD7 transcriptional activation.

Finally, these data suggest that β-catenin could function as a bridge between SF-1 and TCF forming a ternary complex, which would transcriptionally activate StarD7 gene.

Supported by CONICET, FONCyT, MinCyT of Córdoba and SECyT-UNC
Keywords: Wnt signaling, SF-1 transcription factor, StarD7, JEG-3 cells

[P1.46]

REGULATION OF PLACENTAL LEPTIN EXPRESSION BY ESTRADIOL INVOLVES GENOMIC AND NONGENOMIC ACTIONS

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Leptin is a cytokine-type hormone that controls the functional integrity of feto-placental unit, thereby maintaining pregnancy. Leptin is secreted by placenta, where it plays an autocrine trophic role. However, the regulation of leptin production in the placenta is still poorly understood. In the present study, we analyzed the effect of 17beta-estradiol (E2) on leptin expression in human placental cells.

Methods: BeWo choriocarcinoma cells and human placental explants were used. Western blot analyses were carried out to detect leptin expression as well as the phosphorylated form of proteins involved in different signaling pathways. qPCRs were performed to analyze leptin mRNA levels. Transfection assays with reporter constructs and expression vectors were used to determine transcriptional regulation of leptin.

Results: We have found that leptin expression was increased in both BeWo cells and placental explants, evidencing physiological relevance. Maximal effect was achieved at 100 nM E2 in BeWo cells, and was blocked with 10 nM ICI 182,780. The incubation with E2 also enhanced leptin promoter activity. The overexpression of ESRalpha, but not of ESRbeta, increased E2 effect on leptin promoter activity. These effects could be partially explained by membrane ESRs since treatment of cells with E-BSA increased leptin mRNA and protein. This effect was prevented with ICI 182,780. Moreover, the presence of ESRalpha was detected in membrane fraction of placental cells. On the other hand, E2 and E-BSA induced MAPK and PI3K pathways in placental explants. Inhibition of these signaling pathways with dominant negative mutant kinases or pharmacologic inhibitors prevents E2 effect on leptin expression.

Conclusions: All these findings suggest that E2 enhances leptin expression in human placental cells through genomic and nongenomic actions. These results provide new evidence of the mechanisms whereby E2 regulate leptin expression in placenta and confirm the importance of leptin in placental physiology.

Keywords: Leptin, Placenta, Estradiol

[P1.47]

ROLE OF ET-1 IN THE INDUCTION OF PLACENTAL ENDOPLASMIC RETICULUM STRESS IN PREGNANCY DISORDERS

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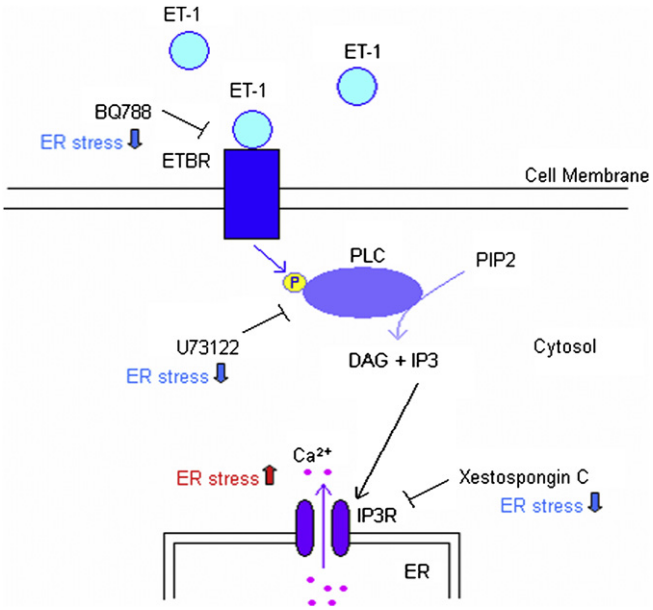
Introduction: Recent evidence implicates placental endoplasmic reticulum (ER) stress in the pathophysiology of pre-eclampsia and intrauterine growth restriction (IUGR). The ER is involved in synthesis and packaging of membrane and secretory proteins, and also serves as a reservoir of Ca2+. Loss of ER Ca2+ homeostasis suppresses post-translational modifications of proteins, triggering ER stress pathways. Endothelin (ET)-1 can induce Ca2+ release from the ER, and is increased in both IUGR and pre-eclampsia. This study therefore investigated whether ET-1 can induce ER stress.

Methods: Immunohistochemistry for ET-1 and the ETB receptor was performed on paraffin-embedded villous samples collected with informed patient consent (25-40 weeks). Human trophoblast-like JEG-3 choriocarcinoma cells were treated with different doses of ET-1 for 1 hour with or without inhibitors. Activation of signalling pathways was assessed by immunoprecipitation and Western blots.

Results: Immunohistochemistry confirmed the presence of both ET-1 and the ETB receptor in the syncytiotrophoblast. Immunoreactivity of both was increased in IUGR and pre-eclamptic samples compared to normal controls. JEG-3 choriocarcinoma cells treated with ET-1 displayed increased expression of ER stress markers, GRP-78 and GRP-94, in a dose-dependent fashion. Immunoprecipitation with anti-phospho-tyrosine antibody showed that ET-1 induces phospho-activation of the ETB receptor. That ET-1 acts via the ETB receptor was confirmed by treating cells with BQ788, an ETB receptor antagonist (Figure), which inhibited the induction of ER stress. ET-1 also stimulated p-PLC levels, which could be inhibited by U73122, providing a mechanism by which ET-1 can induce Ca^{2+} release from the ER. A third inhibitor, Xestospongin-C, which acts on the IP3 receptor, also inhibited induction of ER stress by ET-1.

Conclusion: These data show that ET-1 is able to induce ER stress via the ETB receptor by initiating signalling through the PLC/IP3 pathway. The results suggest an adverse autocrine action of ET-1 on the syncytiotrophoblast in pathological pregnancies.



[P1.48]

RELAXIN BOOST MAY BE FACILITATED BY FORMATION OF INTRACELLULAR VACUOLES IN LUTEAL CELL OF THE PORCINE OVARY: AN IMMUNOCYTOCHEMISTRY AND TRANSMISSION ELECTRON MICROSCOPY STUDY

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Introduction: Relaxin is best known as a reproductive peptide hormone essential during late pregnancy as it promotes growth and softening of the uterine cervix, thereby ensuring rapid and safe delivery and secondly it also promotes growth and development of the mammary apparatus. New physiological functions for this hormone in implantation, vascularization and placentation is emerging, but with great diversity in the physiology and biological effect among species. In the pig the the corpus luteae synthesize this hormone early in gestation with a progressive increase from day 20 to reach a peak at late gestation and then a markedly decline at the onset of parturition. The pattern of accumulation in luteal cells ensuring the boost was the topic for this investigation.
Methods: Corpus luteum (2-3 per stage) from13 stages were investigated, 5 stages (day 33-95) were fixed in glutaraldehyde and processed for electron microscopy (TEM) and 8 (day 55-90 and one nonpregnant in early luteal phase) were fixed in Bouins fixative for immunocytohemistry using a polyclonal relaxin antibody (Immunediagnostic) both by rutine laboratory methods. All controls were negative.
Results and discussion: In the nonpregnant a very weak reaction was shown, and no apparent vacuoles were seen. Cellular vacuoles increased in number per field to day 95. The Relaxin reaction often surrounded these vacuoles and in 10μm sections reaction products were also seen in the lumen of the vacuoles. By TEM the vacuoles varied in size from 1,2μm to 25μm and shape – round, oval or irregular and electron dense granules and their products seen in the lumen. A basal lamina surrounded all lutein cells, but were never seen lining the vacuoles. The lutein cell wall arround parts of the vacuoles were very thin, 0.1μm, often seen close to capillaries.
Conclusion: The vacuoles are storing relaxin facilitating fast and important boost prior to parturition.
Keywords: relaxin, ovary, preparturition effect

[P1.49]

LEPTIN ENHANCES CELL PROLIFERATION AND SURVIVAL IN PLACENTAL CELLS

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Fetal-maternal dialogue during implantation involves multiple regulators such as leptin. This 16KD protein plays diverse roles in placental growth and survival.
Previous results from our group demonstrated that leptin increases cell proliferation and survival in JEG-3 and BeWo cells. We also demonstrated that leptin expression is tightly regulated by different placental regulators. The aim of the present work is to study the mechanisms involved in placental proliferation and apoptosis.
Methods: BeWo and Swan cells, and human term placental explants were used. Western blot analyses were carried out to detect leptin, Bcl-2, Bax and p53 expression. Cell proliferation was determined by cell counting and 3H-thymidine incorporation. Transfection assays with reporter constructs were used to determine leptin effect on different transduction pathways.
Results: Leptin treatment in Swan cells increased cell proliferation up to 3 times. Maximal effect was achieved with 100 ng leptin/ml at two days of incubation. Caspase-3 activation was determined by Western blot. Leptin diminished the proteolysis of caspase-3 in a dose dependent manner. Moreover the diminution in endogenous leptin by treatment with an antisense oligonucleotide (2-4 μM) increases cellular apoptosis measured by caspase-3 activation. Bcl-2 and Bax levels were determined after leptin treatment and the relationship between them calculated. The expression of the key cell cycle regulator p53 was also determined. Slightly changes were observed
Conclusions: All these results reinforce the notion of leptin as a placental cytokine with the function of promoting growth and survival of placental cells.
Keywords: leptin, placenta, proliferation, apoptosis

[P1.50]

LOCALIZATION OF MIF RECEPTORS AT THE MATERNAL-PLACENTAL INTERFACE IN MICE

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Maternal-fetal interaction is crucial to the pregnancy success and involves molecular dialogues between regulatory factors. One of these molecules is the Macrophage Migration Inhibitory Factor (MIF), a pro-inflammatory cytokine released by activated leukocytes and immune-competent cells. MIF is expressed during pregnancy, particularly by trophoblast cells, in different species. The intracellular MIF signaling pathway is triggered by binding to the membrane receptor CD74 and phosphorylation of CD44 resulting in an effective intracellular kinase response and induction of MIF-dependent gene expression. Objective: Knowing the target cells to MIF at the maternal-fetal interface to facilitate the understanding of the downstream cascade and roles played by this factor during pregnancy. Material and Methods: Immunolocalization and gene expression of MIF receptors at implantation sites and placentas of females at gestation days 7.5, 9.5, 13.5 and 17.5. Results: The immunoreactivity for CD74 and CD44 antibodies was observed in decidua and leukocytes in all gestation days studied. At gd 10.5, 13.5 and 17.5 trophoblast giant cells and endothelial-like cells were also reactive for CD74. The immunolocalization of CD44 was showed in decidual cells and trophoblast giant cells on 10.5, 13.5 and 17.5 gd. Endothelial-like cells were reactive only on gd 13.5 and 17.5. The *Mif* mRNA expression for both receptors was found during all gestational periods in the decidua, peaking on gd 10.5. In trophoblast and fetal placental compartment relevant expression were seen only for CD74, exclusively on gd10.5. In conclusion, the expression of both MIF receptors in decidual cells associated with previous results showing MIF expression by trophoblast cells, suggest a paracrine mechanism. Studies to determine the functions associated to this putative dialogue at the maternal-placental interface are now in progress in our laboratory.

Financial support: CNPq.

Keywords: MIF, CD74, CD44, Placenta

[P1.51]

DIFFUSIVE OXYGEN FLUXES TO CAPILLARIES WITHIN THE HUMAN PLACENTA

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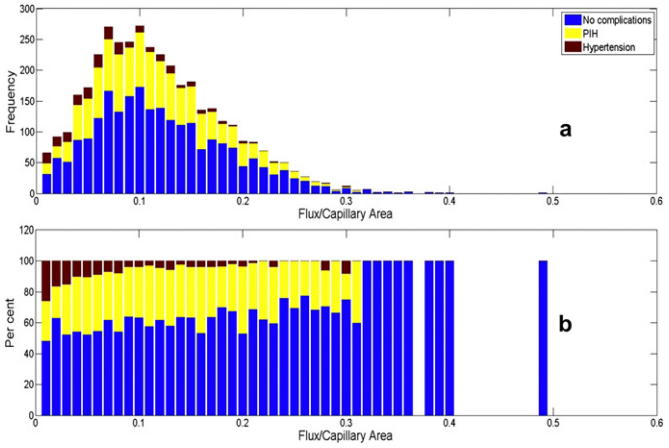
Goal/Background: The mature placenta has a complex vascular network extending to the capillary beds of the terminal villi, the sites of all oxygen and nutrient exchange between the mother and fetus. Respiratory transfer across the placenta to the fetus occurs in three steps: (i) maternal blood bathes the chorionic villi in oxygen, (ii) oxygen permeates the villus surface and diffuses into the fetal capillaries, and (iii) oxygen is transported to the fetus by fetal blood.

Materials/Methods: Step (ii) has been modelled as oxygen diffusion from the villous membrane to the fetal capillaries. The stationary oxygen concentration $c(\mathbf{x},t)$ within each villus is the solution of Laplace's equation in two dimensions, $c_{xx}+c_{yy} = 0$, with a fixed value at the villous surface and a Robin boundary condition at the capillary boundary: $D\partial c/\partial n = Kc$, where $\partial/\partial n$ is outward normal derivative, D is the oxygen diffusion constant and K is the permeability of the capillary. These equations are solved within regions between the villi and capillary boundaries obtained from digitized images.

Results: The diffusive current of oxygen across the capillaries is influenced by many factors, including the shapes of villi and the number and spatial arrangement of the capillaries. Distributions of oxygen transport characteristics determined from our calculations reveal systematic trends and reveal the effect of certain complications. Panel (a) shows the capillary flux per unit area for all calculations, with a breakdown of the distribution and percentages [panel(b)] into normal placentas and those with pre-existing and pregnancy-induced hypertension (PIH). The fluxes of PIH Placentas are skewed toward lower values, which is qualitatively different from the other placentas.

Conclusions: The statistics of oxygen transport characteristics within capillaries of the human placenta are capable of identifying certain abnormalities. Further work should clarify the extent to which this can be generalized to other conditions.



Keywords: Oxygen diffusion, placental function, villous capillary

[P1.52]
IDENTIFICATION OF CHEMOKINE EXPRESSION PROFILES IN CHORIODECIDUA FROM WOMEN IN PRETERM AND TERM LABOUR

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Introduction: Current therapies for preterm labour (PTL) focus on arresting myometrial contractions. These are not effective therefore alternative therapeutic targets need to be identified. Leukocytes infiltrate the gestational tissues during labour and have been implicated in these processes. We hypothesize that chemokines mediate leukocyte recruitment in PTL and term labour (TL), and therefore are potential targets for preventing PTL.
Methods: Women were recruited into 4 study groups: TL, term not in labour (TNIL), PTL and PTL with infection (PTLi) (n=8-10/group). Choriodecidua was collected and RNA extracted. Pooled RNA from the 4 groups was subjected to a pathway specific PCR array for chemokines. Differential expression of candidate genes was validated by real time RT-PCR. Chemokine proteins were localised in fetal membranes using immunohistochemistry.
Results: 25 chemokines were upregulated (2-120 fold change) in choriodecidua from TL compared to TNIL. A similar pattern was detected in PTL, with 18 of the same chemokines upregulated (2-51 fold change) and 2 chemokines downregulated. However a distinct cohort of chemokines was expressed in PTLi, with 14 genes differentially expressed between PTL and PTLi. 10 genes were selected for further study: CCL2, CCL4, CCL5, CCL8, CCL18, CXCL1, CXCL8, CXCL9, CXCL10, CX3CL1. The same expression patterns were observed by real time PCR on both pooled and individual samples. Most chemokines were expressed by both chorion and decidua, localised to decidual stromal cells, chorion trophoblast and infiltrating leukocytes.
Discussion: These data provide compelling evidence that chemokines regulate choriodecidual leukocyte recruitment during labour. The similarities between chemokine profiles in TL and PTL suggest the same processes are occurring, but are triggered precociously. Distinct chemokine profiles in PTL and PTLi is consistent with differences in leukocyte subpopulations present and confirm different aetiologies. Chemokines may represent novel therapeutic targets to prevent PTL.
Keywords: Chemokines, Leukocytes, Decidua, Fetal Membranes

[P1.53]
IMPLICATION OF ORAL BACTERIA IN PLACENTAL INFECTIONS

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Intrauterine infection is a leading cause of adverse pregnancy outcome ranging from preterm birth to stillbirth. The current paradigm indicates that intrauterine infection predominantly originates from the vaginal tract, with the organisms ascending into the otherwise sterile uterus. With the improvement in technology, an increasing number of microorganisms have been identified in intrauterine infection that do not belong to the vaginal microbiome. Studies in both humans and animals have demonstrated that intrauterine infection can originate from the oral cavity following hematogenous transmission. We have recently reported a case of human term stillbirth in which the same *Fusoabcterium* clone that killed the fetus was found in the mother's oral flora but not in her vaginal or rectal floras. Our animal studies have demonstrated that oral *Fusobacterium* causes fetal death by stimulating TLR4-mediated placental inflammatory responses, a mechanism which can be shared by other organisms. Thus, we began to systemically address what proportion of the oral microbiome can translocate to the placenta and cause infection. Pooled saliva and subgingival plaque samples were injected into pregnant mice through tail veins to mimic bacteremia, which occurs frequently during periodontal infection. The organisms colonizing the murine placenta were detected using 16S rRNA-based PCR and clone analysis. A diverse group of organisms were identified, including *Aggregatibacter, Campylobacter, Capnocytophaga, Eikenella, Erysipelothrix, Fusobacterium, Gemella, Granulicatella, Leptotrichia, Microbacterium, Neisseria, Parvimonas, Peptostreptococcus, Prevotella, Porphyromonas, Streptococcus, Selemonas, TM7* phylum, and *Veillonella*. Interestingly, many of them have been associated with adverse pregnancy outcome in humans although their sources of infection were not determined. The majority of these species were oral commensal organisms. This may be due to a dose effect but may also indicate the role of commensal organisms in intrauterine infection. A number of species were selectively “enriched” during the translocation with a higher prevalence in the placenta than in the pooled saliva or subgingival plaque samples. These observations indicate that the placental translocation was species specific. This study provides the first insight into the diversity of oral bacteria which may be involved in intrauterine infection. Oral bacteria play a previously unrecognized role in placental infections.
Keywords: oral bacteria, placenta, inflammation, colonization

[P1.54]

MONOCHORIONIC TWIN PREGNANCY IN SPONTANEOUS AND ASSISTED REPRODUCTION: A FAVORABLE OUTCOME

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Introduction: to assess obstetric complications and neonatal outcome in spontaneous and by assisted reproductive technology (ART) monochorionic twin pregnancies.
Methods: Obstetrical data and neonatal outcome of monochorionic twin pregnancies were retrospectively analyzed between January 2006 and December. Twin pregnancies were divided into spontaneous, ART and corionicity. In monochorionic pregnancy biometrical and Doppler data were performed weekly.
Results: Among the 196 twin pregnancies studied, there was a statistically significant difference between the number of bichorionic (BB) and monochorionic biamniotic (MB) pregnancies (41 patients) as expected.
There were no statistically significant differences of gender and frequency of some obstetric diseases. There was in 10 cases a Twin to Twin Transfusion Syndrome (TTTS) with subsequent death of 4 fetuses for severe prematurity. In the monochorionic non ART group were observed a case of diaphragmatic hernia and two with heart disease. In 97.5% of cases was performed a Caesarean section, in most cases for programming assistance (41%), TTTS (24%), beginning of labour (17%), IUGR (7.3%) and other reasons (10.7%), with the same distribution between the groups. There was also a vaginal delivery a term. The infants were comparable for gestational age at delivery, birth weight and Apgar at 5 minutes. There was a statistically difference in incidence of SGA fetuses in utero and at birth. The neonatal outcome was comparable with the others groups.
Conclusions: monochorionic biamniotic pregnancies do not have an unfavourable outcome compared to bichorionic biamniotic pregnancies in terms of maternal and neonatal morbidity.
Keywords: spontaneous twin pregnancies, twin pregnancy in assisted reproduction, neonatal outcome, management

[P1.55]

ADIPONECTIN IN FIRST TRIMESTER OF PREGNANCIES THAT LATER DEVELOP PREECLAMPSIA: RELATION TO PROGNOSIS AND ADIPOKINES

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Background: Adiponectin is a major insulin sensitizing adipocytokine. In pregnancy, elevated as well as reduced concentrations of adiponectin have been reported in serum from pregnancies complicated by pre-eclampsia. We assessed whether serum adiponectin was a first trimester screening marker of PE and explored the relation to clinical course of PE as well as the relation to other adipokines in maternal serum.
Design, Setting & Patients: Case control study with first trimester, gestational week 10^{+3} – 13^{+6}, serum samples from the Copenhagen First Screening Trimester Study. There were 123 pregnancies that developed PE and 285 control pregnancies.
Results: The maternal serum adiponectin concentration was significantly reduced in pregnancies that later developed PE, median 3.8 ug/mL (range: 1.6 – 10.2 ug/mL) compared to controls, median 4.5 ug/mL (range: 1.4 – 17.3 ug/mL) ($p < 0.001$). There was no significant correlation between the adiponectin level and clinical severity of PE, parity, gestational age or birth weight. Adiponectin dicrimnated significantly, albeit poorly, between PE and control pregnancies. No correlation was found between maternal weight corrected adiponectin and neither TNFα nor free leptin index.
Conclusions: Maternal serum adiponectin is decreased a first trimester of pregnancies that develop PE. This may reflect a metabolic disturbance predisposing to or causing PE.
Keywords: adipokines, adiponectin, first trimester, metabolism

[P1.56]

HYPOXIA INDUCES THE RELEASE OF SFLT-1 AND SENDOGLIN FROM CHORIONIC VILLI VIA A HIF-1-DEPENDENT MECHANISM THAT IS REGULATED BY THE NO MIMETIC GLYCERYL TRINITRATE

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There is evidence that placental anti-angiogenic factors such as soluble fms-related tyrosine kinase 1 (sFlt-1) and soluble Endoglin (sEndoglin) induce systemic maternal endothelial dysfunction thereby contributing to the pathophysiology of pre-eclampsia. Studies have shown that the release of these molecules by the placenta is due to hypoxia resulting from inadequate perfusion. We previously demonstrated that the nitric oxide (NO) mimetic glyceryl trinitrate (GTN) inhibits hypoxia/re-oxygenation (H/R)-mediated apoptosis in the syncytiotrophoblast of chorionic villi explants from term pregnancies. This study sought to determine whether treatment with GTN inhibits the release of sFlt-1 and sEndoglin from chorionic villi exposed to hypoxia. Results showed that the syncytiotrophoblast is a potential source of sFlt-1 and sEndoglin. Whereas exposure to hypoxia (0.5% O2 *vs*. 20% O2 for 24 h) increased the secretion of sFlt-1 and sEndoglin, as well as their expression at both the protein and mRNA levels, treatment with GTN (10 nM and 1 μM) significantly attenuated this response to hypoxia. Treatment with GTN also decreased the levels of HIF-1α protein in explants exposed to 20% or to 0.5% O2. Additionally, siRNA-mediated knockdown of HIF-1α inhibited the hypoxia-induced secretion of sFlt-1 and sEndoglin. These findings reveal that hypoxia induces the release of sFlt-1 and sEndoglin from chorionic villi via a HIF-1-dependent mechanism that is regulated by the NO mimetic GTN. The study provides support for the use of GTN as potential therapy for pre-eclampsia. (Supported by the Heart and Stroke Foundation of Ontario).
Keywords: sFlt-1, sEndoglin, Hypoxia, glyceryl trinitrate

[P1.57]

DIFFERENTIAL EXPRESSION OF VE-CADHERIN AND FLK-1 IN THE SYNCYTIOTROPHOBLAST OF PREECLAMPTIC PLACENTAS COMPARED TO HEALTHY CONTROLS

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Introduction: VE-cadherin is promoting intercellular adhesion of endothelial cells thereby regulating endothelial integrity and permeability. Moreover, VE-cadherin is involved in the regulation of Flk-1 receptor activity and thus cell cycle regulation. Preeclampsia has been associated with impaired syncytial function and altered trophoblast turnover. Therefore we investigated whether altered VE-cadherin and Flk-1 expression might be associated with preeclampsia.
Methods: Biopsies of placentas from 19 patients with late onset preeclampsia and 24 healthy term deliveries as well as 20 cases of early onset preeclampsia and 20 preterm controls were stained for VE-cadherin and Flk-1.
Results: All biopsies showed VE-cadherin and Flk-1 expression in placental vessels and in the syncytiotrophoblast. VE-cadherin expression in the syncytiotrophoblast was significantly higher in late onset preeclamptic cases and flk-1 expression was less pronounced compared to term controls. Whereas in early onset preeclampsia VE-cadherin was significantly less and flk-1 significantly more expressed compared to preterm controls.
Conclusion: Differential expression of VE-cadherin and Flk-1 might contribute to the etiopathologic events at the fetomaternal interface in preeclampsia. Since reduction of VE-cadherin and increase of flk-1could lead to pronounced cell activation in the syncytiotrophoblast these changes than would enhance necrotic shedding instead of apoptotic shedding. The syncytial material released into the maternal system on necrotic shedding is believed to cause the maternal syndrome of preeclampsia. Thus overexpression of VE-cadherin and downregulation of flk-1 would represent a more stable situation in the syncytiotrophoblast and could possibly display a compensatory mechanism in the late onset cases.
Keywords: Preeclampsia, syncytiotrophoblast, VEGFR2/flk-1, VE-cadherin

[P1.58]

PHAGOCYTOSIS OF APOPTOTIC SYNCYTIAL KNOTS PREVENTS ENDOTHELIAL CELL ACTIVATION: AN IMPORTANT ADAPTATION FOR NORMAL PREGNANCY

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Background: Preeclampsia is characterised by an exaggerated inflammatory response and maternal endothelial cell activation. Syncytial knots are multinucleated fetal cells shed from the placenta in large numbers during all pregnancies which may be phagocytosed by endothelial cells. Our previous studies showed that phagocytosis of necrotic but not apoptotic syncytial knots led to endothelial cell activation. It is known that phagocytosis of apoptotic cells leads to active tolerance of immune responses and in this study we questioned whether phagocytosis of apoptotic syncytial knots leads to suppression of the endothelial cells ability to be activated.
Methods: Syncytial knots were harvested from 1st trimester placental explants. Monolayers of endothelial cells were pre-treated with apoptotic syncytial knots for 24 hours. After washing, the endothelial cells were treated with the endothelial cell activators LPS, PMA, IL-6, or necrotic syncytial knots for 24 hours. In some experiments the inhibitor of phagocytosis, cytochalasin D, was added into the cultures along with apoptotic syncytial knots. Endothelial cell-surface ICAM-1 was measured using cell based ELISAs.
Results: Expression of ICAM-1 by endothelial cells that had phagocytosed apoptotic syncytial knots prior to treatment with LPS, PMA, IL-6, or necrotic syncytial knots was significantly ($p=/<0.003$) reduced, compared to control endothelial cells that had not phagocytosed apoptotic syncytial knots. Inhibiting phagocytosis of apoptotic syncytial knots with cytochalasin D abolished this protective effect.
Conclusions: Our data suggest phagocytosis of apoptotic syncytial knots results in the suppression of the ability of endothelial cells to be activated by a number of potent chemical activators, as well as by the physiologically relevant activator, necrotic syncytial knots. This work suggests that the release of apoptotic syncytial knots from the placenta during normal pregnancy may be a mechanism by which the fetus attempts to protect the maternal vasculature against activation.
Keywords: phagocytosis, trophoblast deportation, endothelium activation

[P1.59]
DO SYNCYTIAL KNOTS UNDERGO SECONDARY NECROSIS?

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Introduction: Preeclampsia is triggered by a factor derived from the placenta and syncytial knots shed from the placenta may be a triggering factor. We previously reported that endothelial cells phagocytose syncytial knots and that phagocytosis of necrotic, but not apoptotic syncytial knots caused activation of endothelial cells. It is known that increased numbers of syncytial knots are shed in preeclampsia and it has been suggested this may overburden the clearance mechanism for syncytial knots allowing apoptotic syncytial knots to undergo secondary necrosis. Therefore we investigated whether apoptotic syncytial knots would undergo secondary necrosis.
Methods: Apoptotic syncytial knots/trophoblasts collected from our 1st trimester placental explant model were exposed to either monolayers of endothelial cells or placed into control culture wells with no endothelial cells. After 24 hours unphagocytosed syncytial knots were recovered and exposed to additional endothelial cells for 24 hours. Endothelial cell surface ICAM-1 was determined by ELISA and activity of caspases 3&7 in the unphagocytosed syncytial knots determined by FLICA observed by confocal micrscopy.
Results: The activity of caspases 3&7 in unphagocytosed syncytial knots was apparently reduced compared to freshly shed syncytial knots. The level of ICAM-1 expression by fresh endothelial cells that were exposed to "unphagocytosed" syncytial knots that had been previously exposed to endothelial cells was significantly increased ($p < 0.001$) compared to endothelial cells exposed to freshly shed syncytial knots or control syncytial knots that had been placed into endothelial cell-free culture wells.
Conclusion: Our data suggest that if syncytial knots are not rapidly cleared from the maternal vasculature they can undergo secondary necrosis that is induced by contact with endothelial cells. This endothelial cell-dependent induction of secondary necrosis might how an increased burden of apoptotic syncytial knots might lead to endothelial cell activation in preeclampsia.
Keywords: trophoblast deportation, apoptosis, necrosis

[P1.60]
ELEVATED CALRETICULIN IN MATERNAL PLASMA IN PRE-ECLAMPSIA ALTERS TROPHOBLAST CELL FUNCTION

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Pre-eclampsia (PE) is a multisystem disorder of human pregnancy that involves abnormal placentation (insufficient trophoblast cell (TC) invasion of the maternal spiral arteries). Throughout pregnancy, the endovascular extravillous TCs are continually exposed to maternal blood. The calcium-binding protein calreticulin is significantly increased in peripheral blood in PE compared to normotensive pregnancy (Gu *et al.* Molec. Human. Repro. 2008, 14; 309-315). Although calreticulin mRNA has been shown to increase in human TC with the induction of differentiation (Morrish *et al.* Placenta. 1996, 17; 431-441), little is known about the role of extracellular calreticulin on TC function.
This study determined the effects of exogenous calreticulin at concentrations relevant to normotensive pregnancy (2μg/ml) and to PE (5μg/ml) on the human extravillous TC cell line, HTR8. Cell migration was measured by scratch assay, changes in cell number were measured by the MTS assay (Promega) and cell adhesion measured on different extracellular matrices (Millipore).
The results showed that calreticulin at 5μg/ml did not stimulate HTR8 cell number (control 68044±24542 cells, with calreticulin 72810±30673 cells, $P>0.05$, $n=3$) after 48 hours but it did significantly inhibit migration of the cells by 48±11% ($n=4$), compared to the control by 26 hours (t-test $P<0.02$). Calreticulin at 5μg/ml did not alter HTR8 adhesion on Collagen I, Collagen IV, fibronectin, vitronectin or laminin. Calreticulin at the lower concentration did not alter TC functions.
In conclusion, calreticulin at a concentration consistent with that found in maternal blood with PE was shown to alter TC migratory activity during *in vitro* culture. These results indicate that circulating calreticulin may inhibit TC migration *in vivo* and contribute to the mechanisms that generate the abnormal placental-decidual interface that is the basis of PE.
Keywords: Trophoblast cell function, Calreticulin, Cell migration

[P1.61]

PRE-ECLAMPSIA IS ASSOCIATED WITH LOWER ADENOSINE A_{2A} RECEPTOR RESPONSE IN UMBILICAL VEIN ENDOTHELIAL CELLS

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Pre-eclampsia is associated with high adenosine plasma levels in both maternal and feto-placental circulation. Moreover, adenosine A2A receptor expression is reduced in placental endothelium from pre-eclampsia. Since, the angiogenesis process is partially controlled by A2A receptor stimulation in several cellular models; we wonder whether the changes observed in pre-eclampsia could be associated with reduced proliferation and migration of fetal endothelium

Methods. Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase (0.25 mg/ml) from normal (n=6) and pre-eclamptic pregnancies (n=6) and cultured under standard condition (37°C, 5% CO_2) up to passage 2. The experiments were performed after overnight serum-deprivation and cells incubation with and without adenosine A2A receptor agonist (CGS-21680, 30nM) and/or the antagonist (ZM-241385, 10nM) during further 48 hours. In proliferation assays; cell number was determined using a hemacytometer and they were seeded at a density of 50 x 103/ml. After treatment cells were counted and proliferating cells were estimated. In parallel, monolayers of confluent cells were scratched with a sterile cell scraper, and then cells crossing the wound were counted per unit time.

Results. Demographic characteristic of pregnant women were similar. Newborn from pre-eclamptic pregnancies showed reduced birth weight compared with newborn from normal pregnancies ($p<0.05$). HUVEC from pre-eclampsia exhibited lower proliferation (~42%) and migration (~34%) compared with cells from normal pregnancies. CGS-21680 increased the proliferation (~2-fold) and migration (~1.5-fold) only in normal pregnancies derived cells. These last effects were blocked by ZM-241385 co-incubation. In addition, ZM-241385 alone reduced cell proliferation (~15 and 35%) and migration (~25 and 20%) in both pre-eclamptic and normal derived cells, respectively.

Conclusion. Reduction in fetal endothelium proliferation and migration observed in pre-eclampsia is associated with lower adenosine A2A receptor response. These phenomenons coincide with reduced adenosine A2A receptor expression in placental endothelium from pre-eclampsia and would be contributing in the reduction of placental angiogenesis and lower blood flow toward pre-eclamptic fetuses.

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Keywords: Adenosine, Adenosine receptors, Fetal Endothelium, Angiogenesis

[P1.62]

THE ENDOTHELIN / ENDOTHELIN RECEPTOR SYSTEM IS HIGHLY UPREGULATED IN PREECLAMPSIA WITH OR WITHOUT FETAL GROWTH RESTRICTION IN CONTRAST TO GESTATIONAL DIABETES

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Introduction: In addition to its vasoregulative function, in the human placenta endothelin-1 (ET-1) also regulates cell differentiation, proliferation, invasion and apoptosis. ET-1 effects are signaled through two receptor subtypes ETR-A and ETR-B. We tested the hypothesis that the expression of ET-1 and ETRs is altered in preeclampsia (PE), fetal growth restriction (FGR) and in gestational diabetes (GDM) and differs between early (gestational week $\leq$ 34) and late (GW >34) third trimester pregnancies.

Methods: The study included women (GW 28-41) with PE (blood pressure >140/90 mmHg, protein >300mg/24hrs; n=16), with FGR (<10th birthweight centile and pathological umbilical blood flow; n=7) and PE+FGR (n=5) and with GDM (±insulin treatment n=21), as well as age-matched controls (n=20). ET-1, ETR-A and ETR-B mRNA and ETR-A and ETR-B protein were quantified in placental tissues by real-time PCR and immunoblotting.

Results:

Table 1: mRNA expression in third trimester pregnancies:
Fold changes versus age-matched controls (p-values)

	GW $\leq$ 34			GW > 34		
	ETR-A	**ETR-B**	**ET-1**	**ETR-A**	**ETR-B**	**ET-1**
PE	2.6 (0.04)	3.0 (0.01)	3.5 (0.01)	0.6 (0.05)	2.0 (0.02)	0.4 (0.05)
PE+FGR	5.1 (0.05)	3.4 (0.04)	6.9 (0.003)	-	-	-
FGR	n.s	n.s.	3.8 (0.02)	0.6 (0.05)	n.s.	n.s.
GDM	-	-	-	0.5 (<0.001)	0.8 (0.05)	0.4 (<0.001)

-: not determined, because no material available, n.s.: not significant.

In early third trimester pregnancies ETR-A protein was upregulated (+26%) only in PE. There were no changes in ETR-B protein. In late third trimester pregnancies ETR-A (-17%) and ETR-B protein (-33%) were downregulated in GDM. ETR-B protein was also downregulated in FGR (-23%) and PE (-35%).

Discussion: The upregulation of the ET/ETR system in PE is correlated with the severity of the disease: mild-late<severe-early<PE+FGR). The ET/ETR system is downregulated in GDM.

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Keywords: endothelin / endothelin receptor system, preeclampsia, fetal growth restriction, gestational diabetes

[P1.63]

PREECLAMPSIA AND NKA AND PMCA ACTIVITIES IN BOTH BASAL AND MICROVILLOUS MEMBRANES OF THE HUMAN TERM PLACENTA

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The human placental syncytiotrophoblast (hSCT) layer is the primary barrier for transplacental exchange of Na^+ and Ca^{2+} between the mother and the growing fetus. There is a clear experimental evidence showing the presence of the Na,K-ATPase (NKA) and the plasma membrane Ca-ATPase (PMCA) in both basal (BM) and microvillous (MVM) plasma membranes. Alterations in membrane composition might be involved in a variety of pregnancy complications such as preeclampsia and this might affect the activity of the membrane transport proteins such as NKA and PMCA. In the current study we evaluated if preeclampsia can modify the NKA and PMCA activities of both BM and MVM of the hSCT. METHODS: The hSCT plasma membranes of term placentas obtained from normotensive and preeclamptic women were assayed for NKA and PMCA activities using a colorimetric method. These membranes were also assayed for lipid peroxidation level by measuring TBARS. The number of ATPase molecules was evaluated in hSCT plasma membranes and tissue sections by using Western blots and immunohistochemistry, respectively. RESULTS: The hSCT plasma membranes from preeclamptic women show a significant diminution of the NKA and PMCA activities and a significant rise in the level of lipid peroxidation. The Western blots of hSCT plasma membranes with specific antibodies against NKA and PMCA, did not show a significant difference between normotensives and preeclamptics. Similar results were obtained for the immunohistochemistry studies suggesting that the number of NKA and PMCA molecules in hSCT plasma membranes does not change with preeclampsia. CONCLUSIONS: The rise in the level of lipid peroxidation of the hSCT plasma membranes during preeclampsia promotes a diminution of the NKA and PMCA activities. This diminution of the ATPase activities of the hSCT does not seem to be related to changes in the number of NKA and PMCA molecules in hSCT membranes.

Keywords: Preeclampsia, PMCA, NKA, ATPase

[P1.64]

UMBILICAL VEIN ENDOTHELIUM ACTIVATION IS ASSOCIATED WITH HIGH MATERNAL LEVELS OF E-SELECTIN, VCAM-1 AND SFLT-1 IN SEVERE PRE-ECLAMPTIC PREGNANCIES

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Background: Severe pre-eclampsia is associated with reduction in placental blood flow, and low nutrient and oxygen delivery toward the fetus. Causes for these alterations are unclear, however, maternal proinflammatory and prothrombic state and feto-placental endothelial dysfunction are a hallmark of pre-eclampsia, we wonder whether endothelial activation markers in the maternal circulation are associated with the nitric oxide (NO) synthesis in fetal endothelium.

Methods: Pregnant women at delivery were recruited at the Hospital Clínico Guillermo Grant Benavente from Concepción, Chile. Blood samples were collected from normal (n=7), mild (n=7) and severe (n=10) preeclamptic pregnancies. sE-selectin, sVCAM-1 and sFlt-1 serum quantifications were performed by ELISA. Endothelial cells were isolated from human umbilical vein (HUVEC) by digestion with collagenase and histamine-induced NO synthesis was detect by fluorometric examination.

Results: Mother with severe pre-eclamptic pregnancies had premature and smaller babies than mother with normal pregnancies ($p<0.05$). This phenomenon was associated with high maternal plasma levels of sVCAM-1 (~2-fold) and sFlt-1 (~2.5-fold) and lower (~300%) histamine-stimulated NO synthesis in HUVEC. Considering the entire group of patients, a positive relationship between systolic blood pressure (SBP) and plasma levels of sE-selectin, sVCAM-1 and sFlt-1. Moreover, high SBP and diastolic BP and plasma levels of sE-selectin, sVCAM-1 and sFlt1 were negatively associated with newborn weight and gestational age at delivery. Women with elevated levels of sE-selectin (>63 ng/mL), sVCAM-1 (>752 ng/mL) and sFlt-1 (>15204 pg/mL), showed high risk (RR 2.05, 1.69 and 3.07, respectively) for preterm delivery, very preterm delivery (RR 1.9, 1.21, and 2.66, respectively), or fetal weigh under 1500 g (RR 2.33, 1.10 and 1.94, respectively) compared with women with low levels.

Conclusions*:* High serum levels of endothelial dysfunction markers are associated with poor newborn outcomes such as growth restriction and pre-term delivery. Severe pre-eclampsia characterized by hypertension and high maternal circulating levels of sVCAM-1 and sFlt-1 is associated with decreased NO synthesis in the fetal endothelium.

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Keywords: Preeclampsia, Endothelial cell, Adhesion molecules

[P1.65]

UNDERSTANDING THE RELATIVE EXPRESSIONS OF 5-HYDROXYTRYOTAMINE RECEPTOR SUBTYPES IN NORMOTENSIVE AND PRE-ECLAMPTIC PLACENTAE

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The distribution of different 5-hydroxytryptamine 5HT receptors in the central nervous system has extensively been studied. However the information on their peripheral distribution is limited. This study has investigated the relative mRNA expressions of different 5HT receptor subtypes in normotensive (NT) and pre-eclamptic (PE) placentae at term.

NT and PE placental samples were obtained after informed consent. Conventional RT-PCR protocols were standardised with primers designed from Primer3 and Primer blast. The same primers were used in quantitative RTPCR to compare the expressions of major 5HT receptors in relation to the mRNA expression of b-actin (as control). Analysis of urinary 5-HT and its metabolite 5-HIAA was carried out using a commercially available ELISA kit.

The results from conventional PCR have shown that many of the 5HT receptor mRNAs are expressed in NT and PE placentae. The important ones include 5HT1B, 1D, 1E, 2A, 2B, 5 and 7. Comparative qRTPCR results between NT and PE placentae have shown no significant differences in the expressions of 5HT2A (11.8±2.7 for NT and 9.8±1.5 for PE) or 5HT7 (9.8±1.5 for NT and 8.6 ±1.7 for PE) (Arbitrary units). However the mRNA expression of 5HT1B receptor in PE placentae was significantly reduced (10.5±2.1 for NT Vs. 8.4±1.9 for PE; $p<0.05$ – Mann Whitney U). Interestingly, PE women had significantly higher levels of urinary 5-HT (124.8 ± 23.6 μg/24 hour urine) compared to NT women (59.5 ± 9.8 μg/24 hour urine). On the other hand the levels of the metabolite 5-HIAA were similar in the two groups. Consequently, the ratio of 5-HIAA: 5-HT in PE women was around half (22.1 ±3.2) of NT controls (45.9 ± 7.8).

The results show that the relative mRNA expression of 5HT1B receptor is reduced in PE. This together with increased circulating 5HT in PE may contribute to pathophysiological changes in PE.

Keywords: 5-hydroxytryptamine, Placenta, pre-eclampsia

[P1.66]

USING FETOMATERNAL INTERACTIONS TO IDENTIFY IMMUNOMODULATORY MECHANISMS IN MELANOMA

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The malignant potential of melanoma depends on its ability to evade host immune responses. In vitro and animal models to study immune modulation in melanoma have focused on isolated processes such as involvement of the microenvironment, angiogenesis, or systemic immune modulation; yet all these mechanisms in concert contribute to melanoma progression. One condition that mimics this process, including integration of isolated components of immune modulation, is pregnancy. In fact, we believe that the physiologic transient, organ system-based immune modulation in pregnancy, parallels the coordinated response to cancer and thus can help identify new mechanisms of immune modulation pertinent to metastatic melanoma. To explore this possibility, we evaluated expression of 79 immunologically relevant genes involved in endometrial decidualization and placentation in benign nevi and melanoma to reveal immune markers associated with pregnancy that are also expressed in cancer. We then assessed the presence of corresponding proteins by immunohistochemistry (IHC) in a 118-patient metastatic melanoma tumor microarray (TMA). Using this approach, we identified two new independent prognostic biomarkers for survival, CD58 and galectin 9, along with an overall pattern of immunomodulatory protein expression associated with survival. In pregnancy, alterations in local immune homeostasis that allow invasion of HLA-disparate trophoblast cells into the maternal decidua are also detectable systemically. One key example is specialized CD16- natural killer (NK) cells that are abundant in the decidua are also expanded in maternal peripheral blood during early pregnancy. In metastatic melanoma, we identified a similar expansion of peripheral blood CD16- NK cells, some of which coexpress CD9, representing the first description of this NK cell phenotype outside of the female reproductive tract. Thus, using pregnancy as a model, we have identified previously unexplored mechanisms of immune modulation in metastatic melanoma.

Keywords: immune modulation, tolerance, metastatic melanoma, CD16-NK cells

[P1.67]

ACTIVATION OF ADENOSINE RECEPTOR INDUCED MOBILIZATION OF HUMAN ENDOTHELIAL PROGENITOR CELLS

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Backgroundx: Endothelial progenitor cells (EPC) are involved in repair processes from damage caused by hypoxia and ischemia and is known that an increase in extracellular adenosine stimulates angiogenesis and vasculogenesis of endothelial cells. Therefore, adenosine and the presence of human endothelial progenitor cells contribute to the regulation and recovery of ischemic injury. However, no studies linking the presence of adenosine and processes of mobilization and angiogenesis of human endothelial progenitor cells (hEPC). In this studies characterized the effect of adenosine in the mobilization and migration of early hEPC.
Methods: The hEPC were obtained from peripheral blood of healthy donors and mononuclear cells were separated by density gradient and then cultured for 3 days. Mobilization studies were performed using transwells (8 μm) in the presence and absence of adenosine (10 μM, 20 hrs) and NECA (0,1 μM, 20 hrs) a specific activator of adenosine receptors. The receptor expression was determined by real-time PCR using specific primers for each of them.
Results: Real-time PCR was possible to identify the receptors A2A, A2B and A3 in hEPC. A3 expression was higher ∼1,2-fold and ∼0,3-fold compared to levels of mRNA for A2A and A2B, respectively. Adenosine, increases (∼1,5-fold) the mobilization of early hEPC compared with the control. NECA, increased mobilization of hEPC (~2,5-fold) whose effect is partially blocked by MRS 1523 (0,0005 – 10 μM, 20 hrs) and MRS 1754 (0,0005 – 10 μM, 20 hrs), inhibitors of A3 y A2B, respectively. The analysis of flow citometry shows that the subpopulation of cells mobilized by adenosine corresponds to the (70%) $CD34^+$/ KDR^+ cells, which express the A2B and A3 receptors.
Conclusions: These results suggest that adenosine is involved in migration and mobilization of the hEPC, mediated by activation of adenosine receptors.
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Keywords: Adenosine receptor, Angiogenesis, endothelial progenitor cell

[P1.68]

DOCOSAHEXAENOIC ACID, 22:6N-3 STIMULATES EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN FIRST TRIMESTERTROPHOBLAST CELLS, HTR8/SVNEO

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Inappropriate placentation during the first trimester of pregnancy is thought to be essential in the development of preeclampsia. Angiogenesis is a key factor in the placentation process and vascular remodelling that involves growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PlGF) and angiopoietin like protein 4 (ANGPTL4/PGAR). Maternal LCPUFAs taken up by the placenta are critical for the growth and development of the fetus. However, very little information is available on whether LCPUFAs can affect the expression of angiogenic factors in first trimester placental trophoblast cells. We therefore investigated uptake and metabolism of LCPUFAs such as arachidonic acid, 20:4n-6 (ARA), eicosapentaenoic acid, 20:5n-3 (EPA), docosahexaenoic acid, 22:6n-3(DHA), peroxisome proliferator-activator receptor (PPAR) ligands, and non-essential oleic acid, 18:1n-9 (OA), and their effects on expression of relevant genes in first trimester trophoblast cells, HTR8/Svneo.
Methods: Uptake of these fatty acids was determined after incubating HTR8/Svneo cells with radiolabeled fatty acids for 3h. Expression of several relevant genes and proteins levels of these factors were quantified after incubating these cells with fatty acids (100mM) for 24h, using real-time qRT-PCR, and ELISA. Cell viability was also examined.
Results: We found that HTR8/Svneo cells take up fatty acids in the following order; ARA >>EPA>>>>>>DHA in contrast to the last trimester trophoblast cells which prefer DHA over other fatty acids. Among all the fatty acids and PPAR ligands tested, only DHA increased VEGF mRNA expression and protein (by 8-fold) compared with control in these cells. OA, EPA, ARA and GW-501516 (a PPARdelta agonist) stimulated ANGPTL4/PGAR mRNA expression and protein levels while DHA had no effect. These fatty acids however did not alter expression of other genes such as FABPs, FAT/CD36, FABPpm, FATP1,2,3,4 &6, CAV-1, ACSL1,3,4 &5, HIF1a, and COX-2.
Conclusions: DHA may influence placentation processes by stimulating the expression of VEGF in first trimester trophoblast cells.
Keywords: Docosahexaneoic acid, Vascular Endothelial Growth Factor, Fatty acid transprt, First trimester trophoblast cells

[P1.69]

PROLACTIN-LIKE PROTEIN A AND UTERINE NATURAL KILLER CELL ROLES IN MODULATING UTERINE VASCULAR RESPONSES TO HYPOXIA IN THE PREGNANT MOUSE ENDOMETRIUM

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Experimental studies provide evidence that oxygen tension impacts placentation and uterine vascular development. The present work evaluated roles for prolactin-like protein-A (PLPA) and uterine NK (uNK) cells on organization and vascularization of the placentation site during normal pregnancy and following exposure to hypoxia. Beginning on gestational day (gd) 7.5, wild type, and $PLPA^{-/-}$ knockout, $IL15^{-/-}$ knockout (uNK depleted), and $PLPA^{-/-}/IL15^{-/-}$ double knockout pregnant mice were exposed to a hypoxic environment (11% oxygen) for 48h or were exposed to ambient conditions (21% oxygen; normoxia). Healthy and abnormally developing conceptuses were counted on gd 9.5 and collected for assessing the distributions of uNK cells (anti-perforin), evaluation of vasculogenesis (anti-endoglin, anti-MECA32), trophoblast development (TROMA1), and the expression of VEGF isoforms. The incidence of abnormal (small/hemorrhagic) placentation sites in wild type pregnancies was 1:30, while in the $PLPA^{-/-}$, $IL15^{-/-}$, and $PLPA^{-/-}/IL15^{-/-}$ pregnancies the frequencies of abnormal placentation sites increased to 1:7, 1:5 and 1:6, respectively. Hypoxia exposure increased the incidence of abnormal placentation sites in wild type animals to 1:16 but did not significantly affect the frequency of abnormal placentation sites in the knockout animals. Endoglin positive endothelial cells (EC) were seen lining the mesometrial capillary sinuses within the decidua of wild type and $PLPA^{-/-}$ null pregnancies. On the other hand, MECA32 positive EC were found primarily in the large arterial and venous blood vessels of mesometrial endometrium near the myometrium. MECA32 immunostaining increased following hypoxia exposure in the $PLPA^{-/-}$ and $IL15^{-/-}$ animals, but not in the wild type mice. VEGF gene expression decreased in the $PLPA^{-/-}$ and $IL15^{-/-}$ endometrium. Hypoxia exposure resulted in an increase of VEGF B and C expression. In conclusion, PLPA and uNK cells positively support pregnancy. In their absence, alternative pathways can be mobilized for successful adaptation to ischemic stress.

Grants: CAPES(4683-08-0) and NIH (HD20676)

Keywords: Hypoxia, Angiogenesis, uterine Natural Killer cells, Prolactin like protein-A (PLPA)

[P1.70]

MELATONIN INHIBITS HYPOXIA/REOXYGENATION-INDUCED APOPTOSIS OF PRIMARY HUMAN VILLOUS TROPHOBLAST

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Introduction: The effects of melatonin on human villous trophoblast survival have never been studied. Melatonin possesses anti-apoptotic and cytoprotective properties. We have demonstrated that human placental villous trophoblast synthesizes melatonin and expresses its MT1- and MT2 receptors. Villous trophoblast homeostasis is essential to pregnancy and fetal development well-being. Stressors, such as hypoxia/reperfusion, damage villous trophoblast mitochondria, triggering apoptosis and necrosis which are increased in pathologic pregnancies, such as preeclampsia. Our goal was to determine the role of melatonin on villous trophoblast mitochondrial apoptosis.

Methods: We tested the effect of ± melatonin (10 μM) on human term villous trophoblast cultures subject to hypoxia/reoxygenation compared to normoxia. Mitochondrial induced apoptosis was determined by assessing the loss of mitochondrial membrane potential by fluorimetry using the JC-1 probe and by assessing the protein expression of BAX, Bcl-2, caspase-3 and 9, cleaved ROCK-I, cleaved PARP1 (poly [ADP-ribose] polymerase 1), VEGF (vascular endothelial growth factor) and HIF-1 (hypoxia-inducible factor 1) by Western blots.

Results: Hypoxia/reoxygenation significantly increased the loss of mitochondrial membrane potential and expression of pro-apoptotic molecules BAX, caspase 3 and 9, cleaved ROCK-I, cleaved PARP1, VEGF and HIF-1 compared to normoxia. Melatonin significantly inhibits the hypoxia/reoxygenation increases in these pro-apoptotic parameters. There is no difference between normoxia and melatonin treatment of hypoxia/reoxygenation.

Discussion: Melatonin prevents hypoxia/reoxygenation induced mitochondrial apoptosis in villous trophoblast. These results demonstrate that melatonin inhibits apoptosis of primary human villous trophoblast, suggesting a protective role for melatonin in pregnancy and fetal development.

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Keywords: Melatonin, Primary villous trophoblast, Hypoxia/reoxygenation, Mitochondrial apoptosis

[P1.71]

THE MATERNAL HYPERGLYCEMIA, REGARDLESS OF DIAGNOSIS OF DIABETES, WITH RESPECT ADVERSE PERINATAL OUTCOMES IN FETAL OXYGENATION

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Introduction: Adverse perinatal outcomes (APO) with significant impairment of fetal oxygenation are frequent in gestational (GDM) or pregestational (PGDM) Diabetes mellitus. These results are associated with quality of glycemic control during pregnancy[1]. Our program has identified a group of women who presents normal OGTT100g and hyperglycemia in glucose profile (GP). Pregnant women in this group are carriers of Mild hyperglycemia (MH) and treated for the control of high glycemia[2,3]. Therefore, we intend to compare the markers of fetal oxygenation between women with GDM, PGDM, MH and non-diabetic (ND).
Method: It was performed a cohort of women with GDM (n=22), PGDM (n=15), MH (n=18) and ND (n=31) and their newborns (NB). The diagnosis of GDM and MH was carried out by OGTT100g, applied in parallel with GP, between 24 and 28 weeks of gestation. The maternal parameters evaluated were glucose mean (GM) and glycated hemoglobin (HbA1c). Markers of fetal oxygenation evaluated were hematocrit (Ht), hemoglobin (Hb), cord pH, Apgar score, total (Bt), direct (Bd) and indirect (Bi) bilirubin, need for phototherapy and hospitalization in ICU. It was used the t test to compare means and chi-square test for proportions ($p<0.05$).
Results: GM and HbA1c: higher values in MH (103.21mg/dL; 5.98%), GDM (109.23mg/dL; 6.37%) and PGDM (121.89mg/dL; 6.94%) compared to ND (81.7mg/dL; 5.55%); hematocrit and hemoglobin: higher values in MH (15.77g/dL; 48.09%), GDM (15.50g/dL; 55.29%) and PGDM (16.78g/dL; 51.82%) compared to ND (13.61g/dL; 46.61%).
Discussion: Alterations on the maternal parameters and markers of fetal oxygenation of women with high glycemia were observed especially in relation to HbA1c, hematocrit and hemoglobin. These results indicate that, regardless of diagnosis of diabetes, the hyperglycemia present in the intrauterine environment increases the APO risk related to intrauterine oxygenation. Maternal hyperglycemia, in any source and intensity, must be controlled.
References:
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Keywords: Hyperglycemia, Fetal oxygenation, Pregnancy

[P1.72]

WHICH PLACENTAL ZONE IS MORE AFFECTED IN CASES OF EXPERIMENTAL INTRAUTERINE HYPOXIA?

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Introduction: In humans, alterations of differentiation and division of trophoblast facing adverse conditions can often cause compensatory pathological changes of the placenta. In experimental models, rats of different strains are commonly used as a model for studies of embryology and reproduction toxicology. Surprisingly, the literature does not provide comprehensive baseline data on placental development of these animals.
Objective: To evaluate the thickness of the placental zones facing the intrauterine growth restriction experimentally induced.
Methods: To induce uteroplacental insufficiency and intrauterine growth restriction in Wistar rats, the right uterine artery was ligated on the 15th day of gestation and euthanasia penformed on the 19th day. The placental zones were morphologically examined using Hematoxylin-Eosin staining. Measurements were made with the use of a video camera coupled to a common light microscope and a computer with an automatic image analyzing software.
Results: The junctional zone was significantly higher in cases with ligation ($p\leq0.001$). There was no significant difference in the thickness of the intermediate and labyrinth zones.
Discussion and Conclusion: The increased thickness of the junctional zone in cases with the ligation may be related to a compensatory mechanism of their cells of glycogen, which would increase in number or functionally to compensate for the lack of energy. Although the labyrinth zone has the largest area of maternal-fetal exchange, its time response can be higher than in the junctional zone, and therefore, its changes only detected after a long period of hypoxia. Further studies are needed to clarify the unbalance of the differentiation of strains of trophoblast cells from the placenta of rodents against hypoxia.
Keywords: intrauterine restriction, hypoxia, morphometry, placental zone

[P1.73]

POMEGRANATE JUICE REDUCES OXIDATIVE STRESS IN TERM HUMAN PLACENTA

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Introduction: Oxidative stress is implicated in several complications of pregnancy.

Pomegranate juice (PJ), a rich natural source of polyphenols, reduces oxidative stress and apoptosis in a murine model of ischemic-reperfusion injury (Loren,D.J., et al., Pediatr Res, 2005. 57(6): 858-64; West, T. et al., Dev Neurosci, 2007. 29(4-5): 363-72). Labor induces placental oxidative stress (Cindrova-Davies, T., et al., Am J Pathol, 2007. 171(4): 1168-79), providing a convenient model to test the ability of PJ to moderate oxidative stress during pregnancy.

Objective: We tested the hypothesis that PJ reduces oxidative stress in term human placenta.

Methods: Proteins were extracted from placentas of women with normal term pregnancies administered 8oz per day of commercially available PJ (POM Wonderful, Los Angeles, CA) in the last two weeks of pregnancy who underwent long labor (>5hrs, n=4) and from women with normal term pregnancies not administered PJ who underwent short (<5hrs, n=4) or long (>5hrs, n=4) labors. Proteins were also extracted from placental explants (n=4) and primary human syncytiotrophoblasts (PHTs, n=4) cultured under hypoxia (<1% oxygen) for 8 and 24 hours, respectively, with and without PJ at a concentration of 1:300. Proteins were separated by SDS-PAGE and levels of heat shock protein 90 (HSP 90) determined by immunoblot analysis.

Results: Median levels of HSP 90 were elevated in both short and long labored placentas, and in explants and PHTs cultured under hypoxia. PJ significantly reduced placental HSP 90 in women who underwent labor ($p < 0.01$) (**Figure 1**). PJ also reduced HSP 90 in explants and PHTs cultured under hypoxia in media with PJ compared to those without PJ.

Discussion: PJ reduces oxidative stress in labored placentas in vivo and in explants and placental trophoblasts cultured under hypoxia in vitro. We speculate that prenatally administered PJ may reduce hypoxia-related perinatal complications.

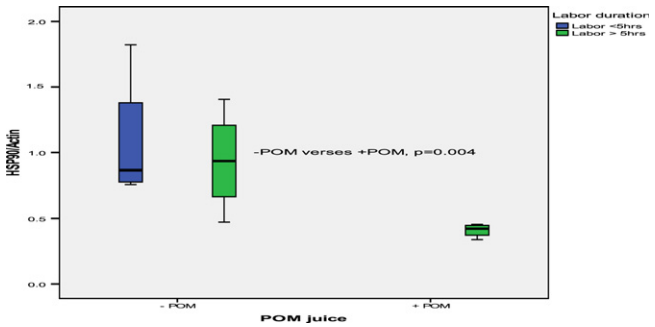

Figure 1. Levels of HSP 90 (normalized to actin) in placentas of women with normal term pregnancies administered pomegranate juice who underwent labor (>5hrs. n=4) and placentas of women not administered pomegranate juice who underwent short (<5hrs. n=4) and long (>5hrs. n=4) labor

Keywords: Pomegranate juice, Polyphenols, Oxidative stress, HSP 90

[P1.74]

PHYSIOLOGICAL REMODELLING OF THE UTERINE SPIRAL ARTERIES DURING PREGNANCY: UTERINE NATURAL KILLER CELLS MEDIATE SMOOTH MUSCLE CELL DISRUPTION

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Introduction: The early stages of spiral artery remodelling are characterised by disruption of vascular smooth muscle cell (VSMC) layers, which is coincident with an influx of uterine natural killer (uNK) cells [1]. Subsequently, extravillous trophoblast (EVT) colonises the vascular wall, replacing the endothelium and VSMC. We hypothesised that soluble factors secreted by uNK cells induce VSMC disorganisation and dedifferentiation, facilitating the entry of EVT.

Methods: uNK cells and EVT were isolated from first trimester decidua and placenta. Unremodelled spiral arteries obtained from non-pregnant, pre-menopausal women undergoing hysterectomy were cultured with conditioned medium (CM; 20% (v/v)) from uNK cells, EVT or uNK-EVT co-cultures for up to 96h. Additional vessels were pre-treated with uNK-CM for 48h, then co-cultured with EVT.

Results: VSMC in arteries cultured with uNK-CM for 48h or more displayed reduced α-smooth muscle actin immunoreactivity consistent with dedifferentiation, became misaligned with the appearance of spaces between layers, and showed altered nuclear morphology, when compared to arteries cultured with control CM, EVT-CM or uNK-EVT co-culture CM. VSMC disruption was localised to discrete areas of the vessel wall and did not cause a significant change in mean arterial diameter or medial area. When seeded onto spiral arteries, EVT colonised the mural tissue after 48h, although the number of invading cells was low within this timeframe. Pre-treatment of arteries with uNK-CM did not increase the number of EVT colonising the vessels; however, the area of the arterial wall exhibiting VSMC disruption was increased, compared to arteries pre-treated with control CM.

Discussion: Soluble factors secreted by uNK cells induced focal disorganisation and dedifferentiation of VSMC within spiral arteries, initiating mural disruption prior to colonisation by EVT. As previously suggested [1], strict spatio-temporal regulation of remodelling events is critical, as uNK - EVT interactions reduced the ability of uNK cells to produce vasoactive factors.

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Keywords: uterine natural killer cells, vascular smooth muscle, remodelling, trophoblast invasion

[P1.75]

EFFECT OF MAGNESIUM SULFATE ON CA-ATPASE ACTIVITY AND LIPID PEROXIDATION OF THE SYNCYTIOTROPHOBLAST PLASMA MEMBRANES ISOLATED FROM EXPLANTS OF HUMAN TERM PLACENTA INCUBATED UNDER HYPOXIA

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Preeclampsia is a clinical syndrome exclusive of the human pregnancy and constitutes the major cause of maternal and fetal morbility and mortality worldwide. It is characterized by high blood pressure, proteinuria, generalized arterial vasospasm and edema; appearing, in general, after the 20th week of pregnancy. It is widely accepted that during preeclampsia there is an abnormal trophoblast invasion, which avoids the formation of the low resistance/high flux circuit in the maternal-fetal axis, which is characteristic of the normal pregnancy. This leads to placental hypoxia and consequently to a rise in the level of lipid peroxidation and a diminution of the Ca-ATPase activity. The standard treatment for severe preeclampsia in the last 60 years has been magnesium sulfate ($MgSO_4$). This treatment blocks the seizures seen during eclampsia, and it has several beneficial effects for both the mother and the fetus.

Objectives: To determine the effect of $MgSO_4$ on Ca-ATPase activity and lipid peroxidation levels in the plasma membranes of explants from human term placenta incubated under hypoxia.

Methods: Explants of full term placentas from normotensive pregnant women were prepared and incubated under normoxia (8% O_2) or hypoxia (2% O_2) for 18h at 37°C. The syncytiotrophoblast plasma membranes were isolated and incubated for 24h at 4°C in the presence and absence of MgSO4. Ca-ATPase activity and TBARS were determined.

Results: It was found an important increase in the level of lipid peroxidation from both the microvillous and basal plasma membranes in placental explants incubated under hypoxia. The Ca-ATPase activity was lower in the plasma membranes of syncytiotrophoblast explants from placenta incubated under hypoxia. Incubation with $MgSO_4$ led to increased Ca-ATPase activity and decreased lipid peroxidation levels.

Conclusions: $MgSO_4$ can reverse both the diminution of the Ca-ATPase activity and the increased of lipid peroxidation levels seen in placental explants incubated under hypoxia.

Keywords: Hypoxia, Lipid peroxidation, Ca-ATPase, Magnesium sulfate

[P1.76]

NAD(P)H-OXIDASE: EXPRESSION AND ACTIVITY IN MOUSE POST IMPLANTATION TROPHOBLAST CELLS

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The complex NAD(P)H-oxidase catalyzes the production of reactive oxygen species (ROS). This plasma membrane-cell enzyme is formed by the membrane-bound components gp91-*phox* and p22-*phox*, the flavocytochrome b558 and, the cytosolic components p47-*phox*, p67-*phox*, Rac1 and p40-*phox*. Implanting trophoblast cells exhibit intense phagocytic activity and ultracytochemical localization of hydrogen peroxide-generating sites suggesting a NAD(P)H oxidase expression at maternal-fetal interface.

Objective: characterize the expression and activity of NAD(P)H oxidase complex in the trophoblast cells under the framework of the implantation process.

Materials Methods: Ectoplacental cones from 7.5-dg embryos grown as primary cultures, stimulated or not with PMA were used to investigate the production of superoxide anion through dihydroethydium oxidation (DHE) in confocal microscopy and immune co-localization assays for detection of the assemble and activation of the enzyme complex . The subunits $gp91^{phox}$, $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and *Rac1* were analyzed by rt-PCR, Western Blotting and IHC.

Results: NAD(P)H subunits were expressed and upregulated after PMA (phorbol-12-myristate-13-acetate) treatment. Confocal microscopy imaging showed co-localization of cytosolic and plasma membrane NAD(P)H oxidase subunits mainly after PMA treatment, suggesting to depend on specific stimuli. Cultured ectoplacental cones produced superoxide in a NAD(P)H-dependent manner suggesting that the ROS production may be associated to the implantation process. DNA sequencing showed high degree of homology between the trophoblast and neutrophil NAD(P)H-oxidase subunits.

Conclusion: Post-implanting trophoblast cells express NAD(P)H-oxidase complex subunits, suggesting a putative role for ROS production in the physiology of implantation.

Financial support: FAPESP, CNPq, CAPES

Keywords: NAD(P)H-oxidase, Trophoblast, implantation, ROS

[P1.77]

HORMONAL REGULATION OF HIF-1 (HYPOXIA-INDUCIBLE FACTOR-1) TRANSCRIPTIONAL ACTIVITY IN THE BEWO CELL LINE

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Introduction: Although HIF-1 has been characterized as a hypoxia-responsive transcription factor there is evidence to suggest that it can be regulated by factors other than oxygen tension. It is possible therefore that HIF-1 levels are the sum of several influences, including hypoxia. We hypothesized that hormones which modulate growth and metabolism would demonstrate regulatory effects on HIF-1 transcriptional activity.
Methods: To measure HIF-1 transcriptional activity we used a BeWo choriocarcinoma cell subclone which stably expresses a luciferase reporter gene coupled to a promoter containing multiple hypoxia-response elements, generated following lentiviral transduction. Cells were pre-incubated for 24 hr in serum-free medium then cultured for 18 hr in the presence of insulin (20 μIU/ml), IGF-I (200 ng/ml), leptin (100 ng/ml), pGH (25 ng/ml) or FBS (10%). A positive control group was cultured in the presence of 0.5 mM DMOG (dimethyloxallylglycine), a hypoxia mimetic. Luciferase activity was measured in quadruplicate using a bioluminescence assay. Data was analysed by ANOVA (Dunnett post hoc test).
Results: Luciferase activity in the control group was significantly above background and was assigned a value of 1.0±0.07 (mean±SEM; n=3). Treatment with DMOG generated an increase in luciferase activity of 8.09±0.39 fold compared to control ($p<0.01$). The other agents showed no effects with the exception of IGF-I; treatment with IGF-I increased luciferase activity by 2.08 ± 0.15 fold ($p<0.05$) compared to control.
Discussion: These data show that there is significant HIF-1 transcriptional activity in the absence of stimulatory agents (>10 fold above background), suggesting a constitutive level of HIF-1 synthesis in these cells. As expected, transcriptional activity was highly upregulated by the hypoxia mimetic. HIF-1 transcriptional activity was also upregulated by IGF-I although not by the other agents. These results suggest that IGF-I may contribute to the regulation of HIF-1 in trophoblast cells *in vivo*, in addition to the previously observed effects of hypoxia.

[P1.78]

A QUANTITATIVE INVESTIGATION OF TRANSCRIPTIONAL ACTIVITY IN SYNCYTIOTROPHOBLAST NUCLEI DURING HUMAN GESTATION

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Introduction: The syncytiotrophoblast (STB) is a terminally differentiated, multi-nucleated syncytium. No mitotic bodies are observed in the STB, which is sustained by continuous fusion of cytotrophoblast cells (CTB). As a result, STB nuclei are of different ages and display varying degrees of heterochromatin. Until recently, it was thought that STB nuclei were transcriptionally inactive, with all mRNAs required by the syncytium being incorporated upon fusion of CTB. However, research now shows the presence of the active form of RNA polymerase II (RNAP) in some STB nuclei[1]. The aim of this study was to quantify the proportion of active nuclei at different gestational ages.
Methods: Paraffin-embedded placentas (n=22), ranging from 13-39 weeks, used for the estimation of STB nuclear number [2] were studied. For each placenta three blocks were selected at random, and adjacent 5μm sections cut. The proportion of RNAP-positive STB nuclei was quantified by the Disector method. Numerical densities of volumes of trophoblast sampled were calculated. Co-localisation of Proliferating Cell Nuclear Antigen and RNAP was used to investigate the relationship between recently fused nuclei and RNAP positivity.
Results: There was no correlation between gestational age and numerical density of RNAP positive nuclei ($r2=0.0765$, $p=0.2248$). The numerical density remained constant throughout gestation.

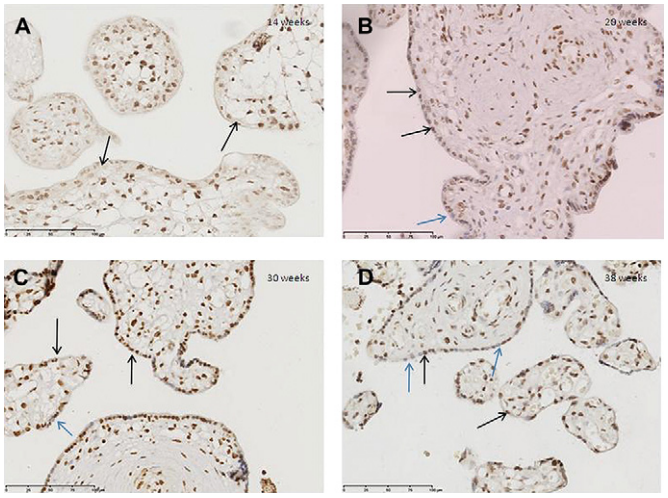

Figure 1. Evidence of RNAP activity in STB nuclei. (A-D) immunohistochemistry for RNAP, where black arrows indicate RNAP positive nuclei and blue arrows highlight negative staining. Gestational ages in weeks are shown in the right-hand colour.

Conclusion: These findings have shown that transcription takes place in a proportion of STB nuclei throughout gestation. Since the number of STB nuclei increases exponentially (0.62×10^{10} nuclei at 13-15 weeks to 5.81×10^{10} at 37-39 weeks) and the numerical density of RNAP positivity remains constant, we conclude the number of transcriptionally active nuclei also increases exponentially. Further research is needed to determine the mechanisms controlling the maintenance of heterochromatin in STB nuclei including investigating chromatin modifications and heterochromatin-binding proteins.
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NF is supported by an ASGBI studentship.
Keywords: Syncytiotrophoblast, Transcription, RNA Polymerase II

[P1.79]

HYPERGLYCEMIA INDUCES OXIDATIVE STRESS IN FIRST TRIMESTER TROPHOBLAST-DERIVED ACH-3P CELLS

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Objectives: Pregnancy is a state of oxidative stress arising from increased production of reactive oxygen species (ROS). ROS formation is often augmented in disease states associated with inflammation such as diabetes.
We hypothesize that diabetes-associated hyperglycemia leads to increased ROS generation in trophoblasts resulting in their altered proliferation potential that can be prevented by antioxidants.
Methods: Trophoblasts (ACH-3P cell line) and explants of the first trimester of pregnancy (wk 6-12) were cultured under hyper- and normoglycemic (HG: 25 vs NG: 5.5mM D-glucose) and different oxygen conditions (2.5, 8 and 21%). ACH-3P proliferation was measured by counting of viable and dead cells (CASY). In explants, the proportion of proliferation-marker (Ki-67) positive cells was counted after immunostaining. Oxidative stress was measured by a fluorescence assay (H2DCFDA) after adapting it to a multiwell format.
Results: ACH-3P cells treated under HG at 2.5 and 8% oxygen produced up to 1.8-fold ($p<0.001$) more ROS than under NG. At 21% oxygen HG led to a 30% reduction of ROS formation ($p<0.001$). Proliferation rates of ACH-3P cells showed no significant alterations by HG under 2.5 and 8% oxygen. At 21% oxygen HG resulted in 60% fewer ($p<0.001$) viable cells. Addition of the antioxidant ascorbic acid and tocopherol (Trolox) did not restore the proliferation potential of ACH-3P by HG and 21% oxygen. Placental explants exhibited decreased proliferation rates up to 35% ($p<0.01$) by HG at 21% oxygen whereas at 2.5% oxygen no significant alteration were observed. At 8% oxygen up to 4-fold ($p<0.001$) more Ki-67 positive cells were counted under HG.
Conclusion: Hyperglycemia leads to elevated ROS generation in ACH-3P cells under 2.5 and 8% oxygen. This is most likely due to enhanced glycolysis. At 21% oxygen the lower ROS levels and decreased proliferation potential under hyperglycemia and 21% oxygen can be a consequence of reduced cell metabolism.
Keywords: oxidative stress, hyperglycemia, trophoblasts, placental explants

[P1.80]

BLOOD AND PLACENTAL OXIDATIVE STRESS ASSESSMENT IN RATS WITH MILD DIABETES

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Introduction: The placenta generates reactive oxygen species (ROS) which may contribute to the oxidative stress seen even in normal pregnancy. However, ROS is increased in pregnancies complicated by IUGR and pregestational diabetes1. Therefore, the purpose of this study was to assess oxidative stress in blood and placentas of rats with mild diabetes.
Methods: Diabetes was induced by subcutaneous streptozotocin (100 mg/kg) in *Wistar* rats (mild diabetes group - MD) on the day of birth. As adults, DM and control (C) rats were mated. The presence of spermatozoa in vaginal smears was used to indicate day zero of pregnancy. Glycemia was measured at days 0, 7, 14 and 21 of pregnancy. On day 21, the animals were anesthetized and killed. Blood was collected for the assessment of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione redutase (GSH-Rd) activity, as well as thiol groups (SH) and malonaldehyde (MDA) concentrations. Placentas were processed for the evaluation of SOD and catalase activity, and glutathione reduced (GSH), MDA and SH concentrations. Statistical analyses were performed using the τ test of Student and normal-inverse gamma distribution ($p<0.05$).
Results: In the DM group, glycemic mean was $>$ 120mg/dL on days 0 and 14 of pregnancy ($p<0.05$) and significantly increased GSH-Px and catalase activity in blood and placental tissue, respectively ($p<0.05$).
Discussion: Glycemic findings at 14 days of gestation were consistent with those reported by Triadou *et al.* (1982) who observed changes in carbohydrate metabolism leading to glucose intolerance. The increased GSH-Px concentration and catalase activity, and the non-exacerbation in MDA concentrations observed in the diabetic groups suggest a maternal protective mechanism against the oxidative damages caused by hyperglycemia and pregnancy. In this study, increasing the production of antioxidative biomarkers was enough to reduce maternal oxidative stress.
FINANCIAL SUPPORT: FAPESP (Number Process: 07/02673-1)
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Keywords: Diabetes, Placenta, Oxidative stress, rats

[P1.81]

ANTIOXIDANT ENZYMES DURING PLACENTAL DEVELOPMENT IN THE RAT

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Introduction: Placental oxidative stress plays a role in maternal disorders, principally preeclampsia and intrauterine growth restriction. However, in normal pregnancy placenta deals with the presence of reactive oxygen species (ROS). ROS accumulation is limited by antioxidant enzymes, including superoxide dismutases (SODs), which catalyse the conversion of superoxide to hydrogen peroxide (H_2O_2) and oxygen, and glutathione peroxidase (GPX), that inactivate H_2O_2.

During pregnancy, an increase in maternal blood flow occurs accompanied by fetal growth and placenta size increase. It is probably during this period that differences in placental antioxidant enzymes distribution get established.

Objectives: To determine the distribution of SOD1 and GPX during placental development in rats.

Material and Methods: Rat placentas were collected on days 14, 17 and 20 postcoitum. We used immunoperoxidase technique to identify SOD1 and GPX in different regions of placenta, which was visualized and captured with a CX81 microscope and DP71 digital camera (Olympus).

Results: Placental weights increased markedly from day 17 to 20 of pregnancy, related to an increase of labyrinth region growth. SOD1 and GPX were observed in all regions of the placenta. However, the distribution in the labyrinth region increases during final stages of pregnancy. In the spongiotrophoblast region only GPX have an increase in their distribution. In the giant trophoblast cells region, both enzymes were observed during all period of study with similar distribution.

Conclusion: Placental distribution of antioxidant defenses increases during final stages of pregnancy in rats, particularly in the labyrinth region, the site of maternal-fetal exchange. This result goes in parallel with a gene expression increment for these enzymes as it has been observed by others and probably this is related to an increase of placental size and blood flow.

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Keywords: SOD 1, Glutathion peroxidase, Placenta, Rat

[P1.82]

CULTURING IN LOW OXYGEN TENSION PROMOTES INVASIVE TROPHOBLAST DIFFERENTIATION AND REDUCES OXIDATIVE STRESS IN EMBRYOS

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Objective: Cell culture experiments suggest that oxygen tension may play a critical role in promoting embryo development. The purpose of this study was to determine the oxygen tension to promote optimal trophoblast proliferation and embryo development.

Methods: Mouse trophoblast stem cells (TSC) were cultured in 2% to 20% oxygen tension and the proliferation rate was determined. Differentiation was induced by removing proliferation factors from the media and assessed by measuring pathway specific gene expression. In parallel experiments, mouse embryos were cultured in low oxygen (2%, 5%, 20%) and assessed for trophoblast proliferation and developmental competence. The effect of oxygen on implantation was measured by evaluating trophoblast outgrowths. Oxidative stress (OS) was assessed by measuring intracellular hydrogen peroxide production; the effect of oxygen on endogenous antioxidant production was measured with real time PCR.

Results: Low oxygen tension induced TSC proliferation by 2.5-fold and promoted their differentiation into spngiotrophoblast cells as determined by increased expression of *tpbp*; optimal differentiation occurred at 5% oxygen. Consistent with these data, mouse embryos in low oxygen had greater rates of trophoblast proliferation as measured by hatching. Also consistent with the stem cell data, embryos cultured in 5% oxygen induced the expression of tpbp. Embryos at 5% oxygen also had better morphology than embryos cultured at either 2% or 20% oxygen. There was no demonstrated effect on invasion. However, there was significantly less oxidative stress (OS) and significantly more antioxidant gene expression at 5% oxygen tension.

Conclusions: Low oxygen tension promotes the proliferation of trophoblast cells in monolayer cell culture and within the blastocyst. Further, 5% promotes healthy morphology and reduces OS. These effects may be do to a protective effect of low oxygen on embryo development. However, the molecular mechanisms of this benefit are unclear and the ideal oxygen tension has not been established.

Keywords: Low oxygen, Embryo morphology, spongiotrophoblast, oxidative stress

[P1.83]

DOES A LOW OXYGEN ENVIRONMENT AFFECT SHEDDING OF SYNCYTIAL KNOTS AND RELEASE OF INFLAMMATORY MEDIATORS FROM THE HUMAN PLACENTA?

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Introduction: Preeclampsia affects 3-5% of pregnancies and is characterised by endothelial cell activation. Syncytial knots shed from placenta into maternal blood circulation are recognised as one of the potential pathogenic triggers of preeclampsia. Poor placental perfusion is associated with some cases of preeclampsia. This had led to the hypothesis that placental hypoxia may have a role in the pathogenesis of preeclampsia, but that hypothesis has been questioned recently. We previously showed that a change in the nature of syncytial knots from apoptotic to necrotic may cause systemic endothelial cell dysfunction. In this study we investigated whether a low placental O2 environment provides a link between the placenta, in terms of the nature of shed syncytial knots and induction of endothelial cell activation.
Methods: 1st trimester placental explants were cultured in 1% or 8% O2 environments and the number of shed syncytial knots quantified. Syncytial knots from the different conditions were added to endothelial cell monolayers, and endothelial cell-surface ICAM-1 expression was measured by ELISA. Cytokines were measured in endothelial cell conditioned medium by commercial ELISAs.
Results: There were no significant differences in the number of syncytial knots shed, nor in the secretion of IL-1β or IL-6 from explants cultured in 1% or 8% O_2. The expression of ICAM-1 by endothelial cells that had been cultured with syncytial knots shed from explants cultured in 1% or 8% O_2 was also not significantly different.
Discussion: Our data suggest that a low placental O_2 environment does not, cause a change in the nature of syncytial knots such that they induce endothelial cell activation, nor in ,in the release of inflammatory mediators from the placenta. Our results add to the growing evidence that a low placental O_2 environment may not an essential component contributing to the pathogenesis of preeclampsia.
Keywords: hypoxia, trophoblast deportation, endothelium activation

Poster session 2 (P2.1 - P2.84)

[P2.1]

DECORIN IS A NOVEL VEGFR-2-BINDING ANTAGONIST FOR HUMAN EXTRAVILLOUS TROPHOBLAST INVASION OF THE DECIDUA

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Introduction and objectives: Extravillous trophoblasts (EVTs) of the human placenta invade the uterine decidua and its arteries to ensure adequate placental perfusion with maternal blood. Poor EVT invasion may result in fetal growth restriction and maternal preeclampsia. We previously identified two decidua-derived molecules TGF-beta, and a TGF-beta-binding proteoglycan decorin (DCN) as independent negative regulators of EVT proliferation, migration and invasiveness. We reported that DCN acts via multiple tyrosine kinase receptors EGF-R, IGFR-1 and VEGFR-2. Since binding of DCN to VEGFR-2 has never been reported earlier, present study explored the nature of this binding, the approximate molecular location of VEGFR-2 binding site in the DCN core protein and its functional role in our human first trimester EVT cell line HTR-8/SVneo.
Methods and Results: Based on far-Western blotting and co-immunoprecipitation studies we report that DCN binds both native (EVT and HUVEC-expressed) and recombinant VEGFR-2 and that this binding is abrogated with a VEGFR-2 blocking antibody indicating an overlap between the VEGF-binding and the DCN-binding domains of VEGFR-2. In a radioligand binding assay conducted with intact EVTs we determined that 125I-labeled VEGF-E (a VEGFR-2 specific ligand) binds EVTs with a Kd of 566 pM, and DCN displaced this binding with a Ki of 5.78 nM, indicating a 10 fold lower affinity of DCN for VEGFR-2. DCN peptide fragments with overlapping amino acid sequences derived from the LRR5 domain of DCN that blocked the binding of DCN to VEGR-2 in EVT lysates also blocked VEGF-induced EVT cell proliferation and migration, indicative of functional VEGFR-2 binding sites of DCN. Finally, DCN inhibited VEGF-induced EVT migration by interfering with ERK1/2 activation.
Conclusions: Our findings reveal a novel role of DCN as an antagonistic ligand for VEGFR-2, having implications for pathophysiology of preeclampsia, a trophoblast hypo-invasive disorder in pregnancy, and explain its anti-angiogenic function. (Supported by grants of the Canadian Institutes of Health Research to PKL).
Keywords: Extravillous trophoblast, Decorin, VEGF-receptor-2, Invasion

[P2.2]

SONOGRAPHIC FINDINGS ASSOCIATED WITH THE DIAGNOSIS OF PLACENTA ACRRETA

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Purpose: A number of sonographic signs have been described as indicative of placenta accreta. The purpose of this study was to determine the relative frequency and accuracy of these signs in predicting invasive placentation.
Methods: We performed a diagnostic accuracy study in which cases of pathology-proven placenta accreta were compared to cases of placenta previa matched by gestational age. Sonographic images were reviewed and ultrasound signs of placenta accreta (previa, loss of myometrial interface, chaotic intraplacental blood flow, 'swiss cheese appearance', placenta bulging into bladder, distance of bulge into the bladder, invasion into the bladder, and color Doppler crossing vessels) were coded as present, absent or indeterminate. The images were independently reviewed by 3 physicians.
Results: Twenty-seven cases of pathology-proven accretas were identified and compared to 54 cases of placenta previa between 2002 to 2008. Of the acceta cases, 14 (52%) were accretas, 4 (15%) were incretas and 9 (33%) were percretas. The frequency of the sonographic signs evaluated are shown in Table 1. The most common and sensitive sonographic finding associated with accreta was loss of myometrial interface. If present all signs were highly specific (90-100%) and had a high positive predictive value (90-100%) for the diagnosis of placenta accreta. Conversely the negative predictive value of each of these signs was high ranging from 68-98%. No combination of sonographic signs could accurately predict all placenta percretas. However, placenta percretas often had more than one sonographic sign.
Conclusions: The presence of a previa with loss of myometrial interface is the most common finding in patients with accreta.

Table 1. Frequencies of the nine sonographic signs associated with placenta accreta.

	Previa	Loss of Myometrial Interface	Crossing vessels	Chaotic Blood Flow	Bladder bulge	'Swiss cheese appearance'	Bladder invasion
All patients (N=81)	98%	30%	23%	28%	25%	21%	19%
Previas (N=54)	100%	0%	0%	0%	4%	0%	0%
Accretas (N=27)	93%	89%	70%	85%	67%	63%	56%

Keywords: Placenta Accreta, Ultrasound, Diagnosis

[P2.3]

IN VITRO STUDY OF METALLOPROTEASES DURING THE HUMAN DECIDUALIZATION PROCESS

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During pregnancy decidua, maternal face, regulates the nutrition of the embryo, the immune response and the restriction of invasion, generating an intra-uterine environment appropriate to the success of pregnancy. The correct formation of the decidua is essential in the process of implantation, placentation and embryonic development. It has been shown the role of metalloproteinases and their inhibitors in the process of decidualization (1, 2, 3, 4). We studied the activity and expression of MMP-2 and MMP-9, as well as the expression of TIMP-2 during human *in vitro* decidualization process. We used primary cultures of human uterine fibroblasts (HuF) from normal term placentae. The cells were cultured and *in vitro* decidualizated in the presence of hormones and cAMP. The activity of MMPs was analyzed by zymography in culture supernatant. We observed a significant increase in proMMP-2 activity, compared to control, during decidualization. On the other hand, no changes were observed in the activity of MMP-2. The low activity of MMP-9 observed in these conditions did not allow analysis by densitometry of gels. The expression of MMPs and TIMP-2 was analyzed by immuno-blot in cell extract. The expression of proMMP-2 was up regulated in treated cells, compared to control. No changes were observed in the expression of MMP-2 and TIMP-2. We neither observed changes in the expression of proMMP-9 and MMP-9. We used a commercial ELISA kit to measure levels of MMP-2 in culture supernatants. The levels of MMP-2 were seen augmented in decidualization, compared to control. These results show the specific regulation of proMMP-2 during the process of decidualization. It is necessary to analyze in further studies the regulation and the participation of the proMMP-2 as an inducer or as a result of this process.
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Keywords: Metalloproteinases, Decidualization Process, Decidua, Placentae

[P2.4]

ACTIVITY AND EXPRESSION OF MATRIX METALLOPROTEINASES IN AN *IN VITRO* CO-CULTURE MODEL

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Successful implantation is the end result of complex molecular interactions between the hormonally primed uterus and a mature blastocyst. Human reproduction is relatively inefficient, of the pregnancies that are lost, 75 percent represent a failure of implantation (1). Ethical restrictions and limited availability of human placental tissue limits the possibilities of human implantation studies (2). *In vitro* culture systems, within their limitations, have proved to be useful in elucidating some of the important molecules involved in implantation and decidualization (3). We proposed to use an *in vitro* co-culture model of human uterine fibroblast (Huf cells) with trophoblast explants to study materno-fetal interaction. The aim of this study was to evaluate matrix metalloproteases' (MMPs) protein expression and activity, because they are principal responsible in remodelling the extracellular matrix during implantation process. In this model HuF cells and trophoblast were obtained from human term plancentae. Western blot analysis was carried out on HuF cells and trophoblast explants to study the protein expression of MMP-2 at 21th day of culture. Gel zymography was used to detect the proteolytic activity of MMP-2 and -9 at the different conditions of culture, at several times: 4, 8, 12, 16 and 20 days. The protein expression of pro-MMP-2 was up-regulated in HuF cells after co-culture with trophoblast compared with controls; on the contrary, protein expression of MMP-2 was down-regulated. In trophobast explants that had been in contact with HuF cells, the protein expression of MMP-2 was up-regulated. The activity of pro-MMP-2 and MMP-2 were up-regulaled in co-culture condition compared with controls ($p<0,005$). In contrast, the activity of MMP-9 was down-regulated ($p<0,005$). The model proposed in this work is a good tool for further studying the role of MMPs during the implantation process.

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Keywords: implantation, co-culture model, matrix metalloproteinases

[P2.5]

DIFFERENTIAL EXPRESSION AND LOCALIZATION OF DECORIN AND BIGLYCAN IN HUMAN NORMAL TERM PLACENTA AND IN HIGHLY INVASIVE PATHOLOGIES

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Introduction: Decorin and biglycan are members of the small leucine-rich proteoglycans (SLRP) family and are important factors in the control of cellular proliferation, migration and invasion. Several placental pathologies include increased invasive activity of the trophoblast. These pathologies can be lethal for both mother and fetus and, they can be developed during gestation as placenta accreta and invasive mole or after gestation, like classic choriocarcinoma. The aim of this study was to characterize the differential expression and localization of decorin and biglycan in normal term placenta, placenta accreta, invasive mole and choriocarcinoma.
Methods: Characterization of the samples were done and the localization of decorin and biglycan was performed using immunohistochemistry.
Results: In normal term placenta, decidual cells presented positivity for decorin whereas the extravillous cytotrophoblast cells (EVT) were negative. Decorin was weakly stained in decidual matrix, however, the matrix-type fibrinoid was not reactive. In placenta accreta and invasive mole, EVT were positive for decorin whereas its surrounding matrix was negative. In choriocarcinoma, only cytotrophoblast cells and metastatic cells were immunoreactive for decorin, including mitotic figures of cytotrophoblast cells. On the other hand, biglycan showed similar results to decorin reaction in almost all cases, with exception of the matrix-type fibrinoid in normal term placenta and the EVT surrounding matrix in placenta accreta, which presented strong staining for biglycan.
Discussion: These results demonstrated that decorin and biglycan are differentially expressed in normal term placenta and in placental pathologies. These results suggest that the expression patterns of biglycan in highly invasive cells from placenta accreta, invasive mole and choriocarcinoma might play a role in modulating trophoblast migration and invasion. Also, the expression pattern presented by decorin suggests that this proteoglycan might exert another role than migration/invasion modulation.
Financial Support: CNPq
Keywords: Biglycan, Decorin, Extravillous Cytotrophoblast, Placental Pathologies

[P2.6]
HYPERGLYCOSYLATED HCG IS A MARKER OF EARLY HUMAN TROPHOBLAST INVASION

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Context: Human chorionic gonadotropin (hCG) is the major pregnancy glycoprotein hormone whose maternal concentration and glycan structure change all along pregnancy. hCG is mainly secreted by the syncytiotrophoblast covering the chorionic villi but little is known about the source of hyperglycosylated hCG (hCG-H) production.
Objective: To analyze expression and secretion of hCG and hCG-H, *in vitro* during human trophoblastic cell differentiation, *in situ* in first trimester placentas and in maternal sera during early pregnancy.
Design: hCG and hCG-H were measured in cell supernatants from primary cultures of first trimester placenta trophoblastic cells, which differentiate *in vitro* into syncytiotrophoblast or invasive extravillous cytotrophoblasts (evct). hCG-H immunodetection were performed on 9 WG placental tissue sections. Total hCG and hCG-H were quantified by chemiluminometric assay in 539 maternal sera collected between 9 and 19 WG during normal pregnancies.
Results: *In vitro*, hCG secretion reached 37 ng/mL/μg DNA during syncytiotrophoblast formation, but contained few hCG-H (2 to 5 % of total hCG). In contrast, hCG secretion (20 ng/mL/μg DNA) in evct supernatants contained 10 to 20 % hCG-H. *In situ*, hCG-H immunostaining was strong in invasive and endovascular evct, weaker in mononucleated villous cytotrophoblasts but negative in the syncytiotrophoblast. In maternal sera, hCG-H concentrations continuously decreased during pregnancy from 406±222 ng/mL at 9 WG to 8±6 ng/mL at 19 WG, whereas total hCG picked at 11WG and then decreased.
Conclusions: This study suggests that the high levels of hCG-H observed in first trimester maternal sera are mainly from invasive evct origin, reflecting the early trophoblast invasion process.
Keywords: hCG, trophoblast, hCG-H, invasive extravillous cytotrophoblasts

[P2.7]
A ROLE FOR NOTCH SIGNALLING IN HUMAN TROPHOBLAST INVASION

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Background: Notch signalling, a highly conserved pathway, regulates numerous cellular processes such as stem cell maintenance, cell fate decision, apoptosis, proliferation and invasion, thus representing an interesting study object in the context of trophoblast differentiation. Activation of Notch signalling occurs via cleavage of Notch receptors (1-4) upon binding to the neighbouring cell associated transmembrane ligands jagged1/2 and Dll1/3/4. Subsequently, the Notch intracellular domain (NICD) tanslocates to the nucleus, where it binds to the transcription factor RBPJ*k* and initiates target gene transcription such as HEY and HES family members. While mouse knock-out studies suggest an important role for Notch signalling in placental development, importance of the signalling cascade in the human placenta remains elusive.
Objective: Firstly, we determined the expression pattern of Notch receptors and ligands in the human placenta by performing several descriptive methods. Secondly, we sought to gain first insights into the activity and function of Notch signalling in human trophoblast invasion.
Methods: Protein and mRNA were isolated from placental villi, decidual tissue and fibroblasts, primary first trimester cytotrophoblasts and extravillous trophoblasts as well as trophoblast cell lines to perform RT-PCR and Western Blot analyses. Moreover, using specific antibodies we analyzed expression patterns of Notch receptors and ligands in tissue sections of first trimester placentae. Notch activity was studied in the trophoblast cell line SGHPL-5 using a luciferase reporter plasmid containing RBPJ*k* binding sites. HES activation was investigated upon overexpression of NICD of Notch1 in SGHPL-5 cells. Migration/invasion was studied by titrating the Notch inhibitor DAPT into invasion assays and differentiating villous explant cultures.
Results: Descriptive analyses revealed a broad expression pattern of both Notch receptors and ligands in placental tissue, primary isolated trophoblasts and trophoblast cell lines. Interestingly, immunohistochemical analyses showed a prominent staining for the Notch receptors 1-3 in cell columns of first trimester human placentae. The RBPJ*k* reporter assay revealed basal Notch activity in SGHPL-5 cells which was further enhanced by overexpression of NICD. Finally, inhibition of Notch signalling by DAPT resulted in an increase of the invasive capacity of both, SGHPL-5 cells and first trimester explants cultures.
Conclusion: Based on our descriptive and functional analyses we propose a novel role for Notch signalling in trophoblast differentiation by negatively regulating the invasive capacity of EVT.
J.P. is supported by grant Nr. P-22687-B13 of the Austrian Science Funds, Austria
Keywords: trophoblast, invasion, Notch signalling

[P2.8]
A NOVEL ROLE FOR PAR6 IN TROPHOBLAST CELL FUSION

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Introduction: Human placental development is dependent upon the establishment of proper trophoblast cell differentiation events including fusion and invasion, shaping proper organogenesis. This study examines the contribution of polarity to trophoblast cell fusion, an area of research which remains elusive, by examining Par6 (Partitioning defective protein 6), a key regulator of cell polarity.
Methods: Human placental tissues across gestation, preeclamptic placentae and age-matched controls were collected. To establish a role for Par6 in trophoblast fusion, primary isolated trophoblast cells were cultured at 3% and 20% oxygen and Par6 expression was examined spatially and temporally in conjunction with a polarity marker, Zona Occludin-1 (ZO-1). Par6 expression was assessed following forskolin treatment in BeWo cells. Par6 siRNA strategy was employed in BeWo cells and ZO-1expression was examined. Additionally, the role of Par6 on trophoblast fusion was assessed by examining fusogenic marker, GCM1 following Par6 silencing.
Results: Early in gestation, Par6 localized mainly to the nuclei of cytotrophoblasts while, with advancing gestation, Par6 expression increased and its localization shifted to the cytoplasm where it was found at the interface between cytotrophoblasts and syncytium. In primary trophoblast cells, Par6 levels were increased after 48 hours of exposure to 3% O2 and localized to tight junctions while at 20%, Par6 expression remained cytoplasmic. Silencing Par6 in BeWo cells resulted in a disruption of ZO-1 localization and this was associated with an increase in GCM-1 expression. Following forskolin treatment, Par6 expression decreased which was associated with an increase in syncytin expression. Interestingly, Par6 expression was decreased in preeclampsia relative to age-matched controls.
Discussion: These findings provide novel insights into a role for Par6 in regulating trophoblast cell fusion via its effect on polarity during human placental development which may be disrupted and contribute to the pathogenesis of preeclampsia. (Supported by CIHR and OWH/IGH).
Keywords: polarity, fusion, oxygen, Par6

[P2.9]
ISOLATION, CULTURE AND CHARACTERIZATION OF EXTRAVILLOUS TROPHOBLAST CELLS FROM TERM PLACENTA: A DIFFERENT APPROACH

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Introduction: The extravillous trophoblast cells (EVT) are a heterogeneous cell population exhibiting different phenotypes and functions. These cells are essentials for the correct attachment and development of placenta, and consequently, for the fetal development as well. In normal term placenta, the majority of the cells that comprise the cytotrophoblast shell at the basal plate undergoes apoptosis and are replaced by fibrinoid. However, in the deeper layer of the term placenta, some viable EVT cells can be found. According to previous studies, these cells are capable of maintaining its invasive and biochemical properties, which could provide a good model system.
Methods: Fragments of the extravillous region of term placenta was carefully dissected from villi, digested and the EVT cells were separated by several centrifugation processes using a Percoll gradient. After, the EVT cells were cultured for 24, 48, and 72h. The characterization of the cultures were done using antibody markers such as human leucocyte antigen G (HLA-G), cytokeratin 7, human chorionic gonadotrophin (HCG), vimentin, placental alkaline phosphatase and placental growth factor (PlGF).
Results: The culture of EVT cells was best suited at 48h, containing cells with typical trophoblast phenotype. The great majority of the cultured cells (95%) exhibited cytokeratin-7, HLA-G, placental alkaline phosphatase and PlGF reactive cells, whereas the remaining 5% were HCG or vimentin positive cells.
Discussion: The current protocol of EVT cell isolation and culture can be considered as an efficient model system that can be useful for the study of third trimester EVT cells, providing an alternative method for villous explant-derived EVT cells. Further studies are ongoing to confirm whether these cells still have an invasive potential. In addition, it is a new opportunity to study EVT cells in countries where the use of first trimester placenta is unlawful.
Financial support: CAPES, CNPq and FAPESP 56780-6/08.
Keywords: Extravillous Trophoblast Cells, Term Placenta, Culture, Cellular Characterization

[P2.10]

INCREASED EXPRESSION OF BCL-2 DURING INTERCELLULAR FUSION OF BEWO CHORIOCARCINOMA

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Development of the human syncytiotrophoblast from mononuclear villous cytotrophoblast results in increased resistance to apoptosis. We previously reported that forskolin-induced differentiation and syncytialization of the BeWo cell line resulted in increased expression of both protein and mRNA for the anti-apoptotic protein Bcl-2 and increased resistance to apoptosis. However, in cells treated with solvent alone approximately 10% underwent spontaneous intercellular fusion, and in forskolin-treated cells approximately 20% did not fuse. We investigated whether increased expression of Bcl-2 was a result of forskolin-induced differentiation or the result of syncytialization. BeWo treated with DMSO or with forskolin for 72 hr were evaluated by immunofluorescence with antibody against E-cadherin to identify fused cells, antibody against Bcl-2, and nuclear staining with DAPI. Staining with anti-Bcl-2 was quantified and expressed as fluorescent intensity. The intensity of staining for Bcl-2 was similar (P = NS) in mononuclear cells from cultures treated with DMSO (77.6 $\pm$ 12.0) or forskolin (75.2 $\pm$ 9.6). Compared with mononuclear cells, staining for Bcl-2 was significantly increased by 17% in spontaneously fused cells in DMSO-treated cultures (90.4 $\pm$ 12.3; $P < 0.01$) and by 30% in fused cells in forskolin-treated cultures (97.5 $\pm$ 15.0; $P < 0.01$). Forskolin treatment enhanced the production of Bcl-2 by 8% in fused cells ($P < 0.05$). Thus, increased production of Bcl-2 in BeWo appears to be related more closely to syncytialization, whether spontaneous or forskolin-induced, than to differentiation.

Keywords: Bcl-2, BeWo, intercellular fusion

[P2.11]

HB-EGF AND HER-1 SIGNALING STIMULATE EXTRAVILLOUS TROPHOBLAST INVASION

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The EGF receptors HER1 and HER2 are differentially expressed during extravillous trophoblast (EVT) invasion. We have previously shown that the ability of Decidual conditioned medium to induce invasive EVT differentiation was abrogated in the presence of the HER-1 antagonist, AG1478 yet the HER1 ligand EGF does not induce migration and invasion of either Jar cells or primary explant EVT. This study examines the role of HER-1 phosphorylation and downstream signaling and the differential effects of EGF and HBEGF in the differentiation and invasion of EVT.

The Jar choriocarcinoma cell line and placental villous explants were used as experimental models and immunohistochemical assessment of protein markers of EVT differentiation (decreased HER-1, Cx40, and increased HER-2) was performed. Western blotting, multiplex phosphorylation and migration assays were performed to assess the role of EGF and HBEGF mediated activation of HER1 and the downstream signaling pathways in trophoblast invasion.

In contrast to EGF, HBEGF treatment downregulates Cx40, regulates the HER-1/2 switch and also induces a potent dose dependant migration of both Jar cells and explant EVT in a manner that is inhibited by both AG1478 or AG825 (HER2 inhibitor). Western blot analysis of HER-1 activation demonstrated that HBEGF stimulates a rapid time dependant phosphorylation of HER1 at both the Tyr992 and Tyr1068 site, leading to sustained downstream activation of the MAP kinase and PI3 Kinase/ Akt signaling pathways. In contrast EGF activates HER-1 Tyr1045 , a site associated with ubiquintination and degradation of HER-1, and does not activate downstream pathways. Real time invasion assays using a panel of signaling pathway inhibitors confirmed that HBEGF mediated migration was dependent on the PI3K/Akt pathway.

These results demonstrate that HBEGF activation of the HER-1 signaling through PI3 Kinase and subsequent activation of Akt and GSK3 a/b is an important component of EVT invasion.

Funded by: CIHR IHD165436

Keywords: HB-EGF, HER-1, Extravillous Trophoblast, Signaling

[P2.12]
OXYSTEROLS INHIBIT TROPHOBLAST SYNCYTIALISATION BY ACTIVATING THE LIVER X RECEPTORS

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Objectives: Oxygenated cholesterol metabolites known as oxysterols display potent biological activities ranging from regulation of lipid homeostasis to cytotoxic and pro-apoptotic effects, and have been linked to lipid disorders such as atherosclerosis. Oxysterols have previously been shown to inhibit invasion of first trimester trophoblasts, an effect which involves activation of the nuclear Liver X-receptor (LXR), suggesting a link between oxysterols and preeclampsia. In this study, we investigated the effects of several oxysterols on trophoblast syncytialisation (differentiation and fusion) in term placental trophoblasts.
Methods: Primary trophoblast cells were isolated from term placentas by dispase enzyme digestion, purified over a discontinuous Percoll gradient, and allowed to syncytialise *in vitro*. Trophoblast cells were treated with various oxysterols [25-hydroxycholesterol (25-OHC), 7-ketocholesterol (7-ketoC), 22(R)-hydroxycholesterol (22R-OHC)] and the synthetic LXR agonist (T0901317) at non-toxic doses, with or without pre-treatment with LXR antagonist geranylgeranyl diphosphate (GGPP). Trophoblast differentiation was monitored by measuring GCM-1 mRNA expression (after 12 hours by quantitative-PCR), hCG secretion (after 48 hours by ELISA) and placental alkaline phosphatase activity (after 48 hours by phosphatase assay), while cell fusion was determined by E-cadherin immunostaining (after 48 hours) and quantification of syncytialised nuclei.
Results: Trophoblast differentiation and fusion were both significantly inhibited by all oxysterols used in this study. GCM-1 expression was reduced between 30–50 %, hCG secretion between 20–35 %, alkaline phosphatase activity between 15–25 % and cell fusion between 30–50 %. The synthetic LXR agonist T0901317 also potently inhibited trophoblast differentiation and fusion. Moreover, treatment with the LXR antagonist GGPP abrogated the inhibitory effects of oxysterols and T0901317 on trophoblast syncytialisation.
Conclusion: These findings suggest that oxysterols impair differentiation and fusion of term trophoblast cells via an LXR-dependent mechanism. Excessive oxysterol exposure may impair placental formation and regeneration via inhibition of syncytialisation, thereby contributing to placental pathologies.
Keywords: LXR, oxysterols, differentiation, syncytialisation

[P2.13]
SYNCYTIN AND ITS RECEPTOR ASCT2 HAVE AN INFLUENCE ON THE FUSION ACTIVITY IN HUMAN PLACENTAL BEWO CELLS

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Introduction: Syncytin, the human endogenous retrovirus envelope proteins, is highly expressed in the placental syncytiotrophoblast layer and contributes to placental trophoblast fusion. ASCT2 is a syncytin receptor and known to be identified as the amino acid transporter B^0. To investigate the role of syncytin and ASCT2 on fusion activity, we use RNA interference technique in human placental BeWo cells.
Methods: BeWo cells was treated with siRNA for syncytin-1 and ASCT2, and then incubated with forskolin to become fusion. The effect of those specific siRNA treatments on the expression of mRNA and protein was evaluated by means of real-time RT-PCR and western blotting, respectively. The cell-fusion activity was evaluated by flow-cytometry and hCG secretion.
Results: The expression of both mRNA and protein of syncytin-1 and ASCT2 was reduced after incubation of its specific siRNA treatment. The secretion of hCG was also reduced after treatment with siRNA for syncytin and ASCT2, but the fusion activity was reduced only after treatment with siRNA for ASCT2 significantly.
Discussion: This result suggests that syncytin has a different effect on fusion activity and hCG secretion compared with ASCT2 in BeWo cells.
Keywords: cell fusion, syncytin, ASCT2

[P2.14]
ADAM12IN MOUSE PLACENTA

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Background: ADAM12 (a disintegrin and metalloproteinase domain 12; meltrin α) is a catalytically active metalloprotease and disintegrin which plays a role in myoblast fusion and osteoclast formation. In the placenta, ADAM12 has multiple functions, one of which seems to be regulation of trophoblast fusion. Therefore, we analyzed the development of mouse placenta from day 5 to 11 of pregnancy.
Methods: We analyzed mouse placenta from different days of pregnancy (5-11). Tissue was fixed in paraformaldehyd and processed for immunoperoxidase staining for detection of ADAM-12 with NovaRed.
Results: ADAM12 was detectable in all samples in maternal tissue. We also detected ADAM12 at day 8 of pregnancy in trophoblast cells invasion starting. At day 11 the signal is in the labyrinth and trohpoblast cell of placenta.
Discussion: ADAM12 is expressed in uterine tissue. It appears in trophoblast cells when invasion is initiated and again in further advanced trophoblast cells, which might be prepared for fusion and loss of invasiveness.
Conclusion: ADAM12 may be involved in regulation of trophoblast behaviour.
Keywords: murine placenta, invasion, ADAM12, trophoblast

[P2.15]
PIAS1/3 EXPRESSION KINETICS IN MOUSE PLACENTA

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Background: The JAK/STAT pathway is one of the important intracellular signaling cascades which is involved in several trophoblast functions including invasion, migration and proliferation. A major group of its negative regulators are PIAS (Protein Inhibitors of Activated STATs). PIAS1 was identified as a specific inhibitor for STAT1 and PIAS3 specifically associates with STAT3. PIAS proteins bind to activated STAT dimers and prevent them from translocation into the nucleus and from binding DNA.
Methods: We analyzed mouse placentae from different days of pregnancy (5-11). Tissue was fixed in paraformaldehyd and processed for immunoperoxidase staining for detection of PIAS1 and PIAS3 with NovaRed.
Results: A low signal of PIAS1/3 is detectable in maternal tissues at all days of pregnancy. At day 11 of pregnancy, PIAS1/3 appears also in the labyrinth of the placenta.
Discussion: PIAS1 and PIAS3 are inhibitors of STAT1 and STAT3 and, thereby, inhibitors of invasion. Their appearance at day 11 in the labyrinth coincides with reduced trophoblast invasiveness.
Conclusion: Our observation of state of pregnancy-dependent PIAS1/3 expression underlines the involvement in regulation of placentation
Keywords: murine placenta, trophoblast, PIAS, JAK/STAT signalling

[P2.16]
PLACENTAL ALTERATIONS IN ACUTE CADMIUM INTOXICATION

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Cadmium (Cd) is an environmental polutant that produces cronic intoxications afecting fetus and placenta. However, accidental exposure of human and domestic animals in industrial areas causes acute intoxications. In the present work the effect of a single 10 mg/kg Cd dose on fetuses and placentas was studied. Uteri, placentas and fetuses from 20 days of pregnancy Wistar rats were employed. CdCl2 single doses at days 4, 7, 10 or 15 of pregnancy were administered. Control females received saline. The number of viable individuals and embrionary resorptions were registered. Placental and fetuses samples were excised to measure Cd concentration. Some placentas were processed for the following techniques: H&E, PAS, Masson, picrosirius and lectinhistochemistry. Fetuses were stained with Alizarine red and H&E. Experimental groups rendered higher values of resorptions and fetal malformations. Cd concentration values were significantly higher in experimental placentas and fetuses. Congestion of laberintic blood vessels and coagulative necrosis were observed in placentas from Cd-treated females, particularly in the seventh day of pregnancy. A colagen type III increase was determined in placentas from intoxicated dams. Regarding lectinhistochemistry, a higher trophoblastic SBA and DBA binding was detected revealing an augmentation of galactose and galactosamine. A fucose decrease was detected by UEA-1. Sections incubated with BSA-1 and microwaved allowed the discrimination of fetal blood vessels and maternal lacunae to perform estereological examination that resulted in a vascular volumen decrease in intoxicated groups. Placental changes found may be the cause of the resorptions and fetal alterations observed. These results clearly demonstrate the risk for female to high Cd concentration exposure during pregnancy.
Keywords: Cadmium, lectinhistochemistry, stereology, rat

[P2.17]
EFFECTS OF CARBAMAZEPINE ON THE PLACENTA AND ON THE EMBRYO DEVELOPMENT

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Carbamazepine (CBZ) is largely used in the treatment of trigeminal neuralgia, affective disturbs and, especially as an anticonvulsive drug, which is prescribed for pregnant women who need receive CBZ continuously to avoid convulsive crises and intra-uterine fetal hypoxia. CBZ passes throughout the hematoplacental membrane and may impairs the embryo development and affects the placenta itself. The aim of this project is to investigate and evaluate some of the morphological alterations on the embryo and the placenta observed on the CBZ treated rats. Normal cycling sexually mature healthy nuliparous Wistar strain rats (UNIFESP, São Paulo, Brazil), three months of age, 250 g of weight, were acclimated under laboratory conditions (12h light, 12h dark, 26°C). They were provided feed and water *ad libitum*. They have been divided into three groups of four animals each and all females have received 20mg/Kg/day, i.p of CBZ as follows: The first group (T8) has daily received CBZ from the 8th to 12th day of pregnancy (dop); the second group (T12) from the 12th to 20th dop and; the third group (T15) from the 15th to 20th dop. Animals from control group have received propylene glycol vehicle. On the 20th dop, uterine horns and ovaries have been collected, weighed and macroscopic analysed. Besides, corpora lutea, embryo implantation sites, dead and alive fetuses and resorption sites have been counted and weighted as well. Placenta has been dissected, weighed, fixed in Bouin's liquid for 24h and conventionally processed for the hematoxylin and eosin staining. Preliminary results point increase of resorption index, decrease of both fetal and placenta weights on the CBZ treated rats from groups T8 and T12. Besides, the survival index for live fetuses has been also diminished and those which survived have not shown external and internal malformations, and visceral anomalies due to CBZ treatment. The placenta morphology from T8 group has shown its deciduas basalis disorganized, in which some decidual cells have presented signs of apoptosis. The CBZ toxicity on the embryo development and, mainly, on the placenta morphology must be better investigated.
Keywords: carbamazepine, decidua basalis, embryo resorption, placenta weight

[P2.18]

IMMUNOHISTOCHEMICAL STUDY OF VEGF EXPRESSION WITHIN THE PLACENTAL LABYRINTH OF MICE CHRONICALLY EXPOSED TO PARTICULATE AIR POLLUTION DURING PREGNANCY

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Within the placenta the fetal vasculature and the trophoblast must develop in such a way as to ensure that sufficient capacity for exchange occurs between fetal and maternal circulations. Thus, factors that affect placental development and function will have impacts on fetal growth and survival. We have previously demonstrated that chronic exposure to particulate air pollution during gestation resulted in morphological changes in the placenta. Among these must be considered smaller maternal blood space volumes and calibers on the maternal side of the placenta and greater surface area of fetal capillaries. Placental VEGF expression promotes endothelial cell proliferation, vascular permeability and is indirectly involved in the induction of trophoblast development. Under hypoxic conditions, placental expression of VEGF is up-regulated. We hypothesized that the observed changes in placental morphology associated to exposure to PM during gestation could be associated to altered placental VEGF levels. To test this, mice were maintained for two generations in exposure chambers situated close to a busy street of traffic in São Paulo. Two groups of 6 females were raised and completed pregnancies in normobaric chambers with exclusively filtered (F) or non-filtered (NF) air. The 24-hr concentration of particulate matter (PM2.5) inside the chambers was determined gravimetrically. At 18 days of gestation, female mice were euthanized and 1 placenta from each female removed, fixed in formalin, embedded in paraffin for microscopical examination. Placental morphology was assessed by stereological methods and VEGF expressions were analyzed via immunohistochemistry. Immunoreactivity was measured as total volume of positive stained tissue within the labyrinth. Results showed that in the labyrinth of animals exposed to particulate pollution the VEGF immunoreactivity are increased (mean (SD) mm^3; F= 0.0062 (0.002), NF= 0.0091 (0.001) mm^3, $p<0.03$), and we did not observe any preferential distribution of VEGF. In conclusion, these preliminary results suggest that the exposure to particulate air pollution during pregnancy elicits an increased expression of VEGF, indicating that the ambient levels of air pollution alter mechanisms that regulate endothelial proliferation.

Keywords: Air pollution, placenta development, VEGF

[P2.19]

GESTATIONAL EXPOSURE TO URBAN PARTICULATE AIR POLLUTION AFFECTS GLYCOGEN CELLS VOLUME IN THE JUNCTIONAL ZONE OF THE MURINE PLACENTA

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Adverse pregnancy outcomes are associated with exposure to urban air pollution (UAP). We have shown that gestational exposure to UAP result in changes in functional morphology of the murine placenta. Decreases in fetal weight of exposed animals were accompanied by decreases in the volume of the maternal blood space, increases in the surface area of fetal capillaries (FC) and in the total diffusive conductance of the intervascular barrier(IVB). The changes seen in pregnancies associated with UAP is surprising in the sense that some seem to be adaptive and others deleterious. Greater surface area of FC, total diffusive conductance and mass-specific conductance of the IVB may be seen as fetoplacental adaptations serving to maintain and expand oxygen and nutrient delivery to the foetus. The fact that foetal weight declines despite these adaptations implies that other factors exert influential effects. Although the function of glycogen cells (GC) in the placenta remains to be fully explained, some authors believe that placental glycogen stores are used as fuel for placental consumption or to attend fetal demand in times of need. Thus we hypothesized that changes in the GC content of the junctional zone(JZ) could also be influenced by exposing pregnant mice to UAP. To test this, mice were raised and completed pregnancies in chambers with exclusively filtered (F) or non-filtered (NF) ambient air. At 18-days gestation, n=4 placentas per group were weighed, fixed, embedded in glycolmethacrylate resin and stained for microscopical examination. Fields of view on vertical uniform random slices were analysed stereologically by point counting. Volume of JZ and CG were estimated. Group comparisons were drawn using Student *t* test. Null hypotheses were rejected at $P<0.05$. Results are summarised in Tables 1.

Table 1. Volume and volume density of JZ and GC of mice exposed to air pollution (NF) or filtered air (F).

	Groups	N	Mean	SD	p
Vol GC (cm^3)	F	4	0.0017	0.0002	0.02
	NF	4	0.0035	0.001	
Vol of JZ (cm^3)	F	4	0.019	0.001	ns
	NF	4	0.021	0.004	
Vol density of GC	F	4	0.092	0.016	0.007
	NF	4	0.166	0.033	

Our data indicated that exposure to air pollution during pregnancy affects the total volume of GC in the juctional zone thus indicating that in these placentas the consumption of glycogen is lower or its accumulation is increased. However, our study design did not allow us to identify which mechanism was responsible for the changes.

Keywords: air pollution, glycogen cells, junctional zone

[P2.20]

PLACENTAL AND FETAL MORPHOLOGICAL CHANGES ASSOCIATED WITH INGESTION OF MYKANIA GLOMERATA

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Background: *Mykania Glomerata*, popularly known as "guaco" is a medicinal plant widely used by Latin American population for the treatment of asthma, bronchitis and rheumatism. Phytochemical composition of the guaco extracts includes flavonoids, tannin and the coumarin, an anticoagulant agent. In spite of the beneficial effects its toxicity has never been assessed during pregnancy in a controlled investigation.
Objectives: This study aims to add baseline information on the biological effects of *Mykania Glomerata* during pregnancy, using as indicators the incidence of embryonic malformation and placental morphology.
Materials and methods: Macroscopic and skeletal analysis of fetuses and microscopic analysis of term placenta from mice treated by gavage with Mykania glomerata extract (600mg/30g) during the gestational days (gd) 7 to 11and 14 to 18, compared to control PBS-treated mice.
Results: When compared to the control group, the treated group exhibited intra-uterine growth restriction and decrease in placental weight ($p < 0.05$); increase in skeletal and cranial-facial birth defects and increase in the mortality rate (0.03 ± 0.05 *vs* 0.26 ± 0.15, $p < 0.05$). Placental morphological changes were suggestive of a functional placental deficit and included reduction in the ratio labyrinthine zone/junctional layer, significant increase of the glycogen cell population, invasion of these cells into the labyrinthine and decidual zones and, increase in the apoptotic index in the junctional layer.
Conclusion: Findings suggest caution in indiscriminate use of this phytotherapic during gestation.
Financial support: FAPESP.
Keywords: mykania glomerata, placenta, birth defects, abortion.

[P2.21]

STEREOLOGICAL STUDY OF THE EFFECTS OF DIESEL EXHAUST EXPOSURE DURING PREGNANCY ON PLACENTAL DEVELOPMENT IN MICE - PRELIMINARY RESULTS

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We have previously shown that maternal exposure to current levels of urban air pollution in São Paulo city may have deleterious effects on murine reproductive health. Maternal exposure before and/or during pregnancy affected fetal outcomes and the observed low birth weight was associated with changes in functional morphology of the placenta. The higher levels of air pollutants in São Paulo city are mainly due to low quality diesel combustion emission (500ppm of sulfur). Therefore in this study we investigated the effects of gestational exposure to diesel exhaust on murine placental development. Animals (5 per group) were daily exposed during pregnancy (0.5 dpc-12 dpc) to either filtered air (F group) or diesel exhaust (DE group) [550 μg/m3 of PM2.5 for 60 minutes/day] using exposure chambers. At 12-days gestation, n=5 placentas per group were prepared for microscopical examination. Fields of view on vertical uniform random slices were analysed stereologically. Volumes (V) of placental compartments were estimated by the Cavalieri Method. No significant differences ($p > 0.05$) were found between groups for any of the placental compartments (table 1).

Table 1. Effects of diesel exposure on placental volumes (mm3)

Group	V Lab	V JZ	V Dec	V CP	V Total placenta	V Total placenta+Dec
F	0.020± 0.01	0.010± 0.004	0.117± 0.025	0.006± 0.003	0.037± 0.016	0.154± 0.033
DE	0.023± 0.008	0.010± 0.004	0.129± 0.045	0.005± 0.003	0.039± 0.016	0.168± 0.058

Lab=labyrinth; JZ=junctional zone; Dec=decidua basalis; CP=chorionic plate
Mice exposure to DE during pregnancy did not affect placental compartments development. However based on previous studies it is too soon to discard the potential effects of DE exposure on placental development. Changes in placenta morphology associated with exposure to ambient air pollution were restricted to the fine structure of the labyrinth. Detailed analysis of labyrinth components and of the interhemal membrane are necessary to confirm or discard the negative effects of diesel exhaust exposure on placental development.

Keywords: Air pollution, gestation, particulated matter, stereology

[P2.22]

EFFECTS OF THE PESTICIDE CHLORPYRIFOS ON *IN-VITRO* MODELS OF TROPHOBLAST CELLS

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Environmental contaminants may disrupt trophoblast cell function and differentiation changing the expression of specific genes. An increased risk of spontaneous abortion, low weight offspring and intrauterine growth restriction in pregnant women chronically exposed to organophosphate (OP) pesticides has been reported. At present, the underlying mechanisms involved in placental toxic effects are not fully understood. Recent studies in other cell models indicate that OP toxic effects could be mediated through alteration of transcription factors implicated in cell replication and/or differentiation. Herein, we investigated whether Chlorpyrifos (Cp), one of the most widely used OP pesticides, has any effect on genes important for placental function. As an initial approach we employed the choriocarcinoma-derived Jeg-3 cell line model. Cellular viability was tested using the MTT assay and cell morphology was analyzed by desmosome immunofluorescencent localization and nuclei staining. Expression of glial cell missing 1 (GCM1) and krüpple-like factor 6 (KLF6) transcription factors, as well as human chorionic gonadotropin β-subunit (hCGβ) were determined by qRT-PCR. Jeg-3 cell viability exposed to increasing Cp concentrations (up to 100 μM) for 24 and 48 h was always greater than 75%. No major effect was observed on cell morphology, except for an increase in the cell number with fragmented and/or condensed nuclei. Cp treatment barely modified KLF6 expression, while it increased hCGβ and GCMa mRNA expression. These molecules are involved in trophoblast differentiation and have been associated with hypoxia-related placental pathologies. Our results suggest that placental Cp toxicity may be due in part to its effect on trophoblast differentiation. We are extending our study to in vitro differentiating cytotrophoblasts and placental explants to confirm this hypothesis. In sum, present data support the idea that Cp modifies placenta gene expression and could have implications for understanding the adverse pregnancy outcomes associated with Cp exposure in humans.

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Keywords: pesticides, gene expression, trophoblast

[P2.23]

PRENATAL EXPOSURE TO SODIUM ARSENITE MODIFIES PLACENTAL GLUCOSE TRANSPORTERS OF BALB/C MICE

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Chronic exposure to inorganic arsenic (iAs) has been associated with disorders in reproductive function, producing spontaneous abortions, premature birth, and intrauterine growth restriction. The aim of this study was to evaluate the effects of prenatal exposure to NaAsO2 on the morphology and the expression of placental transporters GLUT 1, GLUT 3 and GLUT 4. Female Balb/c mice were exposed orally from the 8th day of gestation to 0, 12 and 20 milligrams per liter (mg/L) of NaAsO2. The iAs and arsenic species (MMA and DMA) were quantified in placental tissue by atomic absorption spectroscopy coupled to a hydride generator. Morphologic changes were evaluated in histological sections stained with hematoxylin-eosin and the expression of transporters GLUT 1, GLUT 3 and GLUT 4 was localized by immunohistochemistry and image analysis. Results showed that fetuses and placentas from females exposed to 20 mg/L had a significant decrease in weight compared with those of non-exposed group ($p<0.01$). In placentas from exposed groups, the DMA was the most abundant arsenical specie found. Moreover, placentas from group exposed to 20 mg/L showed a significant increase ($p<0.01$) in the frequency of infarctions, as well as in vascular congestion. Regarding to GLUT transporter expression, exposure to 12 mg/L did not modify the expression of the transporter GLUT 1, whereas GLUT 3 transporter expression increased in placentas of the exposed group. By the contrary, GLUT 4 expression was significantly lower ($p<0.05$) in placentas exposed to 12 mg/L, and although GLUT 4 expression in 20 mg/dL was lower than observed in non-exposed group, difference was not significant. These results suggest that prenatal exposure to NaAsO2 induces morphological changes and modifies the expression of GLUT transporters, which could condition the adequate nutrition of fetus and alter their development.

Keywords: Arsenic, glucose transporters, inflammation, growth restriction

[P2.24]

EXPRESSION AND ACTIVITY OF MATRIX METALLOPROTEINASES IN HUMAN SYNCYTIOTROPHOBLAST CELLS EXPOSED TO LEAD IN VITRO

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Several studies have shown that placenta does not represent an effective barrier to lead transport, which can reach the fetus and promote damage. Prenatal exposure to lead causes placental morphological changes which could involve the activity of matrix metalloproteinases (MMPs), enzymes responsible for extracellular matrix remodeling. The aim of this study was to analyze the expression and activity of MMP-2, -3 and -9 in cultures of human syncytiotrophoblast cells exposed to lead acetate and its relationship with the production of IL-1β and IL-10. Primary cultures of human syncytiotrophoblasts isolated from placentas obtained through cesarean procedure performed in women with a healthy pregnancy, were isolated using the methodology described by Kliman et al. Syncytiotrophoblasts of 48 h of culture were exposed to concentrations of 5, 10, 15, and 20 μM of lead acetate during 24 h. The expression of mRNA of MMP-2, -3, and -9 was analyzed by qRT-PCR, the MMPs collagenase activity was quantified by zymography, and the production of IL-1b and IL-10 in culture supernatants was done by immunoassay. The results showed that exposure to lead significantly decreased the expression of MMP-2 (at 15 μM), and in MMP-9 (at 15 and 20 μM), without modifying the expression of MMP-3. The decrease in the expression of MMP-9 was associated with a decrease in cytosolic gelatinolytic activity at concentrations of 10 and 20 μM and for secreted protease by 5μM of lead. No significant changes were observed in the production of IL-1β and IL-10 at any of the concentrations of Pb applied to the cultures. Notwithstanding, the trend of IL-10 suggests an inverse relationship with MMP-9 actitity. These results suggest that, in our model, lead exposure may suppress the expression and activity of MMP-9, effect that could be regulated by the presence of IL-10.

Keywords: lead, syncytiotrophoblast, metalloproteinases, cytokines

[P2.25]

EXPRESSION OF ACETYLCHOLINE MUSCARINIC RECEPTORS M1 TO M5 IN PLACENTA FROM WOMEN EXPOSED ENVIRONMENTALLY TO ORGANOPHOSPHATE PESTICIDES

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Studies in experimental rodents have demonstrated that exposure to organophosphate pesticides (OP) downregulates the expression of M1 and M2 acetylcholine muscarinic receptors (mAChR). The aim of this study was to demonstrate the expression of mRNA mAChR M1 to M5 in human term placenta, analyze its expression and immunelocalization, and the relationship with environmental exposure to OP.

Placenta samples from women without history of metabolic and gestational disease were obtained at delivery (n = 60). Samples were classified according to risk of exposure in high (n = 27; women living in agricultural areas) or low (n = 31; women residing in urban areas). The mRNA expression of M1 to M5 mAChR subtypes was demonstrated in placental tissue by RT-PCR using primers previously reported. Immunelocalization of mAChR subtypes was carried out using specific antibodies; protein expression was quantified using the Image Pro-Plus software. Results demonstrated that the human term placenta expresses the mAChR subtypes M1 to M5, proteins expression were localized in syncytiotrophoblast layer of tertiary villous, and the order of magnitude mAChR protein expression was M2> M1> M3> M4> M5 subtypes. The analysis of mAChR expression according to risk of OP exposure revealed that although, in group of high risk of exposure, the five subtypes of mAChR showed lower levels of expression, only mAChR M1 had a significant decrease, which modified the M3 and M4 position in order of magnitude in high risk population. In analyzing the history of pesticide exposure found that the decrease was associated with women living with family members who work in the OP preparation. In conclusion, exposure to OP can condition a decrease in placental mAChR M1, although the physiological relevance of M1 receptor in the placenta has not been fully studied, the decrease may produce alterations in amino acids transport and on fetal growth.

Keywords: Acetylcholine, organophosphate, pesticides, muscarinic receptors

[P2.26]

IN VIVO AND IN VITRO BLASTOCYST DEVELOPMENT AFTER PERIGESTATIONAL ALCOHOL CONSUMPTION IN MOUSE

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Hypothesis: Periconceptional alcohol consumption alters preimplantational mouse embryo differentiation, probably inducing trophoblast dysfunction.

Objective: to study the effects of periconceptional alcohol ingestion by CD-1 mice on *in-vivo* (day 5 of gestation) and *in-vitro* blastocyst development (outgrowth, migration and differentiation). Adult females were exposed to 10% ethanol in drinking water for 15 days previous to and up to 5 days of gestation (TF) (Control group [CF] - received only water). Recovered hatched blastocysts were cultured and at 0-24-48 and 72h to analyze the developmental dynamics (number [Nr] of cells/embryo), morphogenesis and, trophoblast growth (proliferative rate), differentiation (% diploid [CT] and giant trophoblast cells [GTC]) and migration (trophoblast expansion area ([TE]). Embryos were classified as small, medium and big according to TE area intervals.

Results: TF presented diminished Nr blastocyts/female ($p<0.05$) and elevated percentage of abnormal embryos ($p<0.05$) vs CF. The percentage of small, medium and big embryos was invariable in CF through 48h-culture; at 48h, TF-bigger embryos % increased ($p<0.05$ vs 48h-CF) and small embryos showed an inverted pattern. Trophoblast nuclei Nr decreased in TF ($p<0.05$ vs CF, 72h-culture). TE of TF-small and medium embryos increased at 48 h-culture ($p<0.05$ vs CF). At 0-24h interval, both TE of TF-medium and big embryos decreased 14 and 28% vs controls, respectively. At 24-48 h interval, the same embryos increased 185% and 450% vs controls. TE of TF-small embryo increased in 6.4% and 11% at two intervals. The % of TGC and the mean TGC and CT area in TF group did not change.

Conclusions: maternal alcohol ingestion in mouse lead to implanting embryo losses, morphological anomalies, growth alterations and deregulation of embryo and trophoblastic expansion/migration, suggesting trophoblast damage during implantation.

Keywords: Blastocyst, Trophoblast, Migration, Differentiation, Implantation, Alcohol.

[P2.27]

PRETERM FETAL MALFORMATIONS AND PLACENTAL ABNORMALITIES INDUCED BY PERICONCEPTIONAL ALCOHOL INGESTION DURING THE FIRST HALF OF PREGNANCY IN CD-1 MOUSE

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Gestational ethanol consumption disrupts fetal development increasing the teratogenic outcomes and postnatal growth retardation. The objective was to study the effects of perigestational alcohol ingestion up to first half of pregnancy on preterm fetal and placental growth and external fetal malformations.

Methods: adult CD-1 females were exposed to 10% ethanol for 15 days previous and up to 4 (TF-D4), 8 (TF-D8) or 10 (TF-D10) days of gestation, following replacement ethanol by water up to day 18 of pregnancy. Control groups (CF) were performed with water. Fetus and placentae were weighted, measured and fixed for external malformations and skeletal alizarin red and histology (H-E).

Results: the number of implantation (no.) site (IS) of TF-D8 and TF-D10 was increased vs IS-CF ($p<0.01$), the total and early resorptions were elevated and the birth index (alive fetus Nr/IS Nr) was reduced in all treated groups vs CF ($p<0.05$). Mean placental weight was diminished in TF-D4 and TF-D10 vs CF ($p<0.05$) and histological alterations were found. The mean fetal weight decreased in TF-D10 vs CF ($p<0.05$) without changes in the mean fetal sizes. The % of total malformed fetus was increased in all treated groups ($p<0,001$), whereas the malformations found were: TF-D4: facial (ear implantation defect); TF-D8: facial and members, TF-D10: craniofacial, members and lower abdominal wall, CF: facial anomalies. Cranial skeletal (exencephaly) and member defects were confirmed by Alizarin analysis.

Conclusion: perigestational ingestion of moderate ethanol up to 4, 8 or 10 day of pregnancy induces at term, increased risk of early miscarriages and fetal growth restriction and external craniofacial malformations. Although the period of alcohol exposure more susceptible for fetal growth retardation and dysmorphology was the organogenesis, alcohol ingestion up to preimplantation was able to induce facial defects and suggesting that this early gestational phase in CD-1 mouse is alcohol-susceptible.

Keywords: Fetus, Malformations, Gestational Alcohol, CD-1 Mouse

[P2.28]

PERICONCEPTIONAL ETHANOL CONSUMPTION ALTERS COLLAGENS AND METALLOPROTEINASE-9 EXPRESSION IN CF-1 MURINE IMPLANTATION SITES AT MIDGESTATION

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Periconceptional alcohol ingestion causes organogenic embryo anomalies, growth restriction and increased early miscarriages. The matrix metalloproteinase 9 (MMP-9) cleaves type IV and V collagens, playing a role in decidualization, angiogenesis, trophoblastic migration and its invasion into the decidua.

Objective: To evaluate if periconceptional ethanol ingestion alters the distribution and deposition of collagen types I, III, IV and V, and MMP-9 expression and activity in vascular mesometrial decidua (VMD) and trophoblastic (T) tissues when organogenesis is occuring.

Methods: Adult CF-1 female mice were treated (TG) with 10% ethanol in drinking water for 17 days previously and up to day 10 of gestation, and they were compared to control group (CG). Implantation sites were processed for picrosirius staining for fibrillar collagens, and immunohistochemistry for I, III, IV, V collagens and MMP-9. Decidual tissues (D) were dissected to perform western blotting for MMP-9 and conditioned media from D 24 h-culture was used to zymograms.

Results: In VMD-CG picrosirius staining was observed as a defined and continuous line underlying the region of basal membrane of the endothelium of blood vessel, whereas in the VMD-TG it was discontinuous and faintly stained. Collagens I and III had similar immunostaining in the VMD-CG and VMD-TG, whereas the immunoreaction for type IV and V collagens was increased in VMD-TG. A slight immunoreaction for MMP-9 was detected in both VMD-CG and T-CG. However, the immunoreactivity was significantly reduced in VMD-TG and T-TG. In D-TG, the MMP-9 expression (immunoblotting) and zymographic analysis for the active form in conditioned medium was significantly decreased vs D-CG.

Discussion: Periconceptional alcohol ingestion, alters collagens deposition and collagen fibrils arrangement in the mouse pregnant endometrium, which was correlated with reduced MMP-9 expression and activity. These effects may be related to early anomalies in decidual-placental development and increased miscarriage due to maternal alcohol consumption.

Keywords: Metalloproteinase-9, Collagens, Placentation, Alcohol.

[P2.29]

ETHYL GLUCURONIDE AND ETHYL SULFATE IN HUMAN PLACENTA AND FETAL TISSUES; POTENTIAL BIOMARKERS OF MATERNAL ALCOHOL INTAKE DURING PREGNANCY

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Introduction: Toxicity of ethanol during first trimester of pregnancy in humans is well known, but toxic mechanisms and ethanol effects on the placenta and the fetus development are not yet fully clarified. Toxicokinetics of ethanol and materno-placental and fetal metabolism during early pregnancy could be useful for a deeper understanding of Fetal Alcohol Spectrum Disorder.

The aim of this study was to develop a method for the direct determination of ethyl glucuronide (EtG) and ethyl sulfate (EtS), in placental and fetal human tissues, as potential biomarkers of ethanol exposure during the first trimester of pregnancy.

Methods: Placental and fetal tissues samples were obtained from women undergoing voluntary termination of pregnancy at 12th week of gestation. After addition of D5-EtG and D5-EtS as internal standards, samples were deproteinized with acetonitrile, centrifuged and diluted 1:10 in bidistilled water. Then an aliquot was directly injected in a LC-MS/MS system, operating in negative polarity and in MRM mode, by monitoring two reactions for each analyte. A LOD of 3 and a LLOQ of 5 pg/mg were reached for both metabolites and a six-point calibration curve ranging from 5-1000 pg/mg was used for quantification purposes.

Results: The method was fully validated and applied to 24 placenta-fetal tissue samples. Two out of 24 cases tested positive for EtG and EtS for both placenta and fetal tissues (Table).

	Case 1		Case 2	
	Placental tissue	Fetal tissue	Placental tissue	Fetal tissue
EtG pg/mg	122.2	33.2	1305.8	391.0
EtS pg/mg		50.7		under the LLOQ

Conclusion: For the first time an analytical method was set up and validated for the determination of EtG and EtS in placental and fetal tissues. Preliminary results suggest that these metabolites are present in both tissues of pregnant women and can be further evaluated as specific markers in the diagnosis of alcohol intake during pregnancy.

Keywords: ethyl glucuronide, ethyl sulfate, pregnancy, FASD

[P2.30]

FAT OVERLOAD IN MATERNAL DIET INDUCES PLACENTOMEGALY AND FETAL MACROSOMIA IN PREGNANT RATS: ROLE OF LEPTIN

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Introduction: Fat overload in maternal diet can increase lipid transfer across the placenta to the developing fetus. This may cause fetal metabolic alterations and macrosomia. Placental endothelial (EL) and lipoprotein (LPL) lipases are key regulators of maternal-to-fetal lipid transport. The aims of this study were to evaluate the effects of fat overload in the diet of pregnant rats on maternal, fetal and placental weight, as well as leptin, insulin and lipid profile in maternal and fetal plasma, and to analyze placental expression of lipases, evaluating the role of leptin in the expression of EL and LPL.
Methods: Rats were subjected to a standard diet (control) with 5% fat or a fatty diet (FD) enriched with 25% saturated fat. Also, fetuses from control rats were injected with leptin (200 ng) or vehicle through the uterine wall on days 19, 20 and 21 of gestation. Maternal plasma, placentas, fetuses and fetal plasma were obtained on day 21 of gestation. Plasma insulin and leptin levels were evaluated by EIA, lipid levels by colorimetric assays, and placental expression of EL and LPL by PCR.
Results: Maternal weight gain, and fetal and placental weight were greater in FD group ($p<0.05$) compared to controls. The FD group showed higher triglycerides ($p<0.01$), insulin ($p<0.05$) and leptin ($p<0.01$) levels in maternal and fetal plasma. Also, FD fetuses had increased cholesterol plasma levels ($p<0.01$). Placentas from the FD group and from leptin-injected fetuses showed no changes in EL expression, but, interestingly, the same increase in LPL expression ($p<0.05$), when compared to controls.
Conclusions: Fat overload in maternal diet causes an increase in fetal lipid and leptin levels, which promotes lipid transfer to the fetus by inducing placental LPL expression. Fetal and placental overgrowth can be caused by the increased bioavailability of insulin, leptin and lipids in the developing fetus.
Keywords: placenta, fetus, leptin, lipid

[P2.31]

MATERNAL CIRCULATING LEVELS OF OMEGA (N)3 LONG CHAIN POLYUNSATURATED FATTY ACIDS, EICOSAPENTAENOIC ACID (EPA), DOCOSAPENTAENOIC ACID (DPA) AND DOCOSAHEXAENOIC ACID (DHA) DURING EARLY PREGNANCY ARE ASSOCIATED WITH FETAL GROWTH MEASURES IN LATER PREGNANCY

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Introduction: Although pregnancies complicated by asthma have been associated with reduced fetal growth, certain dietary nutrients such as omega (n) 3 fatty acids are thought to confer a protective effect against the inflammation that occurs in asthma. We therefore examined the relationship between circulating levels and dietary intake of n3s in pregnancies complicated by asthma and related these to fetal growth.
Methods: Peripheral blood was collected from, and a 24 hour food recall questionnaire was completed by asthmatic ($n=15$) and non-asthmatic ($n=21$) pregnant women at gestational weeks (G) 18, 30 and 36. Circulating levels of n3s were analysed using gas chromatography and dietary analysis was conducted using Foodworks software. Fetal growth parameters were analysed by ultrasound at each gestational visit.
Results: Circulating EPA and DPA (but not DHA) were significantly lower in the low (BWC) group than the high BWC group (ANOVA; $p<.05$) at G18. There was no consistent pattern of circulating EPA over pregnancy in the low BWC group in either the asthmatic or control groups. Circulating EPA was systematically reduced over pregnancy in the high BWC group in both the asthmatic and control groups. There were no significant differences between maternal dietary intake of EPA, DPA or DHA, nor of maternal energy intake.
In the asthmatics, maternal circulating levels of DHA (the most abundant n3 in neural phospholipids) at G18, 30 and 36 in the low BWC group were negatively correlated with head circumference (HC) and biparietal diameter (BPD) at G36 ($p<.05$). Irrespective of BWC category, in the asthmatic group, maternal circulating levels of DHA at G18, 30 and 36, as well as EPA and DPA at G36 were all negatively correlated with head circumference (HC) at G36 ($p<.05$). Interestingly, this pattern of results was not found in the control group.
Discussion: Under normal developmental conditions, it seems that the fetus is able to utilise the n3 fatty acids available over the course of pregnancy. It appears that placental uptake and preferential transfer of these n3fatty acids is impaired in fetuses on a low BWC developmental trajectory. Further, these data suggest that if fetal ontogeny develops under asthmatic conditions, it may be either asthma or its treatment which hinders the capacity of the fetoplacental unit to utilise n3s in order to enhance fetal growth. Under such conditions, maternal supplementation with DHA may not improve fetal growth.
Keywords: omega 3 fatty acids, fetal growth, asthma, diet

[P2.32]

PLASMA AND PLACENTAL IL-10 IN HYPERGLYCEMIC PREGNANT WOMEN- CORRELATION WITH PLACENTAL AND FETAL MORPHOMETRY

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Introduction: IL-10 is fundamental for integrated mother-fetus-placental development1. The purpose of this study was to compare placental IL-10 by immnunohistochemistry (using polymer system linked to phosphatase) with maternal plasma concentrations and correlate them with fetal and placental development markers in hyperglycemic pregnant women.
Methods: This cross-sectional study included pregnant women and their newborns (NB) and placentas. Based on 100g-GTT and glucose profile analysis (GP) performed at 24-28 weeks of gestation, subjects were allocated into three groups: Nondiabetic (ND; n=5)- normal 100g-GTT +normal GP, Mild hyperglycemic (MH; n=6) – normal 100g-GTT+abnormal GP, and Diabetic (DM; n=15) – abnormal 100g-GTT (nongestational and gestational)+abnormal GP2,3. Variables assessed: maternal – glycemic mean (GM / glucose-oxydase), glycated hemoglobin (HbA1c / chromatography) and IL-10 plasma levels (ELISA) at the 3rd trimester; placental – IL-10 immunolabeling (stain score: 0- unstained, 1- weak, 2- medium, 3- strong), weight, placental index (placental weight/NB weight), and villous area; NB – weight and ponderal índex (weight / lenght3). Statistical analyses were performed using ANOVA (test F and Tukey test), LSMeans test (gamma error) and Spearman correlation test ($p<0.05$).
Results: GM (mg/dL) and HbA1c (%) values were: 78.59±11.54 / 4.86±0.59 (ND), < 100.30±5.80 / 5.56±0.55 (MH), < 114.97±20.72 / 6.90±1.09 (DM) ($p<0.05$). In MH, plasma IL-10 values were higher, villosity score was 2 ($p=0.019$), fetal and placental weight were higher, and placental index was lower. DM showed the lowest plasma IL-10 values and highest percentage of unstained villosities. Correlations of placental IL-10 with placental weight (r=0.2908; $p<0.0001$) and fetal weight (r=0.1645; $p<0.0001$) as well as between plasma IL-10 and placental IL-10(r=0.8898, $p<0.001$) were observed.
Discussion: GM control promoted both maternal and placental IL-10 production, favoring fetal and placental development in MH 1,4 but not in DM. These findings show that keeping maternal MG≤100.00mg/dL is necessary.

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Keywords: IL-10, placenta, diabetes mellitus, pregnancy

[P2.33]

CHRONIC INCUBATION WITH HIGH EXTRACELLULAR D-GLUCOSE INCREASES THE CONTRACTILITY OF HUMAN UMBILICAL VEIN RINGS

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Chronic incubation with high extracellular concentration of D-glucose induces changes in signaling pathways related with nitric oxide bioavailability in human umbilical vein endothelial cells (HUVEC). However, it has not been determined whether the chronic exposure to high D-glucose induces changes in vascular reactivity of human umbilical veins. We studied whether high concentration of D-glucose increases the response to a vasoconstrictor in human umbilical veins.
Methods: Umbilical vein rings were obtained from normal pregnancies (Ethics Committee approval and informed patient consent were obtained). Rings (~5 mm) were mounted on isometric force transducer and registered highest contractile response to 62.5 mM KCl. The rings were incubated (2-24 hours) with 5 mM D-glucose (control) or 25 mM D-glucose. After incubation, vessels were exposed to U46619 (10^{-9}-10^{-5} M) (thromboxane A_2 analogue).
Results: 25 mM D-glucose induces an increase (~0.25-fold) in the maximal relative response to U46619, without changes in the EC_{50} (23 ± 0.5 versus 12 ± 0.7 nM for 5 versus 25 mM D-glucose; P>0.05, two way ANOVA test). After a period of 4 hours there was no difference in the U46619-dose response curves. However, vessels incubated for 24 hours with 25 mM D-glucose show an increase (~2.6-fold) in the maximal contraction in response to this vasocosntrictor, without changing the EC50 values (23 ± 0.5 versus 12 ± 0.7 nM for 5 versus 25 mM D-glucose; P>0.05, two way ANOVA test)., compared with vessels exposed to 5 mM D-glucose.
Conclusions: chronic incubation with a high concentration of D-glucose induces an increase in the vasoconstrictory response of human umbilical veins. These findings could be determinant in diseases where a pathological plasma D-glucose is chronically exposed to human fetal endothelium, such as in gestational diabetes.
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Keywords: D-Glucose, Vascular reactivity, Umbilical vein

[P2.34]

PEROXYNITRITES AND MATRIX METALLOPROTEINASES IN THE TERM PLACENTA FROM TYPE 2 DIABETIC PATIENTS

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Introduction: Type 1 pregestational diabetes leads to a pro-inflammatory intrauterine environment, characterized by increased reactive oxygen species (ROS), nitric oxide (NO) overproduction and matrix metalloproteinases (MMP) overactivity. The objective of this work was to address evidence of oxidative and nitrative stress in term placentas from Type 2 pregestational diabetes, and to analyze whether peroxynitrites regulate the activity of MMP-2 and MMP-9 in the placenta.
Methods: Term placental villous tissues from healthy and pregestational Type 2 diabetic patients, were isolated and frozen (-80°C) for further determination of TBARS (an index of lipid peroxidation), nitrates/nitrites (an index of NO production), concentrations of nitrated proteins (enzyme immunoanalysis and Western Blot). Placental explants were incubated a) in the presence or absence of FeTPPs (a peroxynitrite catalyst) for further determination of nitrates/nitrites and TBARS, and b) in the presence or absence of peroxynitrites (10-100μM) for further determination of MMPs activity by zymography.
Results: The placenta from Type 2 diabetic patients showed increased concentrations of nitrates/nitrites ($p<0.01$), TBARS ($p<0.01$) and nitrated proteins ($p<0.001$) when compared to controls. The addition of a peroxynitrite catalyst reduced nitrates/nitrites ($p<0.05$) and TBARS ($p<0.05$) concentrations in the diabetic placenta, while no changes were observed in the control tissues. The addition of peroxynitrites increased the activation of both MMP-2 and MMP-9 proenzymes in the control placenta ($p<0.05$), while no changes were observed in the diabetic tissues.
Conclusions: In the human placenta, peroxynitrites can exert damage, in part due to its capacity to induce lipid peroxidation and MMPs overactivity. Markers of both oxidative and nitrative stress are found in term placentas from Type 2 diabetic patients and can be related to MMPs overactivity.
Keywords: Placenta, Peroxynitrites, Matrix Metalloproteinases, Lipid peroxidation

[P2.35]

EFFECTS OF LONG-TERM TYPE 1 DIABETES ON THE MOUSE PLACENTA

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Diabetic pregnancies are associated with a higher incidence of pregnancy loss, malformations and intrauterine growth restriction[1]. It is known that the development of complications in pregnancy is directly related to the severity and the duration of diabetes[2]. Our group has developed a model of pregnancy complicated by long-term type 1 diabetes in mice, whose characteristics are comparable to those in women with poorly controlled type 1 diabetes of long duration, with the presence of vasculopathy[3]. In this study we investigated the effects of long-term diabetes on the structure of the placenta and the deposition of fibrillar collagens. Diabetes was induced by a single injection of alloxan. Diabetic females, with blood glucose levels ≥400mg/dL, were mated with normal males between 80-110 days after diabetes induction. Placentas and fetuses were collected at 18 days of pregnancy, weighed, fixed in Methacarn and processed for paraffin embedding. Placental samples were stained with hematoxylin-eosin and picrosirius. Diabetic female mice exhibited the pathophysiological features of human type 1 diabetes, characterized by hyperglycemia, hipoinsulinemia, polyuria, glycosuria, polyphagy, polidipsia and decreased body weight. Moreover, the number and weight of the fetuses were significantly lower, whereas the placental weight was significantly higher, when compared to the control group. Placentas from diabetic mice showed morphological changes, characterized by an increase and disorganization of the spongiotrophoblast, diminution of the labyrinth, dilatation of their blood vessels and increased collagen deposition. As far as we are concerned, this is the animal model of pregnancy complicated by type 1 diabetes with the longest duration reported in the literature. Our results indicate that poor fetal development is correlated to placental alterations. Moreover, structural alterations and increased collagen deposition in the labyrinth, first described herein, may contribute to alter maternal and fetal exchanges and consequently impair fetal development.

[P2.36]

GENDER-DEPENDENT REGULATION OF LIPID METABOLIZING ENZYMES IN THE FETAL HEART FROM CONTROL AND DIABETIC RATS

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Introduction: In maternal diabetes, the fetal heart is exposed to the increased lipid transfer through the placenta. PPARα regulates the expression of several enzymes of lipid metabolism such as acylCoA oxidase (ACO) and carnitine palmitoyltransferase-1 (CPT-1), respective rate limiting enzymes in peroxisomal and mitochondrial fatty acid β-oxidation.
Objective: To evaluate lipid concentrations and PPARα, ACO and CPT-1 expression in the fetal heart from control and diabetic rats, and to analyze whether PPARα activation regulates PPARα, ACO and CPT-1 expression in hearts from female and male fetuses from control and diabetic rats.
Methods: Diabetes was induced in rat neonates by streptozotocin administration. On day 21 of gestation, fetal hearts were explanted and lipid concentrations (TLC and densitometry) and PPARα, ACO and CPT-1 expression (RT-PCR) were analyzed. Also, fetuses from control and diabetic rats were injected through the uterine wall with the PPARα agonist leukotriene B4 (LTB4, 0.1μM) on days 19, 20, and 21 of gestation. On day 21 of gestation, fetal heart expression of PPARα, ACO and CPT-1 were evaluated.
Results: Male and female fetal hearts from diabetic rats showed increased concentrations of triglycerides ($p<0.001$), cholesterol ($p<0.05$) and phospholipids ($p<0.05$) and no changes in cholesteryl ester concentrations. PPARα ($p<0.01$), ACO ($p<0.05$) and CPT-1 expression ($p<0.001$) were reduced in male fetal hearts, while only CPT-1 ($p<0.05$) was reduced in female fetal hearts.
In diabetic animals, fetal treatment with LTB4 increased PPARα in male and female fetal hearts ($p<0.05$), increased ACO in male and female fetal hearts ($p<0.01$) and increased CPT-1 only in male fetal hearts ($p<0.01$).
Conclusions: Overaccumulation of lipids in the fetal heart is probably related to reductions in the expression of the transcription factor and enzymes studied that regulate lipid oxidation. This expression can be positively regulated by fetal administration of a natural PPARα agonist in a gender-dependent manner.
Keywords: Diabetes, Fetus, Lipids, Heart

[P2.37]

RELATIONSHIPS BETWEEN MATERNAL SERUM ADIPONECTIN, PLACENTAL ADIPONECTIN MRNA AND UMBILICAL CORD SERUM ADIPONECTIN CONCENTRATION IN UNCOMPLICATED PREGNANCY

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Aims: Adiponectin (APN) may play a role in adapting energy metabolism at the materno-fetal indifference. The potential effects on the placental APN expression of the APN serum concentration in umbilical cord blood and maternal blood have not been assessed so far. The aim of the study was to investigate the relationship between placental APN mRNA expression, maternal serum APN concentration and umbilical cord serum APN concentration in full-term healthy newborns.
Methods: We analyzed the mRNA expression profile of APN in 22 placentas and the APN serum concentration in 23 cord blood samples from healthy newborns (gestational age 37.0 to 41.5 weeks, birth weight 2800 to 4120 g, birth length 46 to 54 cm) and 23 maternal blood samples. The mRNA transcript levels of APN were quantified by RT-RCR. The APN concentration was measured by enzyme-linked immunosorbent assay.
Results: The highest levels of APN serum concentration were fund in umbilical cord blood. Mean concentration ± SD of APN was 34.88 μg/ml ± 12.8 (umbilical cord) vs. 6.6±2.3 (maternal circulation, $P< 0.001$). There is no significant correlation between maternal APN and cord APN concentration. Otherwise, cord APN concentration was positively associated with birth weight ($r=0.55$; $p=0.01$). APN gene expression was found in 8 out of 22 placentas in a very low concentration. There is no correlation between placental APN mRNA and umbilical cord serum APN or maternal serum APN concentration.
Conclutions: This study suggests that high serum APN concentration in umbilical cord blood and maternal blood are not regulated by placental APN mRNA gene expression. In contrast to the findings in adults, these results implicate that the cord APN concentration increases with the fetal fat.
Keywords: adiponectin, placental mRNA expression, cord blood, maternal serum

[P2.38]

PLACENTAL SECRETION OF THE GROWTH HORMONES ADIPONECTIN, GHRELIN, INSULIN-LIKE GROWTH FACTOR 1 AND 2 AND THEIR BINDING PROTEINS IN THE DUALLY PERFUSED HUMAN TERM PLACENTA

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Objectivs: Adiponectin, an adipocytokine, and ghrelin, an orexigenic acylated peptide, as well as insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are likely involved in the fetal growth. The placental share of the fetal production has not been proven yet. The aim of our study was to determine the placental release of adiponectin, leptin, IGFs and IGFBPs into the maternal and fetal circulation.
Methods: We performed dually recirculated perfusions of isolated cotyledons from 9 term human placentae as previously described (Schneider and Huch, Contr. Gyn. Obstet. 13:40-47, 1985). The concentrations of adiponectin, ghrelin, IGF-1 and -2, IGFBP-1,-2 and -3 were measured in fetal and maternal compartment by immunoassay.
Results: Adiponectin, IGF-1, IGFBP-1 and IGFBP-2 were measured both on the fetal and maternal side of the dual in vitro perfused placenta. For each hormone, the maternal release preceeded the release on the fetal side. IGF-2, IGFBP-3 and ghrelin were only detectable in the maternal circulation. There is a closed correlation between IGF-2 und ghrelin and all measured IGFBP's in the maternal side.
Discussion: Contrary to ghrelin, IGF-2 and the IGFBP-3, placental release of adiponectin as well as IGF-1 and IGFBP-1 and -2 might directly contribute to the fetal pool of these hormones.
Keywords: adiponectin, dually placenta perfusion, ghrelin, insulin-like growth factors

[P2.39]

DIETARY FATTY ACID INTAKE IN PREGNANCY AND IMPACT ON FETAL LEVEL OF ESSENTIAL AND *TRANS* FATTY ACIDS AND BASAL CYTOKINE LEVELS

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Introduction: The intake of dietary fatty acids by the pregnant mother may have an impact on duration of pregnancy and fetal development. While it is known that long-chain polyunsaturated fatty acids (LC-PUFA) are essential for the fetus and their metabolites may influence immune reactions, *trans* fatty acids (tFA) likely disturb these physiological processes. In contrast, a special group of tFA occurring in milk fat, conjugated linoleic acids (CLA), are suggested to be preferentially transferred to the fetus and have some modulatory effects on the immune system.
Methods: To analyse a possible correlation between the long term dietary intake of fatty acids, especially n-3 and n-6 LC-PUFA, CLA and tFA, and the level of cytokines in cord blood of term born infants, cord and maternal blood (n=110) were collected and prepared for gas chromatographic lipid analysis in erythrocyte membranes and analysis of spontaneous production of 11 Th1/Th2 cytokines in cord Plasma by cytometric bead array.
Results: While n-3 (e.g. EPA, DHA) and n-6 (e.g. AA) LC-PUFA are transferred preferentially from the maternal circulation to the fetus, CLA, as well as tFA, are not. We found a positive correlation of tFA and CLA between the maternal and fetal circulation indicating a passive transfer of those fatty acids to the unborn. Additionally, there was an inverse correlation between trans fatty acids and LC-PUFA and an inverse correlation between AA in the maternal circulation and the n-3 LC-PUFAs in the fetal circulation.
All analysed cytokines can be detected in cord plasma but mostly at a low level. However, we found some correlations between high levels of cytokines and the level of different fatty acids.
Discussion: Our results give a preliminary evidence for subtle modification of neonatal cytokine levels by maternal nutrition even without supplementation of n-3 LC-PUFA or fish oil.
Keywords: LC-PUFA, trans fatty acid, cytokine, cord

[P2.40]

SMOKING DURING PREGNANCY REDUCED CORD BLOOD ADIPONECTIN LEVEL

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Objectivs: Fetal growth restriction caused by maternal smoking during pregnancy may related with effects on fetal growth hormone synthesis. The aim of the study was to compare the cord blood levels of fetal metabolic hormones after uncomplicated pregnancies at term in smoking and non-smoking women.
Methods: Adiponectin, ghrelin, IGF-1, IGFBP-1, IGFBP-3 and insulin were measured in the venous cord blood of 77 term newborns of non-diabetic women (38 girls and 39 boys; median (interquartile range) gestational age 39 (38-40) weeks and birth weight 3310 (3095-3310) g. 16 pregnant women reported smoking of 5 to 10 cigarettes per day, whereas 55 non-smokers used as controls (unclear information in 6 cases
Results: Cord blood adiponectin was correlated positively with gestational age and as IGF-1 and insulin with the birth weight. Insulin level was correlated with the placental weight too, but negative associated with umbilical artery pH (Pearson correlation, all $p< 0.05$). Smoking during pregnancy reduced significantly cord blood adiponection levels as well as neonatal BMI and head circumference (Mann-Whitney test; all $p<0.05$).
Conclusion: Smoking during pregnancy may influence fetal growth dynamics by affecting fetal endocrine metabolic regulation indicated in lowering adiponectin secretion.
Keywords: smoking during pregnancy, fetal growth hormones, adiponectin

[P2.41]

PLACENTAL IMMUNOSTAININGS OF VEGF, COX-2 AND CASPASE-3 IN PREGNANCIES COMPLICATED BY DIABETES OR MILD HYPERGLYCEMIA - RELATIONSHIP WITH HYPERGLYCEMIA AND UMBILICAL DOPPLER

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Introduction: The results on human placental angiogenesis and hyperglycemia are few and conflicting.[1,2,3,4] Also, they are rare and controversial, those who evaluated apoptosis in placentas from pregnancies associated with diabetes[5,6]. The aim was to evaluate correlations between average glucose (AG) and doppler cord (Umbilical PI) with placental immunostainings for VEGF, COX-2 and caspase-3 in pregnancies complicated by diabetes mellitus (DM), or mild hyperglycemia (HL).

Methods: cross-sectional samples of placentas of 41 pregnant women, divided into three groupos – DM (N = 20) HL (N = 7) and ND (N = 14). The samples were processed for immunohistochemistry using polymer system linked to peroxidase. Slides were randomized to blinded evaluation in image analysis system. We evaluated AG, PI and umbilical placental immunostainings for VEGF, caspase-3 and COX-2, in semi and quantitative analysis. The statistical analysis used were ANOVA (F test, followed by Tukey test), LSMeans test and Spearman correlation, $p \leq 0.05$.

Results: The AG differed in the groups ND (79.60±3.40 mg / dl) and HL (87.97±4.64 mg / dl) from DM (113.91 ±2.81 mg / dl) ($p < 0.000$) but did not correlate with the placental immunostainings. Umbilical PI was similar in ND (1.03±0.20) and DM (1.05±0.20), lower in the HL group (0.89±0.11) ($p=0.003$) and not correlated with VEGF. Umbilical PI correlated inversely with caspase-3 and the same trend in relation to COX-2. At HL group, in the majority of vessels positive for VEGF were strongly marked, the other two groups predominantly moderate intensity.

Discussion: The MG, around 110mg/dl, was a limiting factor for the proposed correlations. The results of umbilical PI were partial and inverse defined by the conditions of apoptosis and cellular regeneration of the placentas. These results are not defined, but emphasize the validity of maternal glycemic control.

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Keywords: placenta, VEGF, COX-2, CASPASE-3

[P2.42]

DIFFERENTIAL EFFECT OF INSULIN ON ARTERIAL VS VENOUS PLACENTAL ENDOTHELIAL CELLS

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Introduction: Maternal diabetes is often associated with fetal hyperinsulinemia activating placental insulin receptors. These are mainly located on the vasculature at term of gestation but their function is unknown. Angiogenesis is a candidate process and may depend on oxygen, extracellular matrix (ECM) and PKB/eNOS activation. We hypothesized that insulin induces pro-angiogenic signaling which may differ between arteries vs veins as they vary in ECM and blood oxygenation.

Methods: Microarray analysis of human primary placental arterial (AEC) and venous (VEC) endothelial cells determined the effect of insulin in 12 and 21% oxygen. Effects of insulin and ECM (collagenI/fibronectin/laminin/gelatin) on angiogenesis (*in vitro* network formation) and proliferation (cell number) were analyzed with or without eNOS inhibitor (L-NAME 200μM) and NO donor (NONOate 200μM). Insulin (10nM) signaling was determined in AEC in 12 and 21% oxygen by immunoblotting.

Results: Microarray analyses revealed differential expression of angiogenesis and ECM-related genes, and distinct insulin effects in AEC vs VEC. Proliferation and survival of AEC were independent on the ECM used, while VEC showed increased proliferation and survival on collagen and fibronectin. Only AEC, but not VEC, were able to form networks. Insulin and NO donor increased the total length and branching points in the AEC network and this was abolished by eNOS inhibitor. No insulin effect was observed on VEC. Insulin induced phosphorylation of PKB and the downstream GSK3 in 21% oxygen, but not in 12%, and was unable to activate Erk1/2 and downstream S6-kinase. Insulin also stimulated eNOS phosphorylation in AEC but not in VEC.

Conclusions: Placental AEC and VEC are different in their angiogenic potential and in their interaction with their environment. In 21% oxygen insulin is able to activate PKB and eNOS in AEC and, hence, induces angiogenesis. Therefore, insulin may contribute to the hypervascularisation present in the diabetic placenta.

Keywords: maternal diabetes, angiogenesis, insulin, ECM

[P2.43]

EFFECT OF ADENOVIRUS-MEDIATED GENE TRANSFER OF INSULIN GROWTH FACTOR -1(AD-IGF-1) ON NUTRIENT TRANSPORT MECHANISMS IN HUMAN TROPHOBLAST (BEWO CELLS)

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We have previously demonstrated in vitro that transfection of trophoblast cells with Ad-IGF-1 significantly increases secreted IGF-1 levels and cell proliferation and in vivo that site-specific placental Ad- IGF-1 corrects fetal weight in mouse, rat and rabbit models of placental insufficiency. To investigate the involvement of altered nutrient transport, we examined the effects of Ad-IGF-1 on BeWo amino acid and glucose transport mechanisms.

Methods: Human BeWo choriocarcinoma cells (CCL-98) were grown in F12 complete medium + 10%FBS. Cells were incubated in serum-free control media ± Ad-IGF-1 or Ad-LacZ for 48 hours. MOIs of 10:1 and 100:1 were utilized. The RNA expression of System A transporters SNAT1 and 2, the System L transporters LAT1, 2 and 2F4hc and the glucose transporters GLUT1 and 3 was analyzed by qPCR. Protein expression was analyzed by Western blot. Data were analyzed by ANOVA and a P value less than 0.05 was considered significant.

Results: Transfection of BeWo cells at an MOI of 10:1 did not alter the RNA expression of any of the transporters compared to non-transfected or Ad-LacZ (MOI 10:1) cells. However, at an MOI of 100:1, Ad-IGF-1 transfection significantly ($p<0.05$, $n=3$ per treatment) increased the RNA expression of SNAT1 and 2, LAT1 and 4F2hc compared to both non-transfected cells and those transfected with AdLacZ by 20-50%. In contrast, LAT2 RNA expression was reduced by 25% following Ad-IGF-1 compared to both non-transfected and LacZ treated cells ($p=0.039$, $n=3$). The glucose transporters GLUT1 and 3 showed no changes in RNA or protein expression with Ad-IGF-1 or Ad-LacZ transfection.

Conclusion: Ad-IGF-1 transfection increases amino acid but not glucose transporter gene expression and may represent one placental mechanism by which IGF-1 corrects fetal growth restriction in the animal models of placental insufficiency.

Keywords: Amino acid transport, IUGR

[P2.44]

DIFFERENCES IN PLACENTAL INSULIN-LIKE GROWTH FACTOR-II DISTRIBUTION IN PREGNANT DIABETIC RATS

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Introduction: Diabetes is associated with complications during the pregnancy in the mother, placenta and/or the fetus. In the placenta (fetal-maternal interface) is where nutrients and wastes exchange occurs. Fetal survival and growth depending on somatotrofic growth factors (IGFs system). Similarly to humans, diabetes in pregnant rats promotes placentomegaly. However, it is not known if diabetes during pregnancy in this species induces changes on the IGFs system.

Objectives: To determine whether maternal diabetes promote differences on placental IGF-II distribution in rats.

Material and Methods: Single injections of alloxan (40 mg kg i.v.) were used to induce diabetes on day 2 of pregnancy in Wistar rats. Placentas were collected on days 14, 17 and 20 postcoitum (dp). We used immunoperoxidase technique to identify IGF-II in placental sections; the reaction was visualized and captured with a CX81 microscope and DP71 digital camera (Olympus). IGF-II positive cells in the labyrinth regions of these placentas were count using Image ProPlus software. Differences were established using Stata 10 software.

Results: In normal and diabetic gestations the presence of IGF-II positive cells in placenta diminished during pregnancy (14, 17 and 20 dp). However, in diabetic placentas we observed a higher number of IGF-II positive cells on days 14dp (72±2 vs 38±2, respectively) and 17dp (36±1 vs 26± 1, respectively) compared to normal gestation.

Conclusion: Fetal demand for nutrients is genetically regulated by the level of growth factors as IGF-II. The high presence of IGF-II in specific regions of the rat placenta observed in our diabetic model could be related to placentomegaly as a compensatory mechanism in growth restricted gestations.

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Keywords: IGF-II, Placenta, Rat, Diabetes

[P2.45]

INSULIN INCREASED ADENOSINE TRANSPORT IN HUVEC FROM GESTATIONAL DIABETIC PREGNANCIES INVOLVE INCREASED EXPRESSION AND ACTIVITY OF HENT1

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Human umbilical vein endothelial cells (HUVEC) from gestational diabetes exhibit reduced adenosine uptake. Expression and activity of the human equilibrative nucleoside transporters 1 (hENT1) are decreased in HUVEC from gestational diabetes, as well as in HUVEC from normal pregnancies exposed to insulin. We studied insulin effect on hENT1 activity and expression and the contribution of insulin receptor isoforms A (IR-A) or B (IR-B) in HUVEC from gestational diabetes.

Methods: Cells were exposed (8 hours) to insulin (0.1-10 nM) and [^{3}H] adenosine transport (4 μCi/ml, 20 s, 22°C) was measured in absence or presence of the ENTs inhibitor nitrobenzylthioinosine (NBTI, 0.01-10 μM, 30 minutes). *SLC29A1* gene (hENT1) transcriptional activity was estimated by firefly/renilla luciferase for pGL3-hENT1^{-3198} and pGL3-hENT1^{-1670} constructs (-3198 and -1670 bp from ATG), respectively. IR-A, IR-B and hENT1 mRNA number of copies were determined by real time RT-PCR. All experiments were performed in absence or presence of *N*G-nitro-L-arginine methylester (L-NAME, 100 μM).

Results: hENT1-mediated adenosine transport, mRNA expression and protein abundance were reduced ($P<0.05$, two way ANOVA test) in gestational diabetic compared with normal pregnancies. Insulin increased hENT1-adenosine transport ($SC_{50} = 0.41 \pm 0.05$ nM), protein abundance ($SC_{50} = 0.18 \pm 0.02$ nM) and mRNA expression ($SC_{50} = 0.23 \pm 0.04$ nM) in gestational diabetes up to values in cells from normal pregnancies. Basal pGL3-hENT1^{-3198} activity was lower (~ 0.7 fold) but pGL3-hENT1^{-1670} was higher (~ 1.3 fold) in cells from gestational diabetic compared with normal pregnancies. Insulin blocked gestational diabetes effect only on pGL3-hENT1^{-3198}, an effect inhibited by L-NAME. Basal IR-A mRNA expression was higher (~ 1.4 fold), but IR-B mRNA expression was unaltered ($P>0.05$) in cells from gestational diabetes compared with normal pregnancies. Insulin blocked gestational diabetes-associated increase of IR-A mRNA expression.

Conclusions: IR-A isoform could be responsible of the gestational diabetes- or insulin-reduced hENT1 mediated adenosine transport in HUVEC, a phenomenon involving NO.

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Keywords: Endothelium, Nucleoside Transporter, Gestational Diabetes, Insulin

[P2.46]

HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS EXHIBIT GLUTS-LIKE TRANSPORT ACTIVITY MODULATED BY INSULIN

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Glucose uptake is mainly mediated by sodium and insulin-independent transport system in endothelial cells. Although insulin-sensible isoform seems to be expressed in fetal human placenta and human umbilical vein endothelial cells (HUVEC). In this study we examined the effect of insulin on D-glucose uptake in HUVEC exposed to normal or high D-glucose concentration.

Methods: Cells were exposed (24 hours) to normal (5 mM) or high (25 mM) D-glucose in sera-supplemented culture medium 199 in absence or presence of insulin (10 nM, 8 hours). 2-Deoxy-D-[^{3}H]glucose (2DG) uptake (1.6 mM 2-deoxy-D-glucose, 1 μCi/ml 2DG, 5 seconds to 5 minutes, 22°C) was measured in the absence or presence of phloretin (GLUTs inhibitor, 100 μM, 3-24 hours).

Results: Initial rate of 2GD uptake was reduced ($P<0.05$, two way ANOVA test, n=8) by high D-glucose (32,00 ± 3,82 versus 16,06 ± 1,77 pmol/μg protein/10 sec for 5 versus 25 mM D-glucose, $p < 0,001$), and blocked by phloretin. Insulin increased initial rate of 2DG uptake in cells exposed to high D-glucose (5.4-fold) reaching values in cells exposed to normal D-glucose in absence of this hormone, an effect that was blocked by phloretin.

Conclusion: These findings show that 2DG uptake is modulated by insulin in HUVEC, suggesting expression of functional phloretin-inhibitable membrane transport GLUTs-like activity. In addition, HUVEC express functional GLUTs-like transport activity that could, at least in part, be due to the activity of an insulin-sensible D-glucose transporter in this cell type.

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Keywords: glucose transport, insulin, high glucose, endothelium

[P2.47]

VASCULAR EFFECTS OF IGF2 ON THE FETO-PLACENTAL ENDOTHELIUM

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Objectives: IGF2 is a key growth factor for fetal development. In maternal diabetes fetal IGF2 levels are elevated. The placental endothelium expresses signalling receptors for IGF2, ie the insulin receptor isoform A and the IGF1-receptor. Here we hypothesized that IGF2 acts on the feto-placental vasculature.

We performed whole genome microarrays to identify IGF2-regulated genes in isolated primary placental endothelial cells. Furthermore, the effect of IGF2 on eNOS phosphorylation and on VEGF expression was determined. As functional endpoints the IGF2-effects on *in vitro* angiogenesis and on electrical impedance as a marker for vascular permeability were measured.

Methods: Placental arterial endothelial cells (6 different isolations) were cultured with or without 10nM IGF2 for 24h. RNA was used for whole genome analysis. eNOS phosphorylation was determined after treatment with 10nM IGF2 for 2, 5, 10 and 45min by western blot analysis. VEGF mRNA expression was measured after culturing the cells with 1, 10 and 100nM IGF2 for 24 and 48h. *In vitro* angiogenesis and electrical impedance were monitored in the presence of 10nM IGF2 in Matrigel angiogenesis assays and in ECIS electrode assay plates (Electric Cell-substrate Impedance Sensing; non-invasive method continuously measuring electrical impedance of cell monolayers), respectively.

Results: IGF2 significantly regulated the expression of 33 genes, of which 6 were related to vascular functions. This was paralleled by the increase of branching points (+22±7.3%, $p<0.005$) but not by tube length in the *in vitro* angiogenesis. Also, IGF2 induced eNOS phosphorylation (+123±70%, $p<0.05$) and stimulated VEGF mRNA expression (+15.1±7.5%, $p<0.05$). Electrical impedance and, hence, permeability was increased by IGF2 (+13.2±.2.9, $p<0.05$).

Conclusion: IGF2 is a potent regulator of vascular functions in placental endothelial cells. These did not only include the stimulation of angiogenesis but also novel effects on vascular permeability. Elevated levels of IGF2 in pregnancies complicated by diabetes may hence cause abnormal vascular development and function of the placenta.

Keywords: growth factor

[P2.48]

THE EFFECT OF ANANDAMIDE ON NITRIC OXIDE SYNTHASE ACTIVITY DEPENDS ON THE PRESENCE OF THE BLASTOCYST.

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Anandamide (AEA), an endocannabinoid, binds to cannabinoid receptor 1 (CB1) and 2 (CB2), besides vanilloid receptors. High concentrations of AEA are toxic for implantation and embryo development, suggesting a relevant role for AEA during early pregnancy. Nitric oxide (NO) is synthetized by nitric oxide synthase (NOS) and modulates vessel formation at the implantation sites. We have previously observed that uterine NOS activity is inversely correlated with AEA production at the implantation period. Thus, the aim of this study was to investigate if AEA modulated uterine NOS activity during implantation.

Wistar rats were sacrificed in days 4, 5 and 6 of gestation. Implantation occurs in the afternoon of day 5. Pseudopregnancy (psp), a model in which the blastocyst is absent, was induced by i.p. administration of PMSG to prepuber rats. Rats on day 5 of psp were sacrificed. NOS activity and CBs expression and localization were determined in the uterus.

AEA (1 nM) inhibited NOS activity in day 5 of psp ($p<0.001$) and this effect was completely reverted by the pre-incubation with SR144528 (0.1 nM), a selective CB2 antagonist. SR141716A (0.1 nM), a selective CB1 antagonist, had no effect. However, AEA (1 nM) increased NOS activity in the uterus from day 5 pregnant rats (8.0±0.3 vs 11.1±0.6 fmoles L-[14C]-citruline/mg ww/15 min, $p<0.001$) and this stimulatory effect was also reversed by SR144528. Finally, both CB1 and CB2 were expressed in the rat uterus in days 4, 5 and 6 of gestation (western blot and real time PCR) and they were localized to the luminal endometrium.

Our results suggest that in the day of implantation, AEA effect on uterine NOS activity depends on the presence of the blastocyst. Besides the expression of CB1, we described for the first time the presence of CB2 in the rat uterus during the peri-implantation period.

Keywords: anandamide, nitric oxide, blastocyst, uterus

[P2.49]

THE REDUCED NOS-DEPENDENT VASODILATION IN IUGR CHORIONIC ARTERIES IS IMPROVED BY ARGINASE INHIBITION

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Vascular dysfunction in Intrauterine Growth Restriction (IUGR) placenta is associated with increased vascular tone and altered nitric oxide (NO) synthesis by the endothelium. Arginase II is expressed in placenta and is implicated in vascular dysfunction through a counteracting activity with NO synthases (NOS). However, whether arginase activity has a role in vascular function in normal and IUGR placental vessels has not been addressed. We studied the vascular effects of arginase activity in normal and IUGR chorionic arteries (CA).

Methods: CA from normal and IUGR placentae were dissected, and vessels rings were mounted in a wire-myograph. Isometric force in response to increasing concentrations of SNP (NO-donor, 10-9-10-5 M), calcitonin gene-related peptide (CGRP, 10-10-10-7 M) with and without the NOS inhibitor L-nitroarginine (L-NA 10-4 M) and the arginase inhibitor *S*-2-boronoethyl-L-cysteine-HCl (BEC 10-5 M) were determined in vessels pre-contracted with KCl (37.5 mM). Responses were expressed as percentage of relaxation relative to KCl-induced contraction (%Kmax) and adjusted to concentration-response curves.

Results: Maximal KCl-induced contraction was similar in normal (2.7 ± 0.6 N/m2) and IUGR (3.6 ± 0.4 N/m2) CA. Relaxation in response to CGRP was higher in control (62.0 ± 8.6 %Kmax) compared to IUGR (32.0 ± 9.5 % Kmax), an effect that was completely prevented by L-NA in both samples. BEC increased the CGRP-induced relaxation in normal (95.6 ± 16.0 %Kmax) and IUGR (96.00 ± 11.0 %Kmax) CA, an effect that was blocked by L-NA. SNP induces a similar relaxation in normal and IUGR CA ($\sim$90 %Kmax).

Conclusions: Arginases acts as NOS-counteracting enzymes in normal and IUGR placental arteries. Since arginase inhibition induced a higher increase in NOS-dependent vasodilation in IUGR ($\sim$3-fold) compared to normal ($\sim$1.5-fold) CA, an imbalance in the NOS/arginase pathways in IUGR placental vessels is proposed.

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Keywords: IUGR, vasodilation, eNOS, arginase

[P2.50]

DIFFERENTIAL MODULATION OF INSULIN RECEPTOR ISOFORMS EXPRESSION AND NOS ACTIVITY BY INSULIN IN HUMAN PLACENTA MICROVASCULAR ENDOTHELIAL CELLS FROM GESTATIONAL DIABETES

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Gestational diabetes is associated with increased placental synthesis of nitric oxide (NO), and umbilical vein and maternal plasma level of insulin. This hormone upregulates NO syntase (NOS) expression and activity in the feto-placental unit. Insulin also activates insulin receptor isoforms A (IR-A) and B (IR-B), for mitogenic and metabolic preferential responses, respectively. We studied insulin effect on IR-A and IR-B mRNA expression and NOS activity in human endothelial cells from placenta microcirculation (hPMEC) from gestational diabetes.

Methods. hPMEC were isolated from normal or gestational diabetic pregnancies. NOS activity was measured by L-[3H]citrulline assay. IR-A and IR-B mRNA number of copies were quantitated by real time RT-PCR.

Results. Basal IR-A mRNA expression was lower ($65 \pm 10\%$, $P<0.05$, two ways ANOVA test), but IR-B was higher (2.0-fold) in gestational diabetic compared with normal pregnancies. In normal pregnancies, insulin did not alter IR-A ($P>0.05$), but increased IR-B (1.9-fold) mRNA expression. However, in gestational diabetes insulin increased IR-A (1.4-fold) compared with normal pregnancies in absence of this hormone. However, insulin reduced ($40 \pm 10\%$) IR-B mRNA expression in this cell type. The fraction of L-citrulline formation inhibited by L-NAME (i.e., indicative of NOS activity) was higher (1.9-fold) in hPMEC from gestational diabetic compared with normal pregnancies. Insulin reduced the gestational diabetes-associated increase of NOS activity to values in cells from normal pregnancies in absence of this hormone, where an increased NOS activity (1.8-fold) in response to insulin was detected.

Conclusion. hPMEC exhibit differential IR-A and IR-B mRNA expression. We suggest that NO is involved in IR-A and IR-B isoforms modulation by insulin in hPMEC from gestational diabetes. However, IR-B, but not IR-A will be under NO-dependent insulin modulation in this tissue from normal pregnancies.

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Keywords: gestational diabetes, microvascular endothelium, insulin, insulin receptor isoforms

[P2.51]

BRADYKININ-POTENTIATING PEPTIDE-10C INCREASES L-ARGININE AND NITRIC OXIDE PRODUCTION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS FROM PREECLAMPTIC PREGNANCIES

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Introduction: Preeclampsia is a hypertensive disorder in pregnancy. The pathogenesis of preeclampsia is not clearly established, but increased vascular reactivity has been reported, possibly due to an imbalance in the nitric oxide (NO) synthesis or bioavailability. The purpose of this study was to analyze the effects of bradykinin-potentiating peptide-10c (BPP-10c), a proline-rich decapeptide from *Bothrops jararaca* venom, on L-arginine levels, Ca2+ influx and NO production in Human Umbilical Vein Endothelial Cells (HUVEC) from normal (HN) and preeclamptic (HPE) pregnancies. This peptide has anti-hypertensive activity independent of inhibition of the ACE and its mechanism of action involves mainly the activation of argininosuccinyl synthase (AsS), a rate limiting key enzyme for the NO production in the endothelium.
Methods: HUVEC were isolated from human umbilical cords using collagenase. Quantification of the NO production and L-arginine, AsS activity, NOS expression, and changes in [Ca2+]*i* transients induced by BPP-10c were determined.
Results: BPP-10c increased the L-arginine production in the HN, probably by activation of AsS. It also induced significantly higher [Ca2+]*i* transients in HPE compared to HN. Regular NO production by HPE was lower when compared to HN, however the treatment of HPE with BPP-10c increased the levels of L-arginine, a substrate of the enzyme NOS, correcting the NO production in HPE.
Conclusion: The fact that BPP-10c induces [Ca2+]*i* response differently in HN and HPE endothelial cells, and correct the NO production by these pathological cells makes this peptide an important tool to study the pathophysiology of preeclampsia, and opens perspectives for the development of drugs.
Supported by FAPESP
Keywords: Preeclampsia, BPP-10c, Nitric Oxide, L-arginine, HUVEC

[P2.52]

DIFFERENTIAL EXPRESSION OF ENDOCANNABINOID SYSTEM IN PREECLAMPSIA: EFFECTS ON NITRIC OXIDE SYNTHESIS.

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Introduction: The endocannabinoid system has been described in human placenta. Anandamide (AEA), a most important endocannabinoid, is synthesized by different pathway and degraded by the action of a fatty acid amide hydrolase (FAAH). AEA exerts part of its effects binding to CB1 or CB2 receptors. Nitric oxide (NO) could produce the observed vascular abnormalities in preeclampsia. Recently has been shown that AEA modulates NO synthesis in various tissues.
Objetive: Our aim was to evaluate the differential expression of the endocannabinoid system in preeclampsia and analyze its involvement in regulating of NO synthesis.
Methods: Placentas were obtained from women undergoing a cesarean section at term (NP, n=14), who exhibietd no pregnancy complications; and women with preeclampsia (PE, n=14) defined as high blood pressure ($\geq$140/90 mmHg after 20 week gestation) and proteinuria ($\geq$ 300mg/24h), after cesarean sections.
Results: We detected the expression of CB1 receptor in NP and PE, and identify a decrease in activity and FAAH expression in PE, suggesting higher levels of AEA in this tissue.
We evaluated the activity of NO synthase (NOS) and found that incubation of NP with AEA (10^{-8}, 10^{-7}M) increased NO synthesis ($p < 0.001$), and this effect was reversed by co-incubation with an antagonist of CB1 (AM251, 10^{-6}M). Later, NP were incubated with URB-597, a inhibitor of FAAH, and NOS activity was stimulated. We found that PE placenta has larger NOS activity and a CB1 receptor antagonist (AM251), was able to decrease the activity of nitric oxide synthase ($p < 0.05$).
Discussion: Our results provide new evidence that AEA is able to regulate NOS activity in human placenta throughout the CB1 receptor and suggesting that increased levels of AEA in PE, due to the sharp decrease in the activity and protein expression of FAAH, could be related with increased NO synthesis observed in this pathology.
Keywords: Preeclampsia, Nitric Oxide, Anandamide, Cannabinoid receptor 1

[P2.53]

PARTICIPATION OF ARGINASES IN THE VASCULAR TONE IN UMBILICAL VEIN FROM IUGR PLACENTAE: ROLE OF THE ENDOTHELIUM AND HYPOXIA

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Intrauterine Growth Restriction (IUGR) is asociated with fetal hipoxia, lower nitric oxide (NO) synthesis by the endothelial NO synthase (eNOS) and altered placental vascular tone. Arginase II (ArgII) has been described to compete with eNOS for their common substrate L-arginine, leading to vascular dysfunction. We studied the role of ArgII in the NOS-mediated vascular effects in IUGR umbilical veins (HUV) and in HUV endothelium (HUVEC) under hypoxia.

Methods: Normal and IUGR HUVEC were isolated by collagenase digestion and cultured to confluence at 37°C (air/CO_2, 95/5); cell cultures were exposed to normoxia (5% O_2) and hypoxia (2% O_2, 24 h) and harvested for further analysis. ArgII, eNOS and p-eNOS (Ser^{1177}) protein levels were determined by western blot, and arginase activity was quantified by urea formation. HUV rings were dissected from placentae and mounted in a wire myograph to determine vasoactive response to insulin (10^{-12}-10^{-8} M), in presence or absence of the arginase inhibitor *S*-(2-boronoethyl)-L-cisteine (BEC 10^{-5} M) and the NOS inhibitor L-nitroarginine (L-NA 10^{-4} M). Responses were expressed as a percentage of KCl-induced contraction (%Kmax).

Results: In normal HUVEC, 24 h of hypoxia increased ArgII protein levels and activity, and reduced p-eNOS/eNOS levels. In IUGR cells under normoxia ArgII level and activity were higher compared to normal HUVEC, whilst there were lower levels of eNOS and p-eNOS, and these results were not different under hypoxia. In IUGR HUV, the NOS-dependent relaxation was decreased (0.1 ± 2.5 %Kmax) compared with normal veins (37.6 ± 3.8 %Kmax). BEC increased the NOS-dependent relaxation in IUGR (18.1 ± 2.5 %Kmax) and normal vessels (57.5 ±3 .1 %Kmax).

Conclusions. The data suggest that IUGR HUVEC has a blunted response to hypoxia. Furthermore, ArgII has a role as vascular modulator in normal vessels, participating in the basal vascular tone as well as in a reduced NOS-dependent relaxation in umbilical veins from IUGR placentae.

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Keywords: Arginase, Vascular Tone, IUGR, Hypoxia

[P2.54]

REDUCTION OF INTRAUTERINE GROWTH RETARDATION (IUGR) IN A HIGH-RISK GROUP WITH THE NO-DONOR PENTERYTHRILTETRANITRAT (PETN) - A PROSPECTIVE RANDOMIZED DOUBLE-BLIND PLACEBO TRIAL

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Background: There is a significant correlation between placental nitric oxide synthesis and the uteroplacental perfusion in IUGR pregnancies. Also it is well known that NO donors can improve decreased uteroplacental perfusion, the major reason of placental insufficiency, without any effects on fetal circulation (Kahler et al. 2004). We present the results of a *randomized, prospective, placebo-controlled, double-blind study* to investigate whether the oral NO-donor Pentalong® (PETN) is suitable as a prophylactic drug in abnormal placentation.

Methods: We included 111 pregnancies presenting with abnormal placental perfusion (bilateral notch or mean RI>0.7) between the 19th to 24th week of gestation (w.o.g.). Further risk factors (high-risk group: history of HELLP/preeclampsia/IUGR/IUFD/placental abruption, type I diabetes mellitus, hypertension, thrombosis/thrombophilia) were identified in 78 study participants. 53 women received PETN while 58 received placebo. Doppler velocimetry measurements of uteroplacental and fetal vessels and fetal growth scans were monitored biweekly with primary endpoints being the occurrence of IUGR, premature birth and / or preeclampsia.

Results: Our data revealed a 33 % relative risk reduction for IUGR (CI 95% -6.5%, 58.3%) in the PETN intervention group compared to placebo. Also PTD was reduced (RRR 63.5%; CI 95% -6.2 to 87.4) in the PETN group. Abruptio placentae was observed in 5 patients of the placebo group (9%) and did not occur in the PETN group. Within the first week of intake, PETN improved uteroplacental perfusion significantly in comparison to placebo (mean PI 1.26 ± 0.36 vs. 1.49 ± 0.44; $p< 0.01$).

Conclusion: These data strongly suggest evidence that PETN is a feasible drug to significantly improve pregnancy outcome in pregnancies at risk for the development of placental insufficiency and subsequent growth restriction and preterm birth

Kahler C, Schleussner E et al Europ J Gynaecol Obstet RB 2004; 115: 10-14
Lees C et al. Ultrasound Obstet Gynecol. 1998 Nov;12(5):334-8

Keywords: NO donor, IUGR, Preterm delivery, abnormal placental perfusion

[P2.55]

DECIDUAL IL-8 STIMULATES NEUTROPHIL INFILTRATION AND DIFFERENTIATION IN THE SECOND TRIMESTER

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The maternal decidual leukocyte populations play crucial roles in the decidual vascular remodeling accompanying normal placentation. Our preliminary FACS study of these populations over the 1st and 2nd trimesters identified a novel population of neutrophils specific to the 2nd trimester. The aim of this study was to further phenotype the decidual neutrophil (DN) population in comparison to peripheral blood (PBN) and to determine if the decidua could drive neutrophil recruitment from the peripheral circulation.

2nd trimester leukocytes were isolated from either decidual tissues or peripheral blood by mechanical mincing or histopaque centrifugation respectively. FACS analysis using Anti CD66b, CD15 (neutrophil markers), CD45 (common leukocyte antigen), CD181, CD182, CD183 and CD184 (chemokine receptors) was performed. Matching decidual biopsies were immunostained for CD66b, neutrophil elastase, and cytokeratin and HLA-G. We also tested the potential of decidual derived IL-8 to stimulate extravasation of primary 2nd trimester PBN in vitro.

FACS analysis showed that 10-20% of the total CD45+ cells (leukocytes) in the second trimester decidua are neutrophils. These DN differ from PBN in that they express high levels of CD66b whilst the IL8 receptors CD181 and 182 are decreased. Conversely CD183 and 184 are increased in DN. Immunostaining confirmed the presence of neutrophils within decidua basalis. Aggregates of neutrophils were seen within the decidual stroma in areas containing EVT. Neutrophils were observed adhered to endothelium and infiltrating the vascular wall. We further showed that 2nd trimester decidua conditioned medium can stimulate PBN to invade and transverse a monolayer of endothelial cells and that this was inhibited by 60% following neutralisation of IL8.

This study indicates the peripheral blood origin of decidual neutrophils and identifies decidual Il-8 as a primary recruiting stimulus for these cells. Recent data suggests that neutrophils contribute to tumour driven angiogenesis suggesting a similar role for these cells in the decidua

Keywords: Neutrophil, Decidua, Invasion, IL-8

[P2.56]

CAN INFLAMMATION INDUCED BY LPS AFFECT MIF EXPRESSION AT MATERNAL-FETAL INTERFACE?

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Introduction: Data from the literature show that serum MIF concentration is positively correlated with the severity of bacterial infection in humans. Moreover, deletion of the MIF gene in mice seems to confer protection against lethal endotoxemia. MIF has also been demonstrated at maternal fetal interface in several species where it is supposed to counter regulate the anti-inflammatory glucocorticoid activities. In this study, we investigated the presence of MIF at maternal-placental interface in pregnant females in which inflammation has been induced by administration of LPS on early gestation in CD-1 mice. We specifically aimed to determine whether the inflammatory conditions change the MIF-producing cellular population at the implantation sites in fetuses that have survived to the infection.

Material and methods: Immunolocalization of MIF was performed in implantation sites from gestation days 7.5 and 10.5 from pregnant females that received LPS at the concentration 0.1μg/ g of body weight or saline as a control.

Results: At maternal-fetal interface, LPS-treated animals exhibited few cells reactive to MIF in comparison with controls. Trophoblast cells when reactive were weakly stained as well as leukocytes and decidual cells.

Conclusions: The fetal survival in LPS-treated pregnant females is certainly a consequence of multiple mechanisms of defense triggered after infection. Our findings suggest that the reduction of MIF at maternal-placental interface may play a role in this intricate survival mechanism.

Financial support: FAPESP, CAPES and CNPq

Keywords: MIF, LPS, Trophoblast cells, inflammation

[P2.57]

THE RETROVIRAL ENVELOPE PROTEIN SYNCYTIN-1 IS IMMUNOSUPPRESSIVE AND HAS HIJACKED THE EXOSOMAL PATHWAY IN THE HUMAN PLACENTA

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The human placenta expresses the endogenous retroviral envelope protein syncytin-1 that promotes fusion of trophoblast cells. Many retroviral envelope proteins are immunosuppressive due to the presence of a highly conserved sequence called the Immunosuppressive Domain (ISD) and syncytin-1 carries a sequence homologous to this ISD. The human placenta also produces and secretes exosomes. Placental exosomes have recently emerged as immune regulators that may help to circumvent maternal immune-surveillance during pregnancy. According to "The Trojan exosome hypothesis", retroviruses may exploit the exosome biogenesis pathway. In light of this information, we hypothesized that syncytin-1 has immunosuppressive properties and is released by the placenta using the exosomal pathway.

A synthetic peptide and the soluble recombinant ectodomain of syncytin-1 which both carry the ISD were tested *in vitro* using human whole blood cultures challenged with LPS or PHA. Supernatants were assayed for TNF-α, IFN-γ and CXCL10 using commercial ELISA kits. Exosomes were purified from the supernatant of placental explant cultures by differential centrifugation followed by sucrose cushion and gradient methods. Exosomes were confirmed by TEM and buoyant density in sucrose gradients. Exosomal proteins were extracted and analyzed by western blotting with two antibodies against syncytin-1.

Both the synthetic peptide and syncytin-1 recombinant protein inhibited in a dose dependent manner the released of TNF-α by human blood cells following maximum stimulating doses of LPS (10μg/ml). At 1μM, syncytin-1 recombinant ectodomain inhibited the release of the inflammatory cytokines TNF-α (50%), IFN-γ (30%) and the release of the chemokine CXCL10 (65%) which is involved in Th1 immune responses and allograft rejection.

The present study shows for the first time, experimental evidence in favor of syncytin-1 having an immunosuppressive role. Also we present novel evidence that syncytin-1 has hijacked the placental exosome pathway. The presence of syncytin-1 in human placental exosomes present a novel mechanism of retroviral mediated immunosuppression that may be relevant in maternal immune tolerance towards feto-placental antigens.

Keywords: Endogenous retrovirus, Syncytin, exosomes, immunosuppresion

[P2.58]

STUDYING A NEW PROTEIN AT MATERNAL-FETAL INTERFACE, SDF-2

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Introduction: Using a Signal Sequence Trap Method (SST) Hamada et al. (1996) have isolated the cDNA encoding of a novel, probably secretory, protein from the stroma cell line ST2 and named it *Stromal cell derived factor 2*, *Sdf2*. They determined the complete nt sequences of this mouse and human protein and showed a high homology between them. The characteristics of the presumptive amino acid sequence are similar of chemokines. Therefore, it is interesting to speculate whether SDF-2 is acting at maternal-fetal interface in diverse aspects of placenta.

Methods: The gene coding for *Sdf2* was subcloned into the pET-3a vector and expressed in *E. coli* strain BL21 Star™(DE3)pLysS. The protein was produced in growing cultures of the *E. coli* strain and was then purified. Anti-mouse polyclonal antibody was obtained from rabbits immunized with the protein associated to Freud's Adjuvant. Western Blot and q-PCR were used for protein and gene expression, respectively.

Results: In mouse, the protein was detected in several tissues and gene expression showed to be temporally regulated during gestational phases, exhibiting high levels of expression during implantational phase. In human, protein was detected by Western Blot during 1st and 2nd trimester of gestation.

Discussion: Our preliminary results showed that Sdf2 is expressed in mouse and human placenta, which may be associated to developmental and dynamic placental functions. However, functional assays are still in progress to support this hypothesis.

Supported by FAPESP and CNPq

Keywords: Trophoblast, SDF-2, Placenta, Blastocyst

[P2.59]

DECIDUAL CELL SURFACE AND SECRETED (PARACRINE) FACTORS REGULATE TROPHOBLAST FUNCTION

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Extravillous trophoblast (EVT) must adhere, migrate and invade through the decidua to form a functional placenta. Abnormal decidualization of endometrial stromal cells (ESC) leads to unregulated trophoblast invasion and pregnancy failure in mice. Recent evidence in humans suggests that preeclampsia is associated with impaired decidualization. The mechanisms by which decidual cells interact with EVT remain largely unknown. We aimed to determine whether decidualized and non-decidualized human (H) ESC regulated EVT adhesion, and identify EVT proteins regulated by decidualized and non-decidualized HESC secreted factors.

Primary HESC were decidualized with cAMP+estradiol 17β+progesterone and prolactin (decidual marker) measured by ELISA. HESC conditioned media (CM) was collected from Days (D) 0-2 (non-decidualized) and D12-14 (decidualized) of treatment. Cytotrophoblast were isolated from 1st trimester placenta and cultured on Matrigel for EVT. EVT were fluorescently labeled and adhesion to decidualized and non-decidualized HESC assayed fluorimetrically. EVT proteins <30kD were purified using size exclusion affinity particles (SEAN) and identified by mass spectrometry.

EVTs showed increased adhesion to decidualized compared to non-decidualized HESC (184.5±45% vs 100% respectively, n=5; $p<0.05$). EVT treated with non-decidualized CM expressed 11 unique proteins, including 4 not previously shown in the placenta and 3 dysregulated in preeclampsia. EVT treated with decidualized CM showed 12 unique proteins, 7 not previously identified in the placenta and 3 dysregulated in preeclampsia. This demonstrates that decidualized/non-decidualized secreted factors differentially regulated EVT proteins. Galectin-7 and profilin-1 (both associated with cell migration) were identified for the first time in EVT and immunolocalized to interstitial EVT in 1st trimester decidua.

These data suggest that cell surface and secreted decidual cell factors regulate EVT function. It also suggests that the extent of decidualization is a critical factor in human EVT function as in mice. In conclusion, adequate decidualization may be critical for appropriate EVT function and therefore placental development in humans.

Keywords: EVT, Decidua, Secretion, Invasion

[P2.60]

CRIPTO-1: A TUMORAL MARKER AT THE MATERNAL-PLACENTAL INTERFACE

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The intense invasive activity of the trophoblast into the mesometrial decidua is a determinant event of placental development. Many signaling factors are likely to play a role in regulating the trophoblast invasion in a similar way to the regulation of metastasis in tumor cells. One of the many factors that have been shown as potential candidate for invasion marker is Cripto 1, early detected in over eighty percent of breast invasive carcinomas and undetectable in normal human breast tissue. In this context, considering the parallel between invasive trophoblast and cancer cells, our research aimed to analyze *Cripto1* (Cr1) gene expression and protein content at the maternal-placental interface.

Methods: Tissue extracts from ectoplacental cones (gestation day 7.5) and placentas (gds 10.5, 13.5 and 17.5) were collected in Ripa buffer and Trizol for Western Blotting and RT-PCR analyses, respectively.

Results: Our results showed that Cr1 was expressed during placental development at the maternal-placental interface. The protein content increased gradually in the fetal compartment as gestation progressed and decreased in decidua after gd13.5. Semi-quantitative evaluations of *Cr1* by PCR were indicative of low expression at the post-implantation and final gestational periods in both, the maternal and fetal compartments. Significant increase was seen at gd10.5 (*vs* gd7.5, $p<0.04$) and mainly at gd13.5 (*vs* gd7.5, $p<0.00$). Cr1 expression at the maternal-placental interface throughout gestation is indicative of a relevant role for this factor during placental maturation, chiefly when several cell types (trophoblast, endothelial and immune cells) are migrating through the decidua. The question of whether Cripto acts on regulatory pathways that particularly mediate the invasive processes of trophoblast cells is the current goal of our research.

Supported by: CNPq and FAPESP

Keywords: Cripto-1, Trophoblast, Decidua, Maternal-placental interface

[P2.61]

SNMCL-1 PROTEOLYTIC ISOFORM REGULATES CELL GROWTH DURING PLACENTAL DEVELOPMENT AND IN PLACENTAL PATHOLOGIES

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Introduction: Mcl-1 (Myeloid cell leukaemia factor 1) is a pro-survival Bcl-2 family member that plays a key role in both placental development and in trophoblast-related pathologies by governing trophoblast cell fate. Recent evidence indicates that a C-terminus truncated Mcl-1 isoform, termed small nuclear Mcl-1 (snMcl-1) is expressed in the nucleus where it interacts with the cell-cycle-dependent kinase CDK-1 thereby preventing its binding to Cyclin B1 thus inhibiting cell cycle progression. Herein, we examined the temporal and spatial expression of snMcl-1 and its association to CDK-1 during placental development and in placental pathologies preeclampsia (PE) and Intra Uterine Growth Restriction. (IUGR).
Methods: Placenta developmental tissues (5-15 weeks, n=21) and placental tissues from PE (n=24), IUGR pregnancies with documented AEDF (n=16) and pre-term controls (PTC, n=14) were used. Protein levels were measured by immunoblotting using CDK-1 and Mcl-1 antibodies. CDK-1/Mcl-1 association and localization were assessed by immunofluorescence followed by deconvolution microscopy.
Results: CDK-1 and Cyclin B1 proteins showed a peak of expression at 5-8 weeks of gestation which inversely correlated to snMcl-1 expression profile. Immunofluorescence revealed strong positive CDK-1 and Cyclin B1 signals in the cytotrophoblastic nuclei during early gestation, while Mcl-1 was mainly localized in the cytoplasm of syncytiotrophoblast cells. With advancing gestation (9-15 weeks), CDK-1/Mcl-1 co-localization was found in the nuclei of cytotrophoblast cells. Notably, while Mcl-1/CDK-1 co-localization was increased in PE cytotrophoblast cells relative to PTC, no association was observed in IUGR tissues.
Discussion: During early placentation high CDK-1/Cyclin B1 levels found in the trophoblastic nuclei may account for increased trophoblast proliferation rates; while, with advancing gestation, increased CDK-1/snMcl-1 nuclear association implicates a novel role for Mcl-1 as an inhibitor of trophoblast cell cycle progression. In preeclampsia, increased CDK-1/Mcl-1 association in trophoblast cells may contribute to altered trophoblast proliferation typical of this pathology. (Supported by CIHR and OWH/IGH).
Keywords: small nuclear Mcl-1, Placenta Development, Preeclampsia, IUGR

[P2.62]

PRESENCE OF T REGULATORY CELLS LAP$^+$ IN RENAL TRANSPLANTED PREGNANT WOMEN

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Introduction and Objective: Immunological tolerance is a key for successful maternal-fetal interaction and transplantation. Regulatory T cells are important components of homeostasis of the immune system, as impaired Treg cell activity is associated with decreased tolerance in these conditions. $CD4^+CD25^+CTLA\text{-}4^+$ T cells have been reported as suppressors of other T cells by cell-to-cell interactions and secretion of cytokines like IL-10 and TGF-β. Previous report showed that $CD4^+CD25^+$ T cells are capable of producing TGF-β either as a secreted or a cell surface protein (LAP). Pregnancy after solid organ transplantation is no longer uncommon, especially in the view of the recent advances in surgery and immunosuppressant. Thus, understanding that the tolerogenic immune microenvironments may shed light on mechanisms of tolerance and strategies of immune modulation that are beneficial for both gestation and the graft survival, in this study we aimed to characterize $CD4^+CD25^+$ $CTLA\text{-}4^+$T cells in pregnant women after renal transplantation, a condition closely related to long-term immunossupression. In addition, the levels of IL-10 and TGF-β were also investigated
Methods: PBMC was obtained from renal transplanted and healthy women pregnancies in the third trimester. Expression of $CD4^+CD25^+CTLA\text{-}4^+LAP^+$ and $CD4^+CD25^+CD127^{low}CTLA\text{-}4^+$ were identified through flow cytometry. IL-10 was measured by CBA and TGF-β levels were obtained by ELISA.
Results and Conclusion: Renal transplanted pregnant women exhibited lower levels of $CD4^+CD25^+CD127^{low}CTLA\text{-}4^+$, IL-10 and TGF-β, but higher levels of $CD4^+CD25^+CTLA\text{-}4^+LAP^+$. These results seem to identify LAP as a putative mechanism of tolerance in these populations.
Keywords: T regulatory cells, TGF-beta, maternal-fetal tolerance, renal transplant

[P2.63]

CLINICAL, IMMUNOLOGICAL AND THROMBOPHILIC FACTORS ASSOCIATED WITH RECCURENT SPONTANEOUS ABORTION AND MULTIPLE IMPLANTATION FAILURE AND THEIR OUTCOMES AFTER TREATMENT

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Introduction: Antiphospholipid antibodies (APA), hereditary thrombophilias (HT) and immunological factors have been treated in reccurent spontaneous abortion (RSA) and multiple implantation failure (MIF).
Objective: To compare clinical, immunological and thrombophilic factors associated with RSA and MIF, describing their live birth rates after intervention.
Methods: Analysis included 175 RSA ($\geq$2 clinical pregnancy losses) and 178 MIF ($\geq$2 fresh embryo transfers) patients and live birth outcome study in 102 and 148 women, respectively. Investigation included APA, HT, paternal lymphocyte crossmatch and NK endometrial cells quantification during the secretory phase (CD56+16). Patients received treatment with paternal lymphocyte alloimunization (PLA) and/or intravenous imunoglobulin (IVIg). Considered significant $P \leq 0.05$ (OR/RR, CI95%).
Results: Groups had same average age, number of previous events and APA prevalence (RSA:21.7% x MIF:18.5%). MIF had higher HT prevalence (RSA:20.6% x MIF:30.3%; P=0.035, OR=1.7[1.0-2.7]). RSA had lower NK rates (RSA:39;7$\pm$16.0 x MIF:46.8$\pm$17.6; P=0.000) and less patients with >60%NK (RSA:10.7 x MIF:22.8%; P=0.003). Overall birth rate was RSA:71.6% and MIF:45.3%. RSA with APA got higher successful rate than negative ones (26.0% x 6.9%; P=0.031, RR=4.7[1.0-21.0]). RSA birth rates for immunological treatment were: PLA:72.4%, IVIg:81.8% and 60.0% with both after previous failure under single treatment. In MIF, 7 natural pregnancies (4.7%) occurred with 85.7% birth. For patients who underwent a new embryo transfer, outcomes were: 43.3% birth, 9.2% abortion and 5.7% biochemical pregnancy. Birth rates were higher in MIF treated with IVIg compared to PLA (80.0% x 38.9%; P=0.004, RR=2.1[1.5-2.9]).
Conclusion: Immunological and thrombophilic causes are common in RSA and MIF, and immunological treatment seems suitable. Physiopathology of both conditions probably has distinct pathways, suggested by the HT and higher endometrial NK cells association with MIF. Despite the lack of strict selection criteria for immunological treatment, IVIg seems to be the choice for MIF patients, needing more controlled trials.
Keywords: antiphospholipid antibodies, pregnancy failure, reccurent spontaneous abortion, multiple implantation failure

[P2.64]

EVIDENCES OF UTERINE-NATURAL KILLER CELL SECRETORY-LYSOSOME GRANULE MEDIATED CYTOLYTIC RESPONSE IN THE MATERNAL-FETAL INTERFACE

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Mouse uterine natural killer (uNK) cell is an unique CD3-CD122+ NK1.1-DX5- subset of cytokine producing NK cell with secretory-lysosome granules that participate in the modulation of homeostasis for successful pregnancy. Contents of cytolytic molecules in the secretory-lysosome granules of uNK cells is well known, but their mobilization in the innate immune type cytolytic response in the pregnant uterus is still speculative. The present work investigated the capability of uNK cells secretory-lysosome granules mobilization as response under stimuli of abnormal pregnancy provoked by embryo disruption. Pregnant mice on gd 9th, were anesthetized and embryos were surgically disrupted introducing needles through the antimesometrial side of uterine wall. Sham operated animals and embryo disrupted (ED) uterine fragments were collected after 15, 30, 60 and 120 minutes of ED and processed for light and electron microscopy. *Dolichos biflorus* lectin (DBA), periodic acid Shiff (PAS) and ponthamine blue (PB) cytochemistry and perforin immunocytochemistry were positive in the core region (secretory compartment) of uNK cells granules, while cathepsin D immunocytochemistry was positive in the cap region (lysosome compartment). Cross analysis of double staining showed heterogeneity of granule contents suggesting phenotype heterogeneity of uNK cells and/or granule maturation step during uNK cell differentiation. The ultrastructure of granules changes as soon as 15' of ED with disruption of membrane, losing of secretory and compactness of lysosomal compartments. Mobilization of granules contents were not equal for all components being the DBA lectin and perforin diffusion seen as soon as 15', while both PAS and PB contents gradually decreased in the core of granules after 30' of ED and latterly followed by cathepsin D of lysosome compartment. In conclusion, this is the first experimental approach showing evidences of uNK cell innate immune type response capability based on commitment of secretory-lysosome granules.
Grants: CNPq and CAPES.
Keywords: uterine Natural Killer cells, secretory-lysosome granule, innate immune response

[P2.65]

IMMUNE CELL CHARACTERISATION IN PREGNANCIES COMPLICATED BY ASTHMA

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Introduction: During pregnancy, we have shown that the asthma exacerbation rate is high, increasing the risk of an adverse neonatal outcome, including intrauterine growth restriction, preterm delivery and still birth. In a majority of cases exacerbations are un-resolvable with inhaled glucocorticoid treatment, suggesting pregnancy changes the asthma phenotype to a form that is non-responsive to asthma treatment. This may be related to maternal adaptations in immune pathways that occur with pregnancy. In normal pregnancy, maternal circulating immune cells undergo modifications in cell concentration, phenotype and function over the course of pregnancy. However little is known about how this adaptation in pregnancy is influenced by the presence of maternal asthma. We aim to characterise leukocyte sub-populations and phenotypes in blood collected from pregnant non-asthmatic and asthmatic women.
Hypothesis: We propose that maternal asthma worsens during pregnancy due to altered leukocyte phenotypes, including increased monocyte CD14dimCD16+ subset and disturbances in T cell subsets particularly Tregs, Th1 and Th2.
Methods: Venous blood was collected from pregnant asthmatic subjects (n =10) and controls (n =10) at 12, 18 and 30 weeks gestation. Peripheral blood mononuclear cells (PMBCs) were isolated at all three time points. Multi-parameter flow cytometry analysis using appropriate antibody pairs was used to determine T cell and monocyte subsets as summarised in the table below.

Panel	Populations defined	Panel	Populations defined
1	naive and memory CD4 T cells	3	central and effector memory CD8 T cells
2	central memory CD4 T cells	9	gamma-delta T cells
3	global TRegs	10	NK subsets cells
4	global TH17	11	DC subsets
5	TH0. TH1 and TH2 Subsets	13	memory subsets

Results: Our preliminary analysis demonstrated that there were no differences in T cell subtypes in control and asthma group as pregnancy progressed. However there were differences in monocyte subsets with differential expression of activation marker HLA-DR ($P < 0.05$) in the asthmatic group. The expression of 'pro-inflammatory' monocyte phenotype CD14dimCD16+ was also indentified in some asthmatic subjects.
Discussion: Circulating monocytes are heterogeneous in phenotype and function. CD14dim CD16+ phenotype expands during infection and inflammatory response. The differential expression of leukocyte subsets in pregnancies complicated by asthma may be part of the mechanism contributing to worsening asthma during pregnancy.
Keywords: immune cells, pregnancy, asthma, activation markers

[P2.66]

RANDOM X INACTIVATION AND EXTENSIVE MOSAICISM IN HUMAN EXTRA-EMBRYONIC TISSUE REVEALED BY ANALYSIS OF ALLELE-SPECIFIC GENE EXPRESSION ALONG THE X CHROMOSOME

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Transcriptional inactivation of all but one X chromosome per diploid cell in females' mammals ensures dosage compensation of X-linked gene products between the genders. In marsupial somatic cells and in murine extra-embryonic tissues, XCI is imprinted and the paternal X is preferentially inactivated. In contrast, in embryonic cells of Eutherian mammals, either the paternal or the maternal X is randomly inactivated. In human extra-embryonic tissues, results concerning XCI patterns are still controversial, and are mostly based on the analysis of only one or two X-linked genes in different cell types. Here we readdressed this issue by performing a robust analysis of allele-specific expression of 22 X-linked genes, including *XIST*, along the entire X through cDNA direct sequencing to investigate the patterns of XCI in 18 human term placenta. The contribution of each allele to X-linked gene expression was quantified using the PickPeaker software1. Six samples presented predominantly monoallelic expression, indicating a completely skewed pattern of XCI. Five placentas, however, displayed biallelic expression in the majority of loci examined, suggesting random XCI. Other seven samples showed skewed XCI, where for almost every gene examined one allele had higher level of expression than the other. As results varied greatly among samples we wondered if the placenta behaves as a mosaic concerning X inactive parental origin. To test this hypothesis, different fragments were collected in non-adjacent regions of three additional placentas, revealing that XCI pattern in this organ is random, and indeed occurs as a mosaic of relatively large patches with either maternal or paternal XCI. The variability found on individual samples in this work can finally explain the lack of agreement among previous studies, and our model of the placenta as a mosaic of large patches proposes a solution to those puzzling results previously reported.
1. Genome Research, 2005. 15:1584-91
Keywords: Placenta, X-chromosome inactivation, Mosaicism, Allele-specific expression

[P2.67]

DNA METHYLATION PROFILING OF HUMAN PLACENTAS WITH PREECLAMPSIA AND INTRA-UTERINE GROWTH RESTRICTION

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Preeclampsia and intra-uterine growth restriction (IUGR) are two of the most common adverse pregnancy outcomes, both of which often show placental pathology with significantly increased perinatal morbidity and mortality. Identification of biomarkers that could be used to diagnose abnormal outcomes during early pregnancy would be a major step forward in antenatal care. While multiple studies have investigated gene expression changes in these disorders, few studies have examined their epigenetic changes. DNA methylation is a chemically stable epigenetic modification of DNA that may provide an alternative approach to identifying potential biomarkers for such disorders. We investigated 1505 CpG methylation sites associated with 807 genes in 26 placentas from early-onset preeclampsia (EOPET), late-onset preeclampsia (LOPET), IUGR and control subjects using an Illumina GoldenGate Methylation microarray. Hierarchical clustering of the placental samples based on their DNA methylation profiles shows that the samples were preferentially clustered by gestational age. For the case-control comparisons, thirty-four loci were hypomethylated (false discovery rate $<$10% and methylation difference $>$10%) in the early-onset preeclamptic placentas, while no and only 5 differentially methylated loci were found in late-onset preeclamptic and IUGR placentas, respectively. Hypomethylation of 4 loci in EOPET was further confirmed by bisulfite pyrosequencing of 26 independent placental samples. Our results suggest that gene-specific hypomethylation may be a common phenomenon in EOPET placentas and it may act as a potential biomarker for the disorder. Furthermore, DNA methylation profile of placenta may change dramatically throughout gestation.

Keywords: DNA methylation, Preeclampsia, Intra-uterine growth restriction, Programming

[P2.68]

EXAMINING GENE: ENVIRONMENT INTERACTIONS IN THE ESTABLISHMENT OF THE PLACENTAL EPIGENOME: GENOME-WIDE ANALYSIS OF PLACENTAS FROM 16 TWIN PREGNANCIES.

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The Developmental Origin of Health and Disease (DoHAD) hypothesis predicts that many adult diseases have their origin *in utero*. This was first proposed to describe the observed link between low birth weight and increased risk of cardiovascular disease in later life 1. Given its location at the fetomaternal interface, the human placenta plays a critical role in mediating environmental effects associated with this programming phenomenon.

Epigenetic modifications, such as DNA methylation, play a major role in controlling gene expression. Mounting evidence suggests an elevated level of inter-individual variation in DNA methylation profile in full term placenta relative to somatic tissues. However, mechanisms underlying this variation remain unclear. We speculate that this results from a combination of both genetic and environmental factors, which combine during pregnancy to modulate the level of methylation present at specific gene promoters.

Studying twins allows researchers to begin to unravel the contributions of genetic and environmental influences on DNA methylation patterns. As part of the Peri/Postnatal Epigenetics Twins Study (PETS), we have collected multiple birth specimens from 250 twin pairs (including placenta), representing one of the largest collections of such tissues internationally. In the current study, DNA methylation profiling of placenta tissue from sixteen twin pairs (50% Monozygotic) was analysed using the Illumina Infinium HumanMethylation27 platform, profiling over 27,000 genomic CpG sites.

Unsupervised cluster analysis revealed that sex was the biggest determinant of overall placental methylation profile. Whereas twin placentas generally show a higher correlation of DNA methylation profile than non-related individuals, there are exceptions, with some twins showing markedly different profiles. Monozygotic twins showed a higher correlation than dizygotic twins, highlighting the contribution of genetic factors to the establishment of the placental epigenome. However, evidence for an environmental and/or stochastic contribution to methylation profile was also apparent.

1 Barker *et al.* BMJ 2010

Keywords: DNA methylation, Twins, Environment, Genetics

[P2.69]

GENOME-SCALE DNA METHYLATION ANALYSIS OF SEVEN COMMONLY USED TROPHOBLAST CELL LINES

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Given the inability of primary trophoblasts to divide in culture, trophoblast-derived cell lines have become an important tool for studying specific aspects of trophoblast function. Several widely used cell lines have been established, each with its own unique functional characteristics and origin. In the current study we compared genome-scale DNA methylation patterns in first trimester cytotrophoblasts to 7 commonly used trophoblast cell lines, with the aim of identifying epigenetic marks involved in conferring specific functional properties of trophoblast cells.

The 7 cell lines used in this study were immortalized HTR8, TEV-7, SWAN and four choriocarcinoma (CCA) lines (AC1M32, BeWo, JAR and JEG-3). Genome-scale DNA methylation analysis was performed using the Illumina Infinium HumanMethylation27 platform, which analyses 27,578 CpG sites covering 14,595 genes. Locus-specific DNA methylation analysis was performed using the Sequenom EpiTYPER platform. Gene expression analysis was performed using the ABI 7300 Real-time platform.

Unsupervised clustering of the most variable Infinium probes separated cell lines into 4 groups 'CCA', 'SWAN and TEV-7', 'Cytotrophoblasts' and 'HTR8'. Most of the highly variable probes showed hypermethylation in CCA relative to other cells, suggesting a role in tumorigenesis rather than trophoblast function.

Specific comparison of the JEG-3 and BEWO cell lines has revealed several DNA methylation mediated gene silencing events in BEWO, some of which may explain the proliferation differences between these cell lines. Current studies are aimed at identifying epigenetic silencing events associated with specific properties of trophoblast functioning.

This study complements earlier studies demonstrating gene expression differences between trophoblast-derived cell lines, and highlights the range of molecular differences between these model cell lines and primary trophoblast cells.

Keywords: DNA methylation, Choriocarcinoma, Trophoblast cell lines

[P2.70]

DIFFERENTIAL GENE EXPRESSION OF FETAL AND MATERNAL MESENCHYMAL STEM CELLS FROM THE HUMAN PLACENTA

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The term placenta is a source of both fetal (1) and maternal (2) mesenchymal stem cells (MSCs). While both types of placental MSCs (PMSCs) have been studied, the differences between the PMSCs have yet to be examined. A major challenge in this field has been obtaining pure fetal MSCs (3). This study investigated the transcription factor expression patterns of pure fetal and maternal PMSCs.

Fetal and maternal PMSCs were prepared from the normal term placenta of a male fetus. Flow cytometry for MSC markers and XY fluorescence in situ hybridisation and karyotyping was carried out to determine the purity of the PMSC populations. RNA was extracted and Taqman homeobox low density arrays were carried out and differences in gene expression were confirmed using realtime PCR. Cardiomyogenic differentiation was carried out using 10uM 5-Azacytidine.

Both populations of PMSCs showed >90% positivity for CD105, CD73 and were negative for CD45. FISH analysis confirmed that the PMSC populations are 100% male (fetal) or 100% female (maternal) respectively. A number of differences in gene expression were identified. *WT1* mRNA was only expressed in maternal PMSCs, while *GATA4* was expressed only in fetal PMSCs. *GATA6* mRNA levels were also much higher in fetal PMSCs. *GATA4* realtime PCR on 5 more samples confirmed that *GATA4* expression was either absent or very low in maternal PMSCs ($p<0.05$, $n=5$). As both *GATA6* and *GATA4 are* important regulators of cardiomyocytes, cardiomyogenic differentiation was carried out in fetal PMSCs. Preliminary results show marked increases in the mRNA level of cardiac markers *Troponin T, GATA4* and *Alpha actinin 1.*

This is the first study to investigate gene expression differences between fetal and maternal PMSCs and demonstrates how such differences could influence future therapeutic applications.

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2. Barlow, S., G. Brooke, et al. (2008). "Comparison of Human Placenta- and Bone Marrow–Derived Multipotent Mesenchymal Stem Cells." Stem Cells and Development **17**(6): 1095-1108.

3. Semenov, O. V., S. Koestenbauer, et al. "Multipotent mesenchymal stem cells from human placenta: critical parameters for isolation and maintenance of stemness after isolation." Am J Obstet Gynecol **202**(2): 193 e1-193 e13.

Keywords: Mesenchymal stem cells, fetal, maternal, gene expression

[P2.71]

TRANSCRIPTOME ANALYSIS OF SYNCYTIAL KNOTS SHED FROM THE HUMAN PLACENTA

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In human pregnancy the surface layer of the placenta is the multinucleated syncytiotrophoblast. Throughout pregnancy portions of the syncytiotrophoblast are shed into the maternal blood as syncytial knots. Currently the mechanisms involved in the shedding of trophoblasts are not well characterised. We hypothesise that syncytial knots contain a limited transcriptome, but that some expressed genes will be important the death or extrusion of the knots. In this study we have begun to determine the transcribed genes (transcriptome) of syncytial knots shed from placental explants.

Eight hundred and sixteen syncytial knots shed from 18 first trimester placentae were collected by direct pipetting from beneath explants cultured in Netwells. RNA was extracted from the syncytial knots and run on the Experion automated electrophoresis system (BioRad). The RNA was transcribed into cDNA and prepared for analysis on the 454 GS FLX high throughput sequencing system (Roche).

A total of 2.30 nanograms of RNA was extracted from the knots, equating to an average of 2.82 picograms of RNA per syncytial knot. The RNA recovered from the knots had a broad electrophoretogram sample peak, indicative of degradation. However, several individual peaks were detected, suggesting intact RNA species were present. Individual transcribed genes have not yet been identified.

Syncytial knots shed from normal placentae are generally considered to be undergoing apoptosis, and therefore to have little ongoing protein synthesis. We found that syncytial knots express low levels of RNA, indicating that they are dying. To our knowledge, this is the first time that identification of the transcriptome of shed syncytial knots has been attempted. Though we have not yet been able to identify specific transcripts our data are beginning to shed light on molecular events involved in trophoblast shedding, and may have implications in diseases associated with the deportation of trophoblasts, such as preeclampsia.

Keywords: transcriptome, syncytial knot, RNA, deportation

[P2.72]

CHANGES IN GENE EXPRESSION ASSOCIATED WITH TROPHOBLAST DEATH AND SHEDDING

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During normal pregnancy, trophoblasts die by an apoptosis-like process and are shed into the maternal blood. In preeclampsia however, it is hypothesised that the trophoblast death process changes to necrosis or aponecrosis, and that this alteration leads to an aberrant maternal response to the dead trophoblasts. Maternal factors associated with preeclamptic pregnancies such as antiphospholipid autoantibodies (aPL) and interleukin-6 (IL-6) have recently been shown by our group to cause an increase in non-apoptotic trophoblast death and shedding *in vitro*. We hypothesised that treating placental explants with aPL or IL-6 would lead to specific gene expression changes. In order to investigate this, we examined the gene expression profiles of first-trimester placental explants that had been treated either with a monoclonal aPL (25μg/mL) or IL-6 (10ng/mL) using Affymetrix HGU133 Plus 2.0 arrays. Fifty-one genes were up-regulated and 66 genes down-regulated in explants treated with the aPL, compared to control-antibody treated explants. Explants treated with IL-6 displayed an up-regulation of 43 genes and a down-regulation of 110 genes. Although there were no regulated genes common to both treatments, several genes involved in apoptotic signalling were dysregulated, including TNFSF10, Fas ligand, and Bcl-x, implicating apoptosis in the trophoblast death and shedding processes. Expression levels of genes previously implicated in the pathogenesis of preeclampsia, such as PTEN and von Hippel Lindau tumour suppressor, were altered in the IL-6 treated explants. This analysis demonstrated that treating placental explants with either IL-6 or aPL resulted in surprisingly little change in the transcriptome of the explants. Other researchers have suggested that cell death can occur in the absence of major transcriptional changes and our results are consistent with that suggestion. Despite the limited changes in the transcriptome our results begin to provide an insight into the genes involved in trophoblast death and shedding.

Keywords: Microarrays, Apoptosis, Antiphospholipid antibodies, Trophoblast death and shedding

[P2.73]
METABOLOMIC PROFILING OF CORDONAL BLOOD IN INTRAUTERINE GROWTH RESTRICTED FETUSES

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Objective. Fetal growth restriction (FGR) is the failure of the foetus to reach its growth potential that continues to be a major contributor to perinatal morbidity and mortality with an increased risk to develop adult-onset disease. The aim of the present study was to investigate the metabolomic profile of FGR fetuses through a non-targeted metabolomic liquid chromatography high-resolution mass spectrometry (LC-HRMS) analysis of cordonal blood collected at birth to identify any alterations in the fetal and/or placental metabolism.
Methods. Cordonal blood samples were collected at birth from 32 FGR neonates and 32 appropriate for gestational age neonates (AGA). Serum samples were deproteinised by mixing with methanol at room temperature and centrifugation. Supernatants were lyophilized and reconstituted in water to analysis. LC-HRMS analyses were performed using an Orbitrap mass spectrometer hyphenated to a Surveyor Plus LC. Samples were injected on a 1.0 x 150 mm Luna C18 column. Spectra were collected in full scan mode at a resolution of approximately 30.000. Data were acquired over the m/z range 50-1,000 with measurements performed in triplicate. To observe metabolic variation between the two sets of samples, we fitted a Principle Component Analysis (PCA) model on the LC-HRMS data.
Results. FGR neonates had a statistically lower birth weight than AGA. A large number of features (ionic species with specific retention times) were shown to differ between the two classes of samples. By comparison with available databases two main differentially expressed metabolites (phenylalanine and tryptophan) were detected. Logistic regression coupled to a Receiver Operating Characteristic curve identified a cut-off value for phenylalanine and tryptophan, which gave an excellent discrimination between FGR and AGA (sensitivity = 100%; specificity = 75%).
Conclusions. The non-targeted metabolomic LC-HRMS analysis of cordonal blood collected at birth appears a promising tool for the identification of potential biomarkers of FGR.
Ramo: proteomica
Keywords: IUGR, metabolomica, phenylalanine, tryptophan

[P2.74]
STUDY OF PROTEINS YOLK SAC BY PROTEOMICS ANALYSIS

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The extra-embryonic membranes are the link of maternal-fetal communication, which will form the placenta, a vital organism for the fetal development and growth. The yolk sac is a membrane which plays an important role in embryonic development, mainly the initial stages of hematopoiesis. While the actual placenta isn't formed yet, during the embryonic development, the yolk sac is the main source of nutrition for the embryo, what makes it a place of transference and synthesis of proteins. These molecules rule almost all cellular functions and are, from the chemical point of view, the biological molecules most complex structured. The proteomic is the large scale study of proteins of a biologically complex sample. The aim of this work was the structural analysis and initial biochemistry of proteins from approximately 23 and 37 days of pregnancy of the yolk sac bovine. From 2D-PAGE gels, the samples of proteins in 37 days of pregnancy yolk sacs revealed about 1230 proteins and the gels of 23 days of pregnancy samples revealed about 970 proteins. In the gels, good reproducibility and high degree of comparison between the two stages of the placenta are revealed. These proteins will be vital to the embryo's development until the formation of the placenta, furthermore they help in the understanding of many mother-fetus relations, in which phase many events may lead to alterations in the development of this membrane and, consequently, to phenomena which harm the placentation and normal development of the embryos.
Keywords: Placenta, Proteins, Yolk sac, Proteomics analysis

[P2.75]

LXR-ACTIVATING OXYSTEROLS SUPPRESS 11-HYDROXYCHOLESTEROL DEHYDROGENASE TYPE 2 IN HUMAN TROPHOBLAST CELLS THROUGH A POST-TRANSCRIPTIONAL MECHANISM INDEPENDENT OF LXR AND PROTEASOME-MEDIATED PROTEIN DEGRADATION

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The placental 11β-hydroxysteroid dehydrogenase type 2 has emerged as a key player in controlling fetal development, but its regulation is incompletely understood. Given that both LXRa and LXRb are expressed in the human placenta where high concentrations of natural LXR ligands oxysterols (also known as hydroxycholesterols) are present, the present study was designed to test the hypothesis that LXR-activating oxysterols regulate placental 11β-HSD2 using primary human trophoblast cells as an *in vitro* model system. We demonstrated that treatment of trophoblast cells for 24 h with 22(R)-hydroxycholesterol (22(R)-HC) resulted in a concentration dependent decrease in 11β-HSD2 activity, such that a 70% reduction was observed at 10 μM. The inhibitory effect of oxysterols on 11β-HSD2 activity was confirmed with the use of another LXR-activating oxysterol, 25-hydroxycholesterol (25-HC). However, the synthetic LXR agonist T0901317 had no effect, suggesting that the effect of oxysterols on 11β-HSD2 in human trophoblast cells is mediated through a novel mechanism independent of LXR. Furthermore, we showed that although 22(R)-HC did not alter the level of 11β-HSD2 mRNA, it caused a significant decrease in 11β-HSD2 protein levels, indicating that 22(R)-HC down-regulated 11β-HSD2 at a post-transcriptional level. In addition, the mechanism underlying this reduction in 11β-HSD2 protein is unlikely to involve proteasome-mediated protein degradation, since treatment of trophoblast cells with the proteasome inhibitor MG-132 did not abrogate the inhibitory effect of 22(R)-HC on 11β-HSD2 activity. Taken together, these findings suggest that LXR-activating oxysterols suppress 11β-HSD2 in human trophoblast cells through a post-transcriptional mechanism independent of LXR and proteasome-mediated protein degradation. Given that high concentrations of oxysterols are present in the human placenta where their function is largely unknown, our present study also reveals 11β-HSD2 as an important target by which oxysterols may regulate human placental function, and consequently fetal development.

Keywords: Fetal development, Placental function, Glucocorticoid actions and metabolism, LXR and oxysterols

[P2.76]

MATERNAL GLUCOCORTICOID EXPOSURE ALTERS PLACENTAL GENE EXPRESSION DIFFERENTLY, DEPENDING ON THE SEX OF THE FETUS

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Introduction: Differences between the sexes have been found with respect to morbidity and mortality during pregnancy, with male fetuses being at higher risk of negative outcomes associated with placental insufficiency, pre-eclampsia, and birth asphyxia. Here we investigated the effects of maternal stress on the placenta, produced by administering dexamethasone (DEX) at the time the placental labyrinth develops in the spiny mouse.

Methods: Dams were exposed to DEX (125μg/kg) or saline (75μl) for 60h via osmotic pump from gestation d20 (term is d39). Placentas were collected immediately after treatment (d23) and processed for histology or qPCR. Relative sizes of labyrinth and junctional zone (JZ) were determined and expression of *Bax*, *Bcl2*, *Vegfa*, *Vegfr2*, *Gcm1*, and *Map2k1* genes was quantified. Differences between groups was analysed by 2-way ANOVA for treatment (PTREAT), and sex (PSEX).

Results: DEX reduced the proportion of labyrinth to JZ in the placenta (PTREAT$<$0.05). DEX caused an increase in *Map2k1*, which was greater in females (PSEX$<$0.0001, PTREAT$<$0.001, PINT$<$0.001). *Vegfa* expression was higher in DEX-exposed placentas, but was higher in females irrespective of treatment (PSEX$<$0.01, PTREAT$<$0.05, PINT=NS). The DEX treatment increased the *Bcl2:Bax* ratio, but significantly more so in female placentas (PSEX$<$0.05, PTREAT$<$.0.0001, PINT$<$0.01).

Discussion: Maternal DEX exposure for 60h altered gene expression and structural development of the placenta. Sexually dimorphic responses were observed in genes important in the expansion of the labyrinth region (*Map2k1*) and the ratio of pro- and anti-apoptosis (*Bcl2:Bax*). Thus, the placenta at mid-gestation is sensitive to a relatively brief exposure to high maternal GC, such as may occur during maternal stress. The consequences of these gene changes on placental function remain to be elucidated.

Keywords: Spiny mouse, Dexamethasone, Labyrinth Development

[P2.77]

ADENOSINE TRANSPORT IS MEDIATED BY DIFFERENT NUCLEOSIDE TRANSPORTERS IN NON-DIFFERENTIATED AND DIFFERENTIATED HUMAN ENDOTHELIAL PROGENITOR CELLS

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Extracellular adenosine removal is via human equilibrative nucleoside transporters 1 (hENT1) and hENT2 in the endothelium, thus regulating adenosine-induced revascularization and angiogenesis. Since human endothelial progenitor cells (hEPCs) promote revascularization, we hypothesize differential expression of nucleoside transporters in hEPCs.

Methods: hEPCs from peripheral venous blood from healthy adults were cultured 3 (hEPC-3d) or 14 (hEPC-14d) days. RT-PCR for prominin 1, CD34, octamer-4, kinase insert domain receptor, oxidized low-density lipoprotein (lectin-like) receptor 1 and tyrosine endothelial kinase was used to evaluate phenotypic differentiation. Flow cytometry was used to estimate $CD34^+/KDR^-$ (non-differentiated), $CD34^-/KDR^+$ (differentiated) or $CD34^+/KDR^+$ (mixed) cell populations. Adenosine transport (0-65 μM, [^{3}H]adenosine, 4 μCi/ml, 20 s, 22°C) was measured in absence or presence of sodium, *S*-(4-nitrobenzyl)-6-thioinosine (NBTI, 1-10 μM, hENTs inhibitor), inosine, hypoxanthine or guanine (0.1-5 mM). hENTs protein abundance was determined by western blot and hENTs, hCNT1, hCNT2 and hCNT3 mRNA expression by real time RT-PCR. Hill coefficient for Na+ dependency of adenosine transport was determined.

Results: hEPC-3d $CD34^+/KDR^-$ population was higher (~7-fold) than hEPC-14d cells, which were predominantly $CD34^-/KDR^+$. hEPC-3d cells exhibit hENT1-like adenosine transport (NBTI sensitive, Na^+-independent), which is absent in hEPC-14d cells. hEPC-14d cells exhibit two transport components: *component 1* (NBTI insensitive, Na^+-independent) and *component 2* (NBTI insensitive, Na^+-dependent, Hill coefficient ~1.8), the latter resembling CNT3-like transport. hEPC-3d cells express hENT1 protein and mRNA, which is reduced (~90%) in hEPC-14d cells, but instead only hCNT3 mRNA is expressed in this cell type. hENT2, hCNT1 and hCNT2 were undetectable in hEPCs.

Conclusions: hEPCs exhibit a differential expression of hENT1 and hCNT3 functional nucleoside transporters related with its differentiation stage.

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Keywords: Adenosine Transport, Adenosine Transport

[P2.78]

DELINEATING THE FIRST STAGES OF MESODERM COMMITMENT DURING DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

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Our understanding of how mesodermal tissue is formed, has been limited by the absence of specific and reliable markers of early mesoderm commitment. We report that mesoderm commitment from human embryonic stem cells (hESC) is initiated by Epithelial to Mesenchymal transition (EMT) as shown by gene expression profiling and by reciprocal changes in expression of the cell surface proteins, EpCAM/CD326 and NCAM/CD56. Molecular and functional assays reveal that CD326negCD56+ cells, generated from hESC in the presence of activin A, BMP4, VEGF and FGF2, represent a novel, multi-potent mesoderm-committed progenitor population. CD326negCD56+ progenitors are unique in their ability to generate all mesodermal lineages including hematopoietic, endothelial, mesenchymal (bone, cartilage, fat, fibroblast), smooth, skeletal muscle and cardiomyocytes, while lacking the pluripotency of hESC. CD326negCD56+ cells are the precursors of previously reported, more lineage-restricted mesodermal progenitors. These findings present a novel approach to study how germ layer specification is regulated, and offer a unique target for modulation of stromal ontogenesis in humans.

Keywords: human embryonic stem cells, mesoderm, endothelium, mesenchymal cells

[P2.79]

ANTI-SINEPTHELIOCHORIAL PLACENTA ANTIBODIES CONSTRUCTED BY PHAGE DISPLAY TECHNOLOGY

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After the first description of construction of a antibodies gene sequences library by phage display by Smith at 1985, a lot of different protocols, and targets, are describe on the literature. In placenta, the first description are made by Schmitz and co-workers (2000) presenting the first anti-human placenta gene library construction. In this work, we present a anti-sinepthelichorial placenta scFv antibody gene libraries (goat, sheep and cow) constructed by this technology. White lineage chickens was three times immunized with placentomes. Fifteen days following immunization, the animals were euthanized and total RNA was isolated from spleen. First-strand cDNA was synthesized and, using specific primers, variable regions on light (V_L) and heavy (V_H) antibodies chains were constructed by polimerase chain reaction . As for V_L and V_H, the scFv antibody fragment were constructed by PCRs, connecting the variable parts of the immunoglobulin via a short linker peptide. The scFv sequences were inserted in the Ph.D.-C7C™ Phage Display Peptide Library Kit. The insert-vector were replicated in ER2738 competent bacterias. Now, researches to identification of epitopes and markers of trophoblast cells are performing like related in others publications.

Keywords: Placenta, Phage Display, Sinepthelichorial, scFv

[P2.80]
ANTI-PLACENTA ANTIBODIES CONSTRUCTED BY PHAGE DISPLAY TECHNOLOGY

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After the first description of construction of a antibodies gene sequences library by phage display by Smith at 1985, a lot of different protocols, and targets, are describe on the literature. In placenta, the first description are made by Schmitz and co-workers (2000) presenting the first anti-human placenta gene library construction. In this work, we present a anti-placenta scFv antibody gene libraries (mouse ectoplacental cone, goat, sheep and cow) constructed by this technology. White Leghorn chickens was immunized with ectoplacental cone tissue and placentomes. Fifteen days following immunization, the animals were euthanized and total RNA was isolated from spleen. First-strand cDNA was synthesized and, using specific primers, variable regions on light (V_L) and heavy (V_H) antibodies chains were constructed by polimerase chain reaction . As for V_L and V_H, the scFv antibody fragment were constructed by PCRs, connecting the variable parts of the immunoglobulin via a short linker peptide. The scFv sequences were inserted in the Ph.D.-C7C™ Phage Display Peptide Library Kit. The insert-vector were replicated in ER2738 competent bacterias. Now, researches to identification of epitopes and markers of trophoblast cells are performing like related in others publications.

[P2.81]
UBIQUITIN-SPECIFIC-PROCESSING PROTEASE 22 (USP22) GENE IS EXPRESSED IN TROPHOBLAST AND CHORIOCARCINOMA CELLS

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Introduction: Proteases perform essential functions in all living organisms. They act as processing enzymes that perform highly selective, limited and efficient cleavage of specific substrates, which initiates irreversible decisions at the post-translational level that influence many biological processes. In an attempt to find proteases that are expressed in different stages of trophoblast development we found USP22 to be highly expressed in trophoblast and chorioncarcinoma cells. USP22 is a ubiquitin hydrolase that is present within the human SAGA transcriptional co-activator complex. Here we show the trophoblast specific expression of this enzyme.
Methods: The expression of USP22 in trophoblasts and chorion carcinomas was determined using RT PCR. The amplified fragment was isolated, cloned and verified by sequencing. This fragment was used for in situ hybridization on placenta sections.
Results: USP22 mRNA could be amplified in both first trimester and term placental tissue as well as in isolated trophoblasts and chorion carcinoma cell lines BeWo, JEG3 and JAR. Using in situ hybridization with a specific probe USP22 could be localized on trophoblasts in first trimester and term placental tissue, with predominance on vilous and extravilous cytrotrophoblasts. No expression could be detected in invasive trophoblasts in the decidua.
Discussion: USP22 is a histone deubiquitinating component of the transcription regulatory histone acetylation complex SAGA. It catalyzes the deubiquitination of both histones H2A and H2B, thereby acting as a coactivator. It is recruited to specific gene promoters by activators such as MYC, where it is required for transcription. USP 22 is also required for nuclear receptor-mediated transactivation and cell cycle progression. USP22 is also discussed to be a potential cancer stem cell marker. We suppose that USP22 and the SAGA complex have an important role in trophoblast development.
Keywords: trophoblast, protease, stemm cell marker, in situ hybridization

[P2.82]
ANTENATAL DEXAMETHASONE TREATMENT LEADS TO CHANGES IN MIF GENE EXPRESSION IN THE DEVELOPING PLACENTA

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Macrophage migration inhibitory factor (MIF) was originally identified for its capacity to inhibit the random migration of macrophages *in vitro*. This cytokine plays special roles as pro-inflammatory factor affecting mainly functions of macrophages and lymphocytes and as the first physiologic counter-regulator of the glucocortocoid effects on the immune response. In spite of being a pro-inflammatory cytokine recent evidences also suggest a critical role for MIF during human and mice implantation and early embryonic development. On the other hand, exogenous glucocorticoids exert many actions that could impact negatively on key aspects of early pregnancy like inhibition of cytokine-prostaglandin signaling, restriction of trophoblast invasion, induction of apoptosis and inhibition of embryonic and placental growth. Therefore, the overall goal of this study has been to determine whether antenatal dexamethasone treatment leads to changes in Mif gene expression in a murine early placenta. For this, a total of twelve pregnant females mice on 10.5 gd were divided in four groups of tree animals each and were injected with intra peritoneal dexamethasone at a dose of 0.25 mg/Kg, 0.5 mg/Kg, 1.0 mg/Kg or received equivalent amount of saline to serve as controls. Thirty minutes after injection animals were sacrificed, placentas were separated from maternal tissues and the samples analyzed by RT-PCR and qRT-PCR. Our preliminary results show that placentas from animals injected with dexamethasone have lower mRNA expression when compared with control samples in an inverse correlation with the used dose (0.25 mg/Kg, $p=0.0024$; 0.5 mg/Kg, $p=0.0022$). This inverse relationship might be indicating that placental Mif does not play roles as a glucocorticoid counter-regulator at maternal-fetal interface, as occurs in other organs.
Financial support: FAPESP, CAPES and CNPq
Keywords: MIF, Cytokine, Trophoblast, Placenta

[P2.83]

EXPRESSION OF FOXO4 IN HUMAN PLACENTA AND FETAL MEMBRANES: EFFECT OF HUMAN LABOUR AT TERM

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Introduction: We have previously identified Forkhead box O1 (FoxO1) proteins in human gestational tissues, and demonstrated a casual link between FoxO1 and rupture of fetal membranes. There is, however, no data on FoxO4 in human intrauterine tissues, or their expression during human term labour. The aim of this study was to characterises the localisation and expression of FoxO4 in human placenta and fetal membranes before and after term spontaneous labour onset.
Methods and Results: The cellular localisation of FoxO4 protein in human placenta and fetal membranes (combined amnion and choriodecidua) was performed by immunohistochemistry. FoxO4 staining in the placenta was moderate and was confined to the syncytiotrophoblast layer. Cytoplasmic FoxO4 staining was also detected in the trophoblast layer of the chorion; more so in the basal layer than the superficial layer. There was no FoxO4 staining within the villous structure, placental endothelium, amnion epithelium and cells within the spongy layer of the fetal membranes. Decidua exhibited weak FoxO4 staining that was both cytoplasmic and nuclear. To determine the effect of human labour on FoxO4 expression, qRT-PCR, Western blotting and immunohistochemistry was performed on placenta, amnion and chorion before and after term labour. Placenta and fetal membranes were obtained at Caesarean section before the onset of labour (pre-labour) and after spontaneous labour and membrane rupture (post-labour). Amnion and chorion were obtained from the SCS in the pre-labour samples and from along the rupture line in the post-labour samples. FoxO4 mRNA and protein expression were significantly lower after labour in placenta and fetal membranes.
Discussion: In summary, human term labour is characterised by decreased FoxO4 mRNA and/or protein expression in placenta and fetal membranes. While the precise role and contribution of FoxO4 in the process of human fetal membrane rupture are unknown, it has been implicated in apoptosis and/or cell cycle regulation.
Keywords: Placenta, Fetal membranes, Human labour, FoxO4

[P2.84]

SILENCING OF CASPASE 8 IN BEWO SUPPRESSES BETA-HCG INDUCTION

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In the human placenta, transition from mononuclear villous cytotrophoblast into multinuclear syncytiotrophoblast is characterized by the induction of hCG and intercellular fusion. The role of apoptotic-related proteins, particularly caspase 8, in the differentiation process has elicited intense investigation. We have reported that a polycaspase inhibitor did not affect syncytialization or hCG production in differentiating BeWo, a choriocarcinoma model for villous cytotrophoblast differentiation. To investigate the role of procaspase 8 we produced stable transfectants that expressed siRNA targeted to procaspase 8; in clone C8-4 procaspase 8 protein and mRNA were diminished (94.8% and 85%, respectively). A control clone was stably transfected with vector alone (vector). Forskolin-induced fusion of clone C8-4 was delayed for 24 hr, but not suppressed. By 48 hr of treatment, fusion (mean % of nuclei in syncytial cells $\pm$ SD) of the vector control cells (47.2 $\pm$ 9.5) exceeded that in clone C8-4 (27.2 $\pm$ 7.8; $P = 0.01$). By 72 hr the vector control had reached the maximum level of fusion (87.5 $\pm$ 5.0), but C8-4 was significantly less (53.2 $\pm$ 15.4; $P < 0.05$). The C8-4 line reached maximum fusion at 96 hr of forskolin treatment (vector control: 87.1 $\pm$ 1.8; C8-4: 85.4 $\pm$ 3.5; $P =$ NS). Expression of hCG was more severely affected; in forskolin-treated C8-4 the level of beta-hCG protein was significantly depressed at all time points (depressed 72% at 24 hr, $P = 0.02$; 62% at 48 hr $P = 0.003$; 70% at 72 hr $P = 0.0005$; 55% at 96 hr, $P = 0.005$) and never reached the levels measured in the vector control. The effects on beta-hCG mRNA expression were less pronounced; mRNA was suppressed by 52% at 72 hr ($P < 0.01$) and by 38% at 96 hr ($P < 0.02$). Thus, the proform of caspase 8 may be an essential participant in the differentiation-related induction of hCG, but plays a minor role in syncytialization.
Keywords: BeWo, intercellular fusion, hCG, caspase 8

Poster session 3 (P3.1 - P3.83)

[P3.1]

HOMEOBOX GENES ARE DIFFERENTIALLY EXPRESSED IN PRIMARY VILLOUS AND EXTRAVILLOUS TROPHOBLAST CELL LINEAGES DURING EARLY PREGNANCY

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During human placental development trophoblast cells differentiate along either the villous cytotrophoblast (VCT) lineage to form the syncytiotrophoblast (ST) or the invasive extravillous cytotrophoblast (EVCT) lineage (1). Abnormalities in early differentiation processes are characteristic of poor placentation, which is associated with fetal growth restriction (FGR) and pre-eclampsia (PE), the major clinical complications of human pregnancy (2). A large family of homeobox gene transcription factors controls "cell-fate decisions" during development (3), but the expression profile and role of homeobox genes in the human trophoblast cell lineages is not well understood. The aim of the study was to determine homeobox gene expression in primary cultures of mononuclear VCT (2h) and EVCT (2h) obtained from first trimester human chorionic villi of 8-12 weeks of gestation and *in vitro* differentiated ST (72h) and invasive EVCT (48h), respectively. The isolation and characterization of freshly isolated VCT, EVCT and *in vitro* differentiated ST and invasive EVCT were performed as described previously (1,4). The homeobox gene mRNA profile was performed using PCR arrays in a pooled sample of VCT and EVCT (n=6 in each group) and further validated by real-time PCR. Homeobox gene expression studies revealed *MSX2* mRNA levels were the highest in VCT (2h) but undetectable in EVCT (2h). Further comparisons of homeobox gene expression in *in vitro* differentiated ST to invasive EVCT showed marked increase in *MSX2, DLX3, DLX4* and *MEIS1* mRNA levels in ST, which are regulators of cellular differentiation in many studies. Homeobox genes *HLX* and *HHEX*, which are implicated in regulating cellular proliferation showed decreased mRNA levels in ST compared to invasive EVCT. Our results demonstrated several known placental and novel homeobox genes are differentially expressed in trophoblast cell lineages. Functional studies of these candidate genes will provide a better understanding of the molecular mechanisms of early placental development.

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Keywords: trophoblast differentiation, villous and extravillous trophoblast lineages, gene expression, placental development

[P3.2]

MICRORNAS ARE DIFFERENTIALLY EXPRESSED IN FIRST TRIMESTER HUMAN PLACENTA

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Placental functional development is characterised by dynamic and co-ordinated changes in expression of genes that drive invasion, differentiation and growth. These changes may arise in part from altered expression of microRNAs (miRNAs) via their regulatory networks. MiRNAs are short, single-stranded, non-coding RNAs involved in the post-transcriptional repression of gene expression. MiRNAs bind to complementary sites in the 3'UTR of target mRNAs to repress or silence translation. MiRNAs have been detected in the mammalian placenta, but their patterns of expression throughout pregnancy have not been systematically characterized.

Using microarrays, miRNA gene expression was compared between two stages (6-8 weeks and 10-12 weeks) in early gestation, in chorionic villi of human placentas. Putative and validated targets of differentially expressed miRNAs were extracted from freely accessible databases, miRBase [1], PicTar [2], TargetScan [3] and miRecords [4].

15 miRNAs were differentially expressed between these gestational ages ($p<0.05$). 11 of these miRNAs were upregulated in 10-12 week villi and 4 were downregulated. Many of the differentially expressed miRNAs are members of the same polycistronic clusters, suggesting that these miRNAs may be co-expressed. Shared targets of differentially expressed miRNAs from the same clusters were assessed using Ingenuity Pathways Analysis, to search for significantly represented molecular networks.

All downregulated miRNAs at 10-12 weeks shared 35 putative targets and were in 1 of 2 clusters, on chromosome 13 or X. Previously validated targets include PTEN [5], Notch1 [6], VEGFA [7], CDKN2A [8] and DHFR [9]. Six of the upregulated miRNAs at 10-12 weeks are members of 3 clusters on chromosome 19, 9 and X. Networks targeted by these cluster members include PTEN, HIF1α and IL-12 signalling. Together all of these processes are active and important in early placentation and their predicted targeting by differentially expressed miRNAs is consistent with an important role in placental development.

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Keywords: microRNA, microarray, first trimester, chorionic villi

[P3.3]

EFFECTS OF PLACENTAL GROWTH HORMONE (PGH) ON FIRST TRIMESTER AND TERM PLACENTAL CELL TURNOVER

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Introduction: Defective placental development has a major impact on fetal growth and is associated with fetal growth restriction (FGR). The placenta produces a variant of pituitary growth hormone, placental growth hormone (PGH), which acts via the growth hormone receptor (GH-R). GH-R mRNA is expressed by the placenta suggesting PGH may have autocrine/paracrine effects upon placental development. In pregnancies complicated by FGR, levels of PGH are decreased compared to normal pregnancies; it is not known whether this contributes to abnormal placental development. We hypothesize that PGH stimulates placental growth and development, acting through GH-R.
Method: First trimester (n=7) and term (n =5) placentas were collected. GH-R was localised by immunohistochemistry. Explants from both gestations were cultured in the presence of PGH (0, 10, 100ng/ml). The proliferative and apoptotic response of cytotrophoblast and stromal cells to PGH was examined by immunohistochemistry for Ki67 and M30 respectively. Lactate dehydrogenase (LDH) release and human chorionic gonadotropin (hCG) secretion were used to assess viability.
Results: GH-R was strongly localised to the syncytiotrophoblast microvillous membrane, cytotrophoblasts and a population of stromal cells in both first trimester and term placenta. PGH had no effect on first trimester cytotrophoblast proliferation; however, stromal proliferation was significantly elevated by 10ng/ml PGH ($p \leq 0.05$). At term, PGH had no effect on cytotrophoblast or stromal proliferation. There was a trend towards decreased cytotrophoblast and syncytiotrophoblast apoptosis with PGH at 10ng/ml at both gestations ($p \leq 0.06$). PGH had no effect on LDH or hCG levels in culture medium.
Conclusion: These data confirm that the placenta is a target tissue for PGH. The stimulatory effect of PGH on stromal cell proliferation suggests PGH may act indirectly to promote placental growth and development via the stromal compartment. More studies are required to investigate the mechanisms of PGH actions on placental development to elucidate its involvement in FGR.
Keywords: Placental growth hormone, Proliferation, Apoptosis, Fetal growth restriction

[P3.4]

GLUCOCORTICOID TARGET GENES IN FIRST TRIMESTER HUMAN PLACENTA

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Introduction: Excessive exposure to glucocorticoids (GC) is associated with reduced fetal growth. Animal studies implicate the placenta in GC-induced FGR. We and others have shown that GCs alter placental cell turnover (reduced proliferation, increased apoptosis), angiogenesis, vascular reactivity and amino acid transport. The molecular mechanisms underlying GC actions have not been delineated. The aim of this study was to identify GC target genes in the placenta.
Methods: First trimester placentas (8-12 weeks) were collected following elective surgical pregnancy termination. Placental explants were cultured for 48 hours in the presence or absence of 1μM dexamethasone. DNA was extracted and fetal sex determined by PCR amplification of amelogenin gene. RNA from placentas where the fetus was female (n=5) was extracted, pooled and subjected to Affymetrix GeneChip Human Exon 1.0 ST arrays in triplicate. Candidate genes were validated by real time PCR.
Results: 44 genes were upregulated (2 to 5.6 fold) and 58 genes were downregulated (-2 to -8.7 fold) by dexamethasone treatment. These included inflammatory mediators, proteases, extracellular matrix, cell adhesion molecules, angiogenic and growth factors, and transporter molecules. Differentially expressed genes were selected for further studies based on their functional properties. Downregulated candidate genes: interleukin(IL)-1, IL-6, CCL2; fatty acid binding protein 3, Na^+/K^+ATPase β polypeptide 1/3, slc7A11 cationic amino acid transporter, bradykinin receptor B1, vascular endothelial growth factor C, heparin binding epidemeral growth factor, transforming growth factorβ-3, sphingosine-1-phosphate phosphatase 2. Upregulated candidate genes: prokineticin 1, calcium-activated potassium channel (BKCa), TIMP4, slc22A3 monoamine transporter, GCM-1, angiotensin II receptor. Validation PCR studies on individual samples are ongoing.
Discussion: These studies have identified GC-target genes in first trimester placenta and provide putative candidates for mediating previously identified GC effects on cell turnover, angiogenesis, vasoconstriction and transport. Future studies include investigation into GC target genes across gestation and will explore any influence of fetal sex.
Keywords: Gluocorticoids, Placenta, Microarray

[P3.5]

COMPARATIVE EXPRESSION OF HCG IN VILLOUS TROPHOBLAST FROM EARLY AND LATE FIRST TRIMESTER OF PREGNANCY

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Context: The human chorionic gonadotropin hormone (hCG) is largely produced by the syncytiotrophoblast, which represents the endocrine tissue of the placenta that baths in maternal blood in the intervillous space. hCG is detected in the maternal blood for the first week of pregnancy, with a peak level at 12 weeks of gestation (GW). During the first trimester the oxygen concentration in the intervillous space changes from about 1-2% (prior to 10 GW) to approximately 6-8% (after 12 GW) due to development of blood flow to the placenta. The association of hCG release and these early gestation events are poorly described.
Objective: To compare *in situ* hCG localization, expression, *ex vivo* secretion and levels of the major known transcription factors involved in hCG regulation in the villous trophoblast prior to and after oxygenation of the intervillous space in early pregnancy.
Design: First trimester chorionic villi from 8-9 and 12-13 GW were used for: i) immunodetection of hCG *in situ*; ii) isolation of fresh within 2h and primary cultures of mononucleated villous cytotrophoblasts (VCT) differentiated in syncytiotrophoblast (ST) *in vitro* from 24 to 72 h. Cell supernatants were collected at 24h and 72h and hCG secretion quantified using immunoassay. Total cell extracts were prepared for Q-PCR analysis and/or nuclear and cytoplasmic extracts prepared for immunoblotting.
Results: hCG was immunodetected in both VCT and ST at 8-9 GW and predominatly in ST at 12-13 GW. hCG secretion was higher in 24h-VCT cultures from 8-9 (1167 U/L $\pm$ 662) than from 12-13 (264 U/L $\pm$ 125). During differentiation into ST, hCG increased 25 fold in primary cultures from late placentas, whereas in cultures from the early ones only a 6-fold increase was observed. hCG immunoblotting of the cytoplasmic fractions confirmed the secretion results. On the same immunoblots, SOD Cu/Zn, an oxygen inducible gene was increased in the late placentas confirming the rise of oxygen in the intervillous space. Finally, we analyzed by immunoblotting the levels of AP2alpha, AP2beta, AP2gamma and Sp1, Sp3, in the protein nuclear fractions. In the ST, AP2alpha strongly decreased in all GW analyzed compared to VCT. For AP2beta and Sp3, the same phenomenon was observed but the expression pattern was less pronounced. In contrast, AP2gamma and Sp1 remained unchanged. These data suggest a possible association of these transcription factor expression patterns with the observed change in hCG in early pregnancy.
Keywords: hCG, trophoblast differentiation, oxygene, transfection factors

[P3.6]

LOW-DOSE ASPIRIN TREATMENT INCREASED THE EXPRESSION OF UTERUS ENDOMETRIAL RECEPTIVELY PROTEINS, LIF OR INTEGRIN BETA 3 IN MICE

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Introduction: Embryo implantation is the processes of developing blastocyst adhere and embed into the receptive endometrium. Changes in the expression of molecules on the cell surface have been seen in the conversion of the endometrial surface from a non-receptive to a receptivity state. Cell adhesion molecules like integrins, or Leukemia inhibitory factor (LIF) that affects cell growth and development have been identified to play an important role during pre-implantation period. Studies have suggested that low-dose aspirin (<100 mg/d) treatment can significantly improve implantation rate and ovarian responsiveness, uterine and ovarian blood flow velocity, and pregnant rates in IVF patients. However the mechanism of low-dose aspirin treatment on increasing pregnancy rate is unclear. Therefore the aim of this study was to investigate whether the low-dose aspirin treatment could alter the endometrial receptively proteins LIF or integrin beta3 expression in mice.
Methods: Female mice were treated with 0.9mg/ml aspirin daily for 15 days and then were mated with male mice. When pregnancy was confirmed, on the gestation day 4, the uterine samples were collected and the level of integrin beta 3 and LIF were determined using immunohistochemistry and RT-PCR.
Results: Immunohistochemistry demonstrated LIF or integrin beta3 expression was substantially increased on uterine endometrium of pregnant mice that were pretreated with low dose of aspirin compared untreated pregnancy mice. In addition, the mRNA level of LIF or integrin β3 on endometrium of pretreated pregnant mice with low dose of aspirin was significantly increased compared to control mice.
Discussion: These data suggested that uterine endometrial receptively protein LIF or integrin b3 expression, which are important for embryo implantation on uterine endometrium of pregnancy mice was increased with low dose aspirin treatment. Increased levels of LIF or integrin beta 3 may lead to higher pregnancy rate.
Keywords: Embryo implantation, integrin beta3, Leukemia inhibitory factor

[P3.7]
CHARACTERIZATION OF CULTURED MOUSE MESOMETRIAL DECIDUAL CELLS

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Two major subpopulations of endometrial stromal cells are present during early implantation in the mouse and rat: the antimesometrial and mesometrial decidual cells, and the mesometrial fibroblasts. Besides, other cell types such as uterine natural killer (uNK) cells and macrophages could be present. The aim of this study was to characterize the mouse mesometrial decidua by immunocytochemistry and histochemistry assays *in vitro*. Female Swiss mice were anesthesized and sacrificed on the 7.5th day of pregnancy at 09h00. Implantation sites have been dissected, and the embryo and anti-mesometrial and mesometrial deciduas have been mechanically separated. Only the mesometrial decidua compartment has been processed for cell culture. After collagenase digestion, approximately, 1-3 x 10^5 cells have been plated onto coverslip surface, cultured during a 72 hour period at 37°C and CO_2 5%. Then, cells have been fixed in either methanol or formalin 10% and immunocytochemistry assay for detection of desmin (decidual cell marker), alpha-2 macroglobulin (mesometrial decidual cell marker), factor VIII (endothelial cell marker) and F-4/80 antigen (macrophage marker), and histochemistry assay for *Dolichos biflorus* agglutinin (DBA) lectin (uNK cell marker) have been carried out. Cultured mesometrial decidual cells have shown positive staining for desmin and alpha-2 macroglobulin, and negative negative staining for factor VIII, F-4/80 antigen and DBA lectin. These preliminary data support the potential of the cultured mesometrial stromal cells grown on glass, which may be particularly useful in the study of mesometrial stroma interactions among different cell types such as uterine natural killer cells, macrophages, endothelial cells and trophoblast cells.

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Keywords: mesometrial decidual cell, cell culture, placenta, mouse

[P3.8]
AMNION REEPITHELIZES THIRD-DEGREE BURNS

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Because of the high statistics of burn victims in the world the experimental production of reproducible cutaneous burns in laboratory animals is essential for any investigation. In this study we demonstrated the potencial of the amnion in burns. Amnion is formed by a simple epithelium, a basement membrane and an avascular mesenchyme. It present antiadhesive effects, bacteriostatic properties, wound protection, pain reduction, epithelialization effects, lack of immunogenicity and niche of stem cell. By these features, our study tested the effectiveness of macro and microscopic response to dermal and epidermal repair in the regenerating/healing process of 3rd degree burn in rats treated with autologous amnion. The burns were caused with a plate of brass ($5cm^2$) in 19 anesthetized rats, followed by ethical standards. The lesions were evaluated daily and at 7, 14 and 21 days the injured, tissues were processed by routine techniques for light microscopy. Animals treated with amnion in the early days showed initial reepithelialization microscopic, but no signs of inflammation around the injured area. In 14 days stand out in borders reepithelization and collagen fibers arranged microscopically but not signs of inflammation were detect. After 21 days the lesions had begun tissue repairing, decreasing the initial size of the injury and reepithelialization of the edges in 80% of the burn length, but with increased thickness compared to the normal epithelium. Lesions in the control group showed only the beginning of tissue repair at 21 days, but with granulation tissue thin, dry aspect, points of bleeding and failure of collagen to fill the entire length of the lesion. We concluded that amnion as an alternative treatment for burns injuries from the early stages of healing may contribute to the efficiency and effectiveness in the treatment of burns, and thereby contribute to greater quality of life of burn patients.

Keywords: amnion, burns, reepithelialization, wounds

[P3.9]

GLYCOPROTEIN SECRETIONS FROM ENDOMETRIAL GLANDS ALTER IN PREGNANCY WITH THE LOSS OF SPECIFIC TERMINAL SIALIC ACID RESIDUES

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Introduction: Histiotrophe is known to be an important feature of early human pregnancy, providing nutrients and growth factors to the developing embryo. Our aim was to examine the glycan composition of histiotrophe from first trimester decidua basalis and parietalis and to compare it with secretions present in endometrial glands in the late secretory phase of the menstrual cycle.
Methods: Twenty samples of decidua from pregnancies terminated between 8 and 11 weeks were processed into epoxy resin and sections stained with a panel of 22 lectins, together with six late-secretory phase endometrial biopsies. Specimens were analysed using a semi-quantitative ranking system and the density of lectin binding to the glandular secretions and the epithelium assessed.
Results: Histiotroph contained terminal ß-galactose, α-N-acetyl galactosamine and N-acetyl lactosamine residues bound by *Arachis hypogaea* (AHA), *Glycine max* (soybean, SBA), *Helix pomatia* (HPA) and *Erythrina crystagalli* (ECA) agglutinins, whereas in late secretory phase endometrial gland secretions terminal non-reducing sialic acid was present, inhibiting lectin binding. This suggests that, in histiotrophe, there is loss of the capping sialic acid residues present in the non-pregnant state. No differences in glycosylation were apparent between decidua basalis and parietalis. Despite these changes, sialic acid could still be detected.
Conclusions: There is suppression of terminal sialylation in specific oligosaccharide chains in early pregnancy, suggesting that endometrial glycan biosynthesis is modified in response to placental signals. These changes may facilitate absorption of histiotrophe by the trophoblast and enhance availability of substrates for degradation
Keywords: Histiotrophe, Endometrium, Decidua, Glycosylation

[P3.10]

VILLOUS STRUCTURAL STUDY BASED ON MULTILEVEL REGISTRATION

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Goal: In this study we analyze various characteristics of a placental tissue using a sequence of digitized hematoxylin and eosin (H&E) stained histology slides. In particular, we study variation in tissue and blood density along a sequence of placental slices. We also extract a villous tree structure from this image sequence.
Methods: To achieve this, registration of these images is a crucial step. This is a large-scale problem, as the size of 'each' histology slide could be as large as 500MB. We proceed with the registration sequentially i.e. we register the (n+1)st slide to the nth slide. To this end, we use 'multilevel affine registration'. In this approach, we scale down the images dyadically and then input the affine-transformation parameters, i.e. translation vector, angle of rotation and scale, to the next level of registration. This significantly reduces computing time. Figure 1 shows an unregistered image, which needs to be aligned, primarily through rotation. The result of the registration is depicted in figure 2. (The result of the entire sequential registration can be downloaded from the following URL: http://tinyurl.com/3xxfoas.)
After registration, we use a novel image analytic algorithm to segment tissue and blood. This technique consists in identifying the tissue and blood based on color segmentation.
Results: In figure 3 we observe that the distribution of the tissue along the sequence is not uniform, with more tissue density at center slices. We note that the blood distribution, shown in figure 4, varies in a close range. Finally, in figure 5 we show a 3D rendering of a skeletonized villous tree that was extracted from the registered images.
Conclusion: We use multilevel approach for registration of large histology images. This serves as the first important step in the analysis of tissue/blood distribution and villous tree extraction.

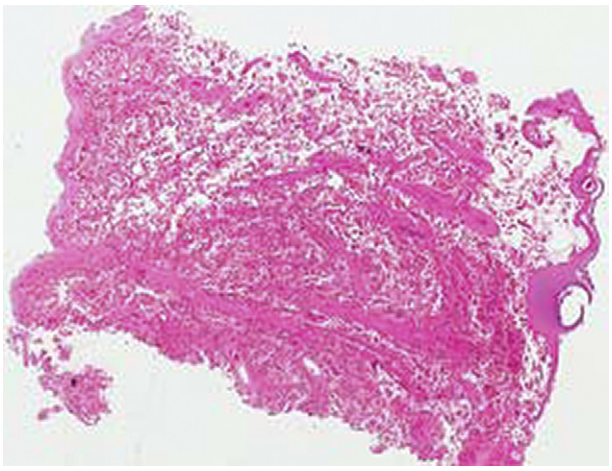

Figure 1. Unregistered image rotation

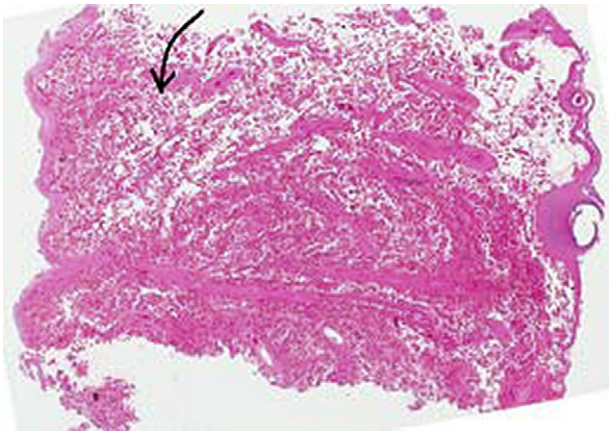

Figure 2. Registered image, rotated

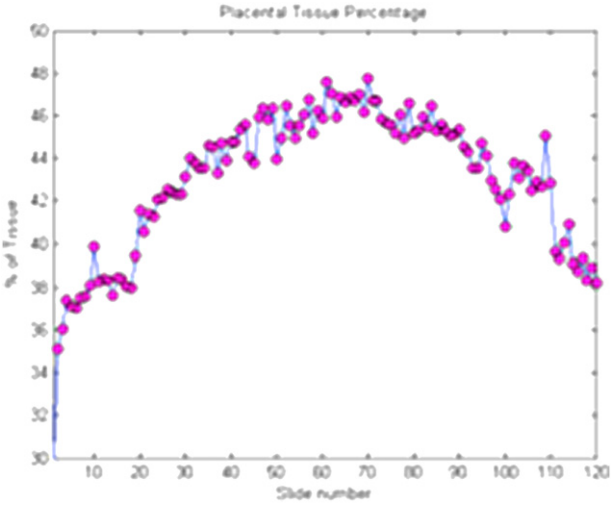

Figure 3. % placental tissue

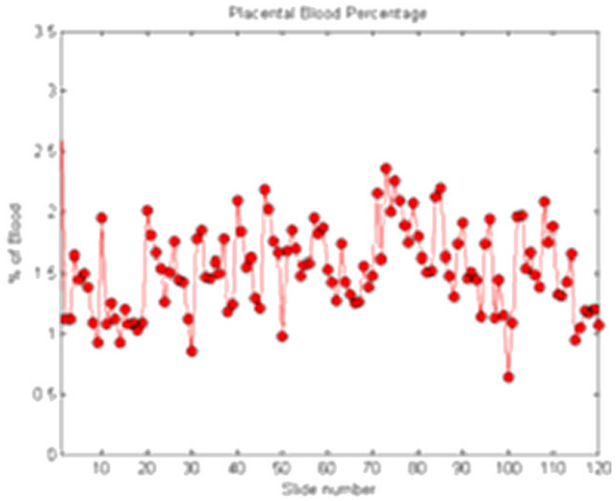

Figure 4. % blood (fetal or maternal)

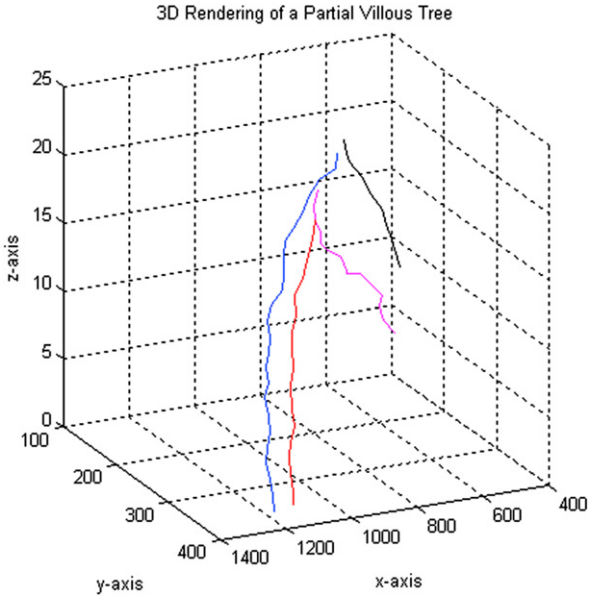

Figure 5. A 3-D rendering of a partial villous tree

Keywords: villous branching, image analysis, image registration

[P3.11]

ANALYSIS OF INFLAMMATION IN REGARDS TO DISTANCE OF NEUTROPHIL MIGRATION IN HISTOPATHOLOGY IMAGES: A MARKER OF INFECTION SEVERITY/DURATION?

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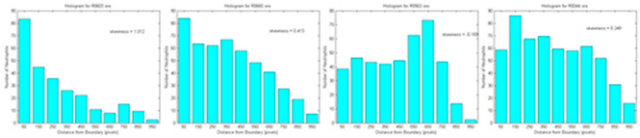
Background: Most pathologists currently rely on a visual examination of hematoxylin and eosin (H&E) stained placenta histology slide. Such qualitative examination fails to accurately determine the extent of an infection. Moreover, merely counting the number of the inflammatory polymorphonuclear leukocytes (neutrophils) does not give a clear understanding of the infection. For a relatively fresh infection, we expect more number of neutrophils near the tissue boundary. As the infection spreads, the neutrophils move and spread into the interior. In this paper we develop an algorithm that quantifies the extent of infection and determine the motion of the neutrophils relative to the tissue-boundary.

Methods: We studied randomly generated regions of interest (ROIs) in histology slides and detect the neutrophils using its morphological features. These inflammatory nuclei are labeled using an efficient run-length implementation. The next step is to measure the minimum distance to the boundary from each neutrophil. This distance also needs to be a 'perpendicular' distance from the boundary. This is essential due to occlusions in the ROI. To efficiently find the minimum and perpendicular distance of a neutrophil from the boundary we develop a novel algorithm, which consists in determining a closest segment on the boundary using only a few periodically sampled boundary points. At the same time, we estimate the angle made by the line joining the neutrophil to this segment. The advantage of using only the coarser sampling is that the angle estimates are smoother, since it does not see the finer oscillations along the boundary.

Results: Neutrophils, which we have previously shown to be reliably segmented using image analysis, can be measured in terms of the distance they have migrated from their site origin, and result expressed as histograms. From left to right, the distance histograms show increasing numbers of neutrophils that have migrated greater distance from their source of origin.



Conclusions: We anticipate such measures will correlation with amniotic fluid proteomics scores, and with infant and childhood outcomes related to presence and severity of intraamniotic infection.

Keywords: image analysis, neutrophils, migration, acute fluid infection

[P3.12]

STASIS-INDUCED FETAL VASCULOPATHY IN PLACENTAS FROM EXIT PROCEDURES

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The ex-utero intrapartum treatment (EXIT) procedure is used to secure fetal airway, cannulate for extracorporeal membrane oxygenation (ECMO), or resect a tumor s during partial delivery in a modification of by cesarean section. This is a retrospective study of placental pathology from EXIT procedures.

Placental reports and glass slides from 36 placentas from EXIT pregnancies (study group SG) and 36 placentas from pregnancies matched for gestational age from pregnancies without perinatal mortality and also delivered by cesarean sections were blindly reviewed. Indications for EXIT procedures were: 11 cervical teratomas, 9 diaphragmatic hernias, 4 pulmonary airway malformations, 4 micrognathias, 3 vascular malformations, 3 CHAOS, and 2 aortic stenoses. 22 clinical and 43 gross and histological placental features were compared using the analysis of variance or Yates Chi-square where appropriate.

The average gestational age in the SG and the CG was 34.9 weeks. Of placental features, statistically significant differences were found in massive dilatation of stem veins (80 v.47%) [p=0.007], focal segmental and/or total cannon ball-like fibrosis of isolated chorionic villi (9.7±7.9 vs 6.1±5.3 villi per placental section), as well as clusters of at least 3 avascular chorionic villi (33 v. 5%), in the SG and the CG respectively. There were no other statistically significant differences in maternal and placental parameters studied.

This is the first, to our knowledge, analysis of placentas from EXIT procedures. The fetal thrombotic vasculopathy indicates an underlying chronic and on-going stasis in fetal circulation due to the presence of conditions which were indications for the EXIT procedures. The possibility of stasis-related increased fetal blood coagulability should be considered in management of the fetuses and neonates.

Keywords: placenta, thrombotic vasculopathy, EXIT procedure, fibrosis

[P3.13]

PLACENTAL SIZE IS REDUCED AND PLACENTAL INFARCTION AND SYNCYTIAL KNOTS ARE INCREASED IN PREGNANCIES COMPLICATED BY DECREASED FETAL MOVEMENTS

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Introduction: Decreased fetal movements (DFM) are associated with increased risk of stillbirth and fetal growth restriction (FGR). DFM is thought to represent fetal compensation to conserve energy due to insufficient oxygen and nutrient transfer resulting from placental dysfunction. There have been no studies of placental morphology in cases of DFM.

Objective: To investigate whether placentas from pregnancies associated with DFM show macroscopic or microscopic evidence of altered placental development and/or damage.

Methods: Placental samples were collected from normal pregnancies (n=25) and DFM (n=18). Placentas were weighed, photographed and three tissue samples taken using an established systematic random sampling method. Placental dimensions and areas of infarction were measured from photographs using image analysis software. Samples were fixed, wax embedded and stained with haematoxylin and eosin for quantification of syncytial knots.

Results: Even in the absence of FGR, macroscopic measurements revealed a significant reduction in trimmed placental weight (DFM 471.2g vs. normal 536.4g; $p<0.05$) and area (DFM 214.1cm2 vs. normal 247.5cm2; $p<0.01$) in DFM compared to normal pregnancies. The proportion of the placenta showing macroscopic signs of infarction was increased in DFM (DFM 3.5% vs. normal 0.6%; $p<0.01$). DFM placentas had a higher density of syncytial knots compared to normal pregnancies (DFM 98.7 vs. normal 33.7 knots/mm2; $p<0.001$).

Conclusion: This study is the first to demonstrate that DFM is associated with abnormal placental morphology and parallels findings from placentas in FGR. Therefore, women presenting with DFM require further investigation to identify placental insufficiency, including ultrasound assessment of fetal growth and liquor volume. One novel avenue of exploration to detect placental dysfunction is placentally-derived hormones; this is currently underway in a pilot study of 300 women.

This study was funded by the Manchester Wellcome Trust Clinical Research Facility

Keywords: Decreased Fetal Movements, Infarction, Placental pathology

[P3.14]

PLACENTAL SUPERFICIAL IMPLANTATION: FROM HISTOLOGY TO CLINICAL ASPECTS

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Introduction: Human blastocyst implantation is a complex process with many changes occurring at the anatomical, hormonal and immunological level. Modification of the maternal spiral arteries represents part of these changes: loss of the muscular vascular wall, invaded by the trophoblasts and replaced by fibrinoid material, resulting in dilatation of the lumen of the vessel represents the final goal of the physiological vascular adaptation during implantation. When the physiological vascular changes do not occur a pathological placental condition defined "placental superficial implantation" (PSI) may be identified at the histological examination.
The aims of our study were to evaluate the frequency of PSI in singleton pregnancies and to examine the anatomical-clinical correlations between the histological lesion and pregnancy outcome.
Material and methods: 1534 consecutive singleton pregnancies who delivered at the San Paolo Hospital Medical School of Milan were retrospectively analyzed. An extensive histological and clinical investigation was performed.
Results: PSI was present in 84 [5.5%] cases. Multivariate analysis showed that maternal body mass index represents the major maternal pregestational factor that can influence implantation and the incidence of PSI (Odds ratio 1.8, IC 95% 1.1-3 in cases with BMI>30 kg/m2). When compared to cases without PSI, cases with PSI exhibited a higher incidence of preeclampsia (10% vs 2%), placental abruption (5.5 % vs 0.3%), preterm premature rupture of membranes (7% vs 1.3%) and intrauterine fetal death (18% vs 2.2%) (p<0,001).
Conclusion: Placental superficial implantation is associated with significant adverse pregnancy outcome. The influence on the woman lifestyle with a search of pregnancy when the ideal body weight is obtained might reduce its incidence.
Keywords: placenta, implantation, pregnancy outcome, spiral artery

[P3.15]

MATERNAL AND PERINATAL OUTCOMES IN WOMEN WITH SICKLE CELL TRAIT

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Introduction: The effect of sickle cell trait (SCT) if any on pregnancy is debated. SCT is relatively prevalent among African Americans (carrier frequency 8-12%). The objective of this study is to evaluate the maternal and perinatal outcomes with SCT compared to controls.
Methods: 96 Black pregnant women delivering a fetus > 16 weeks at NY Methodist Hospital from 12/2007-12/2009 were identified by ICD 9th edition code for "sickle cell trait". The next delivery of a African/Caribbean American pregnant with a normal hemoglobin electrophoresis composed the controls (N=96). Outcome measure included birth weight, preeclampsia, preterm birth, delivery mode, APGAR scores, cord pH, placental weight, signs of placental malperfusion and NICU admission. Groups were compared using the ANOVA test and Mann-Whitney test for non-normally distributed variables.
Results: Infants born to women with SCT had lower birth weights (3180 + 501 g v. 3362 + 408 g, p=0.043), lower placental weights (460+116 g v. 495 +82 g, p<0.038), and a trend to higher fetoplacental weight ratios (7.2=1.8 v. 6.8+1.6, p=0.10). After adjustment for semiquantitative scores of uteroplacental vascular histopathology lesions, the association of reduced birth and placental weights with SCT was abolished. Of secondary outcomes, SCT mothers had increased rates of preeclampsia (p= 0.007), NICU admissions/transfers (p=0.001), and fetal nucleated red blood cells (p < 0.001) No association was found between SCT and preterm birth (p= 0.093).
Conclusions: SCT mothers delivered infants with significantly lower birth and placental weights (that appears to be due to excess placental histopathology related to maternal uteroplacental malperfusion), had increased rates of preeclampsia, and increased NICU admission. The decreased placental weight and malperfusion findings may provide explain the mechanism of poor perinatal outcome.
Keywords: sickle cell trait, placental pathology, birth weight, placental weight

[P3.16]

PLACENTAL VASCULAR INDICES OBTAINED BY A SINGLE CENTRAL SONOBIOPSY CORRELATE WELL WITH THOSE FROM EVALUATION OF THE ENTIRE PLACENTA

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Introduction: Abnormal placentation is associated with several complications of pregnancy. Advances in 3D ultrasound/power Doppler permit quantitative non-invasive assessment of placental vascularization. Vascular indices are generally obtained from evaluation of the entire placenta, a procedure only possible early in pregnancy. Sonobiopsy has been proposed as an alternative when utilization of the entire placenta is not feasible.
Objective: We tested the hypothesis that placental vascular indices obtained from a single central sonobiopsy correlate with those from evaluation of the entire placenta.
Methods: Three-dimensional power Doppler ultrasound examinations were performed in 90 singleton pregnancies at 11-14 weeks' gestation. The VOCAL™ (Virtual Organ Computer-aided Analysis) program was used with 30 degree rotation angles (six planes) to calculate vascularization index (VI), flow index (FI) and vascularization flow index (VFI). The same indices were calculated from a single spherical central sonobiopsy taken from the middle of the image, extending from the basal to the chorionic plates. The two sets of indices were compared for their degree of correlation and agreement.
Results: The mean gestational age was 12.1 $\pm$ 0.6 weeks'. The mean of each of the placental vascular indices obtained from placental sonobiopsy and whole placenta evaluation were not significantly different (14.6 vs. 13.9, p=0.56 for VI; 46.3 vs. 44.9, p=0.53 for FI; 6.6 vs. 6.1, p=0.51 for VFI, respectively). The indices obtained from the two techniques were also significantly correlated (Pearson's r= 0.52 for VI; r=0.50 for VI; r=0.50 for VFI, all p<0.0001).
Discussion: Placental vascular indices obtained from sonobiopsy correlate with those from evaluation of the entire placenta. Sonobiopsy may be a valid alternative for evaluation of the placental vascular tree when visualization of the entire placenta is not feasible.

Figure 1: Comparison of placental vascular indices from whole placenta evaluation and sonobiopsy (N=90)

Vascular Indices	Whole Placenta	Sonobiopsy	P-value	Correlation	
	Mean (95 % CI)	Mean (95 % CI)		Pearson's r	P-value
VI*	13.9 (12.1 – 15.9)	14.6 (11.8 – 18.1)	0.56	0.52	<0.0001
FI	44.9 (43.0 – 46.9)	46.3 (43.9 – 49.0)	0.24	0.50	<0.0001
VFI*	6.1 (5.2 – 7.2)	6.6 (5.1 – 8.5)	0.51	0.50	<0.0001

*Logarithmic transformation required to achieve normal distribution

Keywords: Three-dimensional power Doppler, Placental vascular indices, Sonobiopsy, Correlation

[P3.17]

ABNORMAL MATERNAL IMMUNE ACTIVATION LEADS TO COAGULOPATHY IN A RAT MODEL OF SPONTANEOUS ABORTION

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Introduction: Spontaneous abortion is often associated with an aberrant maternal immune activation and systemic maternal coagulopathies. We have previously shown that activation of the maternal immune system, via administration of lipopolysaccharide (LPS; 100 μg/kg), leads to spontaneous abortion in rats through a TNFα-dependent mechanism. We hypothesize that inflammation-induced pregnancy complications are *causally* linked to maternal coagulopathies.
Methods: Using the LPS model of spontaneous abortion, we characterized the maternal coagulation status in rats (n=5) at 1hr post LPS treatment using thromboelastograpy (TEG), a global haemostatic assay that measures the kinetics of clot formation. To determine a causal link between activation of a maternal inflammatory response and coagulopathies, we assessed the maternal coagulation status following administration of the TNFα inhibitor Enbrel®.
Results: Maternal coagulopathy was evident 1 hr following LPS administration. Two of the five rats tested exhibited stage II disseminated intravascular coagulation (DIC), characterised by excessive fibrinolysis, reduced clot strength, reduced rate of clot formation, and low clotting index as compared to saline controls. The remaining three rats exhibited hypercoagulability alone, showing a reduced clotting time, poor fibrinolysis, and enhanced rate of clot formation as compared to saline controls. Administration of Enbrel® to LPS-treated rats improved the DIC condition in two of the three rats tested as evidenced by reduced fibrinolysis, regained clot strength, increased rate of clot formation, and increased clotting index as compared with LPS-treated rats.
Conclusion: Modulation of maternal immune activation may be useful in the prevention of coagulopathies associated with complications of pregnancy.
Keywords: Coagulopathy, Spontaneous Abortion, Inflammation

[P3.18]

PLACENTAL FORMATION, CHILDBIRTH, AND FIBROID TREATMENT: AN INTEGRATION AND REVIEW OF THE CIRCULATION OF THE PLACENTA AND THE UTERUS DURING A WOMAN'S LIFE-CYCLE.(1)

F Burbank*, Salt Creek International Women's Health Foundation, United States

During pregnancy, mother's blood prepares for an enormous hemostatic event that is 9 months away: the delivery of the placenta - the fetal organ that is the vascular link between mother and child. At childbirth, 1/10th of mother's cardiac output flows through the placenta. When the placenta is sheared from the uterus, 200 large, uteroplacental arteries are ripped apart and bleed profusely into the uterine cavity. For hours following delivery, uterine contractions slow blood flow within the uterus, which then allows the high concentration of clotting factors in mother's blood to solidify throughout the uterus and stop blood loss. Hours later, the tide reverses and most of these blood clots dissolve and blood flow returns to the uterus. For many hours following delivery, the uterus is ischemic and hypoxic. Unlike brain and heart, which can only survive minutes of decreased blood flow, the uterus can withstand dramatically diminished blood flow for hours. In fact, not only can the uterus tolerate low blood flow, it is evolutionally programmed to experience very low blood flow every few years. Uterine ischemia and hypoxia are a natural part of every woman's genetic past and are necessary for uterine health.

In 1995 a group of French physicians discovered that it was possible to emulate the vascular physiology of childbirth by stopping blood flow to the uterus with small, plastic particles. Initially, they injected these particles to diminish blood loss during myomectomy. However, they soon learned that the injection of these particles was therapeutic in-and-of-itself for women with symptomatic fibroids.

Unbeknownst to this French group, earlier, in 1964, an American physician surgically occluded the uterine arteries to treat women without fibroids who had excessive monthly menstrual blood loss. Subsequent physicians have occluded the uterine arteries in various ways to treat a third common disorder, adenomyosis. Finally, these clinical successes suggest that future episodes of endometriosis may be preventable in some women treated with uterine artery closure.

The biological basis of these treatments rests on an understanding of the circulation of the placenta and uterus immediately following childbirth.

(1)Burbank F. Fibroids, menstruation, childbirth, and evolution: The fascinating story of uterine blood vessels. Tucson, AZ: Wheatmark; 2009; ISBN: 978-1-60494-170-8.

Keywords: uteroplacental arteries, hemostasis, fibroids, evolution

[P3.19]

NFB-DEPENDENT INCREASE OF KYNURENINE PATHWAY ACTIVITY IN HUMAN PLACENTA: INHIBITION BY SULFASALAZINE

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Introduction: In the human placenta the kynurenine pathway of tryptophan metabolism is up-regulated by infection, releasing pro-inflammatory and neuroactive metabolites into the fetal circulation. In this study we used the NF-κB inhibitor sulfasalazine (SZS) to determine if activation of NF-κB is involved in the inflammation-induced increase of kynurenine pathway activity.

Methods: Placentas (n=8) were obtained after elective caesarian section at 37-40 weeks gestation and explants (35-40 mg) were prepared from terminal villi and incubated for 24 or 48 h in the presence of 10μg/ml LPS; duplicates of each explant were incubated with or without 5 mM SZS added to the medium. mRNA expression of the kynurenine-forming enzymes indoleamine 2,3-dioxygrenase (IDO) and tryptophan 2,3-dioxygenase (TDO), and the inflammatory cytokines TNFα and IL-6 were measured by RT-PCR. NF-κB activity was measured in nuclear protein using a colorimetric sandwich ELISA. Kynurenine production from tryptophan was measured in the incubation medium by HPLC.

Results: mRNA expression of IDO, TDO, TNFα and IL-6 mRNAs were all significantly increased by the LPS treatment ($P<0.05$), a response significantly decreased by the presence of SZS ($P<0.05$ vs no SZS). Kynurenine released into the culture medium increased with LPS treatment but this was also prevented by SZS. Under control conditions of incubation (i.e., without LPS), SZS significantly decreased NF-κB activity ($P<0.001$), and while LPS treatment significantly increased NF-κB activity($P<0.001$), this was totally prevented by SZS.

Discussion: SZS inhibited both kynurenine and proinflammatory cytokine production induced by LPS in the placenta. Direct measurement of NFκ-B activity showed that SZS decreased NFκ-B activation under both control and LPS-treated conditions. These observations show that kynurenine pathway activity in the human placenta is increased by a NFκ-B dependent pathway, and suggests a new therapeutic strategy for the management of pregnancies with *in utero* infection.

Keywords: Infection, Kynurenine, Cytokines, Sulfasalazine

[P3.20]

IS AN ELEVATED LEVEL OF INTERLEUKIN-1BETA IN POSTPARTUM MATERNAL PLASMA ASSOCIATED WITH POSTPARTUM MOOD DISORDERS?

S.R. Srinivas, Jodi Hudgins, D. Sutherland, V. Ganapathy, S.C. Mobley, P.D. Prasad* et al, Medical College of Georgia, United States

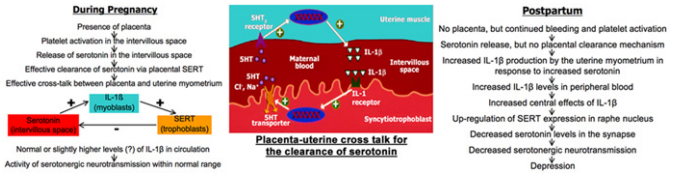

Figure 1. Placenta-brain axis as a link to postpartum mood disorder

Background: Postpartum mood disorders (PMD) are mood disturbances that are experienced by majority of mothers after childbirth. The biochemical etiology of PMD is still largely unclear. The leading biologic theory for the cause of nonpuerperal depression lies in the deregulation of monoaminergic neurotransmission, especially the serotonergic neurotransmission. Studies done in our lab have shown the existence of an efficient mechanism involving a placenta-uterine cross talk for clearing the serotonin (5HT) from the placental intervillous space as shown in Fig. 1. IL-1β and serotonin transporter (SERT) are the primary players in this placenta-uterine cross talk. We hypothesize that a disruption of this cross talk at parturition, as detailed in Fig. 1, will trigger an increase in circulating IL-1β levels in postpartum maternal plasma, which in turn will precipitate PMD. The objective of the present study is to monitor the circulating levels of IL-1β in maternal plasma at various times during the peripartum period.
Study design: Pregnant women who had no complications such as gestational diabetes, preeclampsia, major psychiatric disorders, and history of recreational drug use were recruited. In addition, all women with a history of any infection within one month of blood draw were excluded from the study. Blood was collected in the third trimester before delivery and at 1, 2, 4, and 8 weeks after delivery. IL-1β levels were measured in the plasma using a commercially available ELISA kit.
Results: Preliminary studies from an ongoing study are presented here. Plasma sample collected from 104 women were included in the analysis. Also, not all women provided all the five blood samples. The results obtained are shown in Table 1.

	IL-1β (pg/ml plasma) ± SEM
Prenatal (n=83)	0.194 ± 0.028
Week 1 (n=51)	0.164 ± 0.026
Week 2 (n=72)	0.183 ± 0.028
Week 4 (n=70)	0.149 ± 0.018
Week 8 (n=67)	0.180 ± 0.033

Conclusions: We report for the first time maternal IL-1β levels measured before and at various time points after delivery. Results indicate that there is no significant change in maternal IL-1β values following parturition. However, these results are preliminary with relatively few subject numbers. In addition, analyses of changes in IL-1β values within the same subject also need to be performed before a role for IL-1β in the etiology of postpartum depression can be completely ruled out. (Supported by HRSA grant R40 MC 08967)
Keywords: Postpartum Mood Disorder, postpartum depression, serotonin transporter, interleukin-1beta

[P3.21]

INTERACTIONS BETWEEN VITAMIN D RELATED GENES AND MATERNAL CIRCULATING 25OH VITAMIN D3 IN PREGNANCY COMPLICATIONS IN AN AUSTRALIAN POPULATION

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Introduction: Preeclampsia is associated with vitamin D deficiency at diagnosis. We aimed to determine if pregnancy complications are related to maternal serum 25 hydroxy vitamin D3 (25OHD3) at 15 weeks gestation and with single nucleotide polymorphisms (SNPs) in the vitamin D receptor (VDR) and CYP24A1 (Vitamin D metabolising enzyme) genes in the Adelaide SCOPE cohort.
Methods: 1169 nulliparous couples were recruited prospectively from 2005-2008. Peripheral blood from couples and cord blood from babies were collected. Genotyping was performed using Sequenom MassARRAY. Serum 25OHD3 was measured using the Roche Elecsys Immunoassay. Data for 991 Caucasian couples were analysed. Genotypes, vitamin D concentrations at 15 weeks gestation and other risk factors for preeclampsia (PE n=71), gestational hypertension (GHT n=92), preterm birth (PTB n=69), small for gestational age (SGA n=94), gestational diabetes (GDM n=38) were compared with normal controls (n=450) and analysed using Logistic regression and Chi Square.
Results: Serum 25OHD3 at 15 weeks gestation was <60nmol/L (level of 25OHD3 sufficiency) in 35% of women and <80nmol/L (proposed level of sufficiency) in 75% of women. It was higher in smokers and inversely correlated with BMI ($r=-0.177$, $p<0.0005$) and maternal blood pressures ($r=-0.135$, $p<0.0005$). When adjusted for BMI and month of sampling, serum 25OHD3 was significantly higher in women destined to develop PE but lower in women who later developed GDM (both $p<0.05$). Maternal VDR rs7975232 associates with PE ($p<0.023$) and GDM ($p<0.016$), while maternal CYP24A1 rs2248137 associates with GHT ($p<0.039$). Maternal and baby VDR and CYP24A1 SNPs significantly affect circulating 25 OHD3 in the mother at 15 weeks gestation.
Conclusion: In Adelaide circulating 25OHD3 at 15 weeks gestation is associated with pregnancy complications. SNPs in vitamin D related genes interact with these and BMI in adverse pregnancy outcomes. Ongoing research will explore the vitamin D pathway in early placental function and pregnancy outcome.
Keywords: Vitamin D, preeclampsia, gestational diabetes, genetic polymorphisms

[P3.22]
CAN STEROIDS INDUCE ENDOGENOUS ANALGESIA DURING LABOR?

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Steroid hormones can cross the blood-brain-barrier and induce changes in emotions and behavior at the genome level. These affects, the result of classic steroid hormone function mechanisms, operate relatively slowly over minutes up to an hour *(McEwen BS, 1991)*. Some steroids, however, can rapidly influence neuron excitability, on the order of seconds or even milliseconds. These compounds are known as "neuroactive steroids" (NAS). NASs modulate the function of ion channels, in particular GABAA and NMDA receptors.
Rapidly acting steroids have been known since the 1940s. At that time, we became aware of the anesthetic influence of steroid hormones (*Selye H, 1941)*, which led to the subsequent development of the steroid anesthetics alphaxalone and pregnanolone (Althesin®, Saffan®, Eltanolon®). NASs, especially allopregnanolone and pregnanolone, thus have significant anesthetic effects. In addition, they act as notable anxiolytics, analgetics, and compounds influencing memory *(Beekman M, 1998)*.
NASs are synthesized in the nervous tissues from cholesterol. Such NASs made in nervous tissues are called neurosteroids. NASs are also formed from steroid precursors originating in periphery. During pregnancy, this peripheral NAS production is dominant, particularly from the placenta and fetal liver. The production of NAS greatly increases during pregnancy, reaching up to 100-times higher level around parturition than out of pregnancy *Gilbert-Evans SE, 2005)*. Even though precursors of NASs are largely of fetal origin, the levels of GABAergic NASs are significantly higher in the mother than in the fetus. The ratios of NAS/inactive oxidized precursors are about 2.5 and 5-times higher for allopregnanonolone and pregnanolone, respectively (Fig. 1). In light of the well-known neurophysiologic effects of NASs, the question arises whether there is an increased threshold for pain, or alternatively if in addition to the endogenous opioid system there is a second pathway of endogenous analgesia before birth.

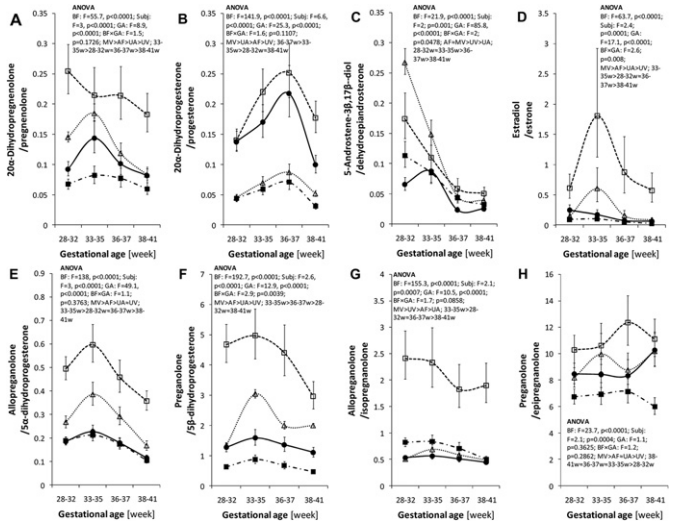

Figure 1. Profiles of the ratios of steroids in reduced forms to the corresponding oxidized forms in the plasma from the umbilical artery (UA), umbilical vein (UV) and maternal cubital vein (MV) and in amniotic fluid (AF) in preterm and normal labor. The symbols with error bars represent re-transformed means with their 95% confidence intervals for individual body fluids (full circles=UA, full squares=UV, empty squares=MV, empty triangle=UA. The 95% confidence intervals are computed using the least significant difference multiple comparisons ($p<0.05$). The confidence intervals, which do not overlap each other, denote significant difference between the respective subgroup means. The horizontal line from the full circles represents the mean level of the steroid in the luteal phase of the menstrual cycle.

Keywords: neurosteroids, placenta, endogenous analgesia, labor

[P3.23]
INTERLEUKIN-1 RECEPTOR ANTAGONIST IN CORD BLOOD FROM PRETERM NEONATES

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Introduction: Increased concentrations of cord blood pro-inflammatory cytokines are associated with a number of adverse conditions in preterm infants, including growth inhibition, chronic lung disease and cerebral damage. The actions of these cytokines are, in part, self regulated through the release of opposing cytokines as well as cytokine antagonists. While decreased levels of the endogenously released interleukin-1 receptor antagonist (IL-1ra) in preterm cord blood have previously been associated with adverse outcomes in preterm neonates, these studies failed to assess simultaneously the balance between IL-1receptor agonists and antagonists. The aim of this study was to characterise cord blood IL-1α, IL-1β and IL-1ra concentrations together in cord blood collected from term (37-41 weeks), preterm (32-36 weeks) and very preterm (24-31 weeks) neonates.
Methods: Plasma was separated from cord venous blood from 12 term, 20 preterm and 8 very preterm infants. Concentrations of IL-1α, IL-1β and IL-1ra, were determined by Luminex multiplex system.
Results: IL-1ra was detectable in 75% of term and 78% of preterm infants. IL-1α and IL-1β were detectable in 25% of term infants, and 14% of preterm infants. Cord blood concentrations of IL-1ra decreased across gestation, with significantly higher levels observed in very preterm compared to preterm, and preterm compared to term infants ($p< 0.05$ in both instances). IL-1ra concentrations were significantly correlated with IL-1β levels in preterm ($r=0.422$, $p=0.05$) but not term cord blood. IL-1α and IL-1β concentrations were not significantly different in preterm compared to term, but were significantly correlated with each other ($r=0.554$, $p=0.001$). No sex specific differences in these cytokines were observed.
Discussion: Given that IL-1ra functions to regulate the agonist effects of IL-1α and -β in normal biologic processes, the significant correlation between IL-1ra and IL-1β in preterm cord blood suggests an attempt towards self-regulation in preterm parturition. The gestation specific decrease in cord blood IL-1ra levels suggests a greater role for this protein in protecting against inflammation in early development and in preterm compared to term infants.
Keywords: Preterm infant, cord blood, cytokine, IL-1 receptor antagonist

[P3.24]

ALLERGEN INDUCED CYTOKINE RELEASE IN HUMAN PLACENTAE AND IT'S POSSIBLE ROLE IN FETAL PROGRAMMING OF ALLERGIES

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Introduction: Previous animal experiments indicated the transmissibility of general allergic diathesis from the mother to the fetus. All major cell types known to be involved in allergic processes, including dendritic cells, T cells, B cells and mast cells are present in the decidua. Thus, allergen induced mediator release may prime the fetus for easier sensitization in later life. To investigate possible differences in cytokine production from placental tissue of atopic and healthy mothers after contact with a relevant allergen, we developed a one sided open placenta perfusion model with an internal standard to perfuse two separate cotyledons from the same placenta simultaneously with and without addition of relevant allergens
Methods: Pregnant mothers were asked for allergic symptoms or known allergy against birch pollen or apple. Reported allergy was confirmed by CAP/RAST-Test. In placentae, obtained directly after birth, two cotyledons were chosen for open perfusion. Cotyledon "A" was perfused with normal medium for 1 hour followed by a 5 hour perfusion with apple allergen-containing medium (mal d 1). Cotyledon "B" resembles the internal control and was only perfused by allergen-free medium for 6 hours. Outflow samples were collected and analyzed for immune mediator release. Histamine concentration was determined spectrophoto-fluorometrically after extraction and derivatization with o-phthalaldehyde. The cytokines IL-2, IL-4, IL-6, IL-10, TNF-alpha and IFN-gamma were analyzed by using a cytometric multiplex bead array.
Results: After application of apple allergen in perfusion medium, a significant time-dependent release of TNF-alpha and IL-6 was detectable in placentae of mothers suffering apple allergy compared to those from healthy mothers. The expression of the other interleukins and histamine was not remarkably altered.
Discussion: Allergens can induce allergy related effects, which may disturb the immunological balance at the feto-maternal interface and might prime the developing fetal immune system for facilitated later susceptibility for allergic sensitizations.
Keywords: placenta, perfusion, allergy, cytokine

[P3.25]

CORRELATION BETWEEN PLACENTAL ABNORMALITIES AND SIGNS OF FETAL INTRAUTERINE GROWTH RESTRICTION IN CASES SUBMITTED TO TRANSCUTANEOUS ELECTRICAL NERVE STIMULATION

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Introduction: In humans, studies demonstrated that the Transcutaneous Electrical Nerve Stimulation (TENS) can be used for therapeutic purpose in cases with placental insufficiency. The current literature, however, do not provide comprehensive data regarding the influence of placental vascular abnormalities on fetal internal organs in cases submitted to TENS.
Objective: To evaluate placental vascularization and intrauterine growth restriction induced in an experimental model, by morphometric analysis of the internal organs, demonstrating or not the viability of the use of TENS during pregnancy.
Methods: To induce uteroplacental insufficiency and intrauterine growth restriction in Wistar rats, the right uterine artery was ligated on the 15th day of gestation. TENS was applied from the immediate postoperative period until euthanasia. To analyze placental vessels, factor VIII was identified by immunohistochemistry. There was performed the morphometric analysis of the placental vessels and common fetal organs related to intrauterine restriction, as brain, lung and liver. Results: the caliber of placental vessels was smaller in cases stimulated ($p=0.02$), with the ligation ($p=0.001$) and in interaction of stimulus and ligation ($p<0.001$). The number of vessels was smaller in cases with ligation ($p<0.001$). In cases stimulated, the measures of brain were smaller ($p=0.045$), and liver measures tended to decrease ($p=0.085$). There was a negative correlation between the caliber of placental vessels and measures of the brain ($r=-0.488$, $p=0.0533$), and negative and significant correlation between the caliber of these vessels and measures of lung ($r=-0.538$, $p=0.307$).
Discussion and Conclusion: Our data indicated a decrease in the caliber of placental vessels associated with TENS, contradicting the hypothesis of vasodilatation associated with this current, and also, reduction of measures of fetal internal organs typically affected by intrauterine growth restriction, suggesting caution about its use during pregnancy.
Keywords: intrauterine restriction, morphometry, placenta, Transcutaneous Electrical Nerve Stimulation

[P3.26]

THE EXPRESSION OF SYNDECAN PROTEOGLYCANS IN FETAL GROWTH RESTRICTION

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Fetal growth restriction (FGR) is a leading cause of perinatal morbidity and mortality. FGR pregnancies are associated with abnormal umbilical artery Doppler velocimetry and histological evidence of placental thrombosis. Heparan sulphate proteoglycans (HSPGs) are highly expressed in the placenta and contain heparan sulphate side chains which interact with antithrombin. This interaction may prevent thrombosis within the placental circulation. We hypothesised that altered expression of the HSPGs, syndecans 1-4, may result in dysregulation of coagulation within the placenta, and therefore contribute to the development of FGR.

The aim of this study was to determine the expression of syndecans 1-4 in FGR-affected placentae compared with gestation-matched controls. RNA obtained from 28 FGR and 28 control placentae were reverse transcribed into cDNA. Real-time PCR was used to determine the mRNA expression of syndecans 1-4, relative to the housekeeping gene, GAPDH, according to the 2-ΔΔCT method [1]. Western immunoblotting and immunohistochemistry was performed to determine the protein expression and cellular localisation of syndecans 1-4, respectively. Data are represented as mean±SEM with statistical analysis by the Student's t-test.

The table shows the relative mean mRNA expression of syndecans 1-4 in FGR-affected placentae compared with gestation-matched controls.

Mean mRNA expression (mean±SEM)

Syndecan	Control	FGR	P Value
Syndecan 1	1.47±0.17	0.91±0.14	0.02
Syndecan 2	1.04±0.09	0.54±0.1	0.0009
Syndecan 3	0.96±0.1	0.78±0.08	0.19
Syndecan 4	1.18±0.1	0.88±0.1	0.06

Western immunoblotting supported these findings and immunohistochemistry confirmed that all syndecans were located in close proximity to either fetal or maternal blood (i.e.: localised to fetal vascular endothelial cells or syncytiotrophoblast).

These data demonstrate reduced expression of syndecans 1 and 2 in FGR-affected placentae compared with controls. However, the expression of syndecans 3 and 4 is not different between the two groups. This suggests that even though the syndecans belong to the same family, the role of each syndecan may be different. Altered expression of syndecan may contribute to the pathogenesis of FGR. Understanding the precise mechanism by which this occurs may lead to future therapies for this disorder.

1. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.

Keywords: Syndecan, Proteoglycan, Fetal growth restriction, placenta

[P3.27]

HYPERHOMOCYSTEINEMIA MOUSE AS AN ANIMAL MODEL FOR PRE-TERM BIRTH

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Introduction: Folic acid deficiency increases the risk for neural tube defects in fetuses. Recent studies indicate that folic acid deficiency may also increase the risk for pre-term birth. Hyperhomocysteinemia is a consequence of folic acid deficiency. Therefore, hyperhomocysteinemia may be a pathogenic factor in pre-term birth.

Methods: Cystathionine-beta-synthase knockout mouse was used as a model of hyperhomocysteinemia. Homozygous mice do not survive beyond 3 weeks of age. Heterozygous mice survive and are fertile. Heterozygous females were mated with heterozygous males, the day of conception noted, and the number of days for delivery recorded. Wildtype females mated with wildtype males served as controls. Gene expression in wildtype and homozygous placentas was studied by microarray. The effects of oxytocin and homocysteine on myometrial contraction were investigated using uterine tissues from wildtype and heterozygous pregnant mice.

Results: The gestational period in wildtype females was 19.5 +/- 0.07 days. In contrast, the gestational period in heterozygous females was 16.6 +/- 0.13 ($p < 0.001$). Plasma levels of homocysteine in heterozygous mice were 4-fold higher than in wildtype controls. Homozygous fetuses/placentas weighed significantly less than wildtype and heterozygous fetuses/placentas ($p < 0.01$). Several genes were expressed differentially in placenta as a consequence of hyperhomocysteinemia. One of the genes upregulated markedly in hyperhomocysteinemic placenta was cyclooxygenase 2. The upregulation was evident also in uterine tissue. The expression of the proteins responsible for placental transfer of folic acid was not altered in hyperhomocysteinemic mouse placentas. Homocysteine did not affect uterine contraction caused by oxytocin in wildtype mice. In contrast, homocysteine itself caused uterine contraction and also potentiated oxytocin-induced contraction in uterine tissues from hyperhomocysteinemic pregnant mice.

Discussion: We conclude that elevation of circulating levels of homocysteine causes pre-term birth in mice. Therapeutic strategies to reduce homocysteine levels may be effective to prevent pre-term birth in humans.

Keywords: pre-term birth, folic acid deficiency, Hyperhomocysteinemia, Cyclooxygenase 2

[P3.28]

CHARACTERISATION OF FETAL MEMBRANE AND DECIDUAL LEUKOCYTE SUBPOPULATIONS DURING TERM AND PRETERM LABOUR

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Introduction: There is considerable evidence for inflammatory processes during normal labour at term, with leukocyte infiltration of the cervix and myometrium1. However, there are conflicting data regarding inflammatory events in the fetal membranes and decidua1,2. The decidua is a major immunological tissue in early pregnancy and is ideally placed to coordinate inflammatory events during parturition. We hypothesized that leukocyte infiltration of the amniochorion and decidua occurs with preterm and term labour.

Methods: Women were recruited into 4 study groups: term labour (TL), term not in labour (TNIL), preterm labour (PTL) and PTL with infection (PTLi) (n=8-10/group). Fetal membranes were sampled at delivery. Immunohistochemistry was performed for leukocyte common antigen (CD45), macrophages (CD68), neutrophils (n-elastase) and uNK cells (CD56). Leukocyte densities were quantified using image analysis.

Results: Leukocytes were more abundant in decidua (10-fold higher) than amnion and chorion. There were no differences in overall CD45+ leukocyte numbers between study groups. However significantly greater numbers of macrophages were present in decidua in TL and PTL compared to TNIL (56.5 and 43.0 vs. 21.0/mm2; $p<0.05$). Neutrophil infiltration was negligible in TL and PTL, but significantly elevated in PTLi (4.9 and 7.1 vs. 345.1/mm2; $p<0.05$). Decidual uNKs were significantly greater in PTL (76.1/mm2) compared to other groups (0-5/mm2; $p<0.05$).

Discussion: This study confirms that decidual macrophage infiltration increases in PTL and TL. This is consistent with our data from animal models3 and suggests a role for macrophage-derived mediators in the labour process. Higher numbers of uNK cells in PTL may reflect the earlier gestation. These data identify the decidua as a major player in inflammatory events during labour at preterm and term labour.

[1]Osman et al (2003) Mol Hum Reprod 9, 41-45; [2]Keski-Nisula et al (2000) Hum Pathol 31, 841-846; [3]Hamilton et al (2010) Reprod Sci, 178A.

Keywords: Leukocytes, Decidua, Labour, Fetal Membranes

[P3.29]

PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) REPLICATION IN FETAL IMPLANTATION SITES

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PRRSV is considered to be the most important viral pathogen in swine production worldwide. Reproductive failure due to PRRSV is characterized by an increase in late-term abortions, early farrowing, number of dead and mummified fetuses and weak-born piglets. Sialoadhesin (Sn) and CD163 are markers for PRRSV susceptibility of macrophages [1]. It has been postulated that induction of apoptosis in human fetal membranes facilitates reproductive failure [2, 3]. Because Sn+ and CD163+ cells are present in endometrium/placentas from healthy sows [4] and PRRSV-infected lung macrophages die by apoptosis [5], we hypothesized that PRRSV can replicate and induce apoptosis in endometrial/placental macrophages. The objective of the present study was to localize and quantify PRRSV-positive and apoptotic (TUNEL+) cells in endometrium/placentas. Three sows were inoculated with PRRSV at 90 days of gestation, euthanized at 10 days post-inoculation and sampled (uterus with placenta corresponding to every fetus; fetal sera; organs). Non-inoculated sows served as controls. Transplacental PRRSV spread was detected in all inoculated sows. Histopathological changes were not found in endometrium/placentas from inoculated and healthy sows. Only one PRRSV-positive fetus was found dead and degeneration of the fetal placental mesenchyme was observed. Using immunofluorescence staining, single PRRSV-positive cells were found in the maternal stroma corresponding to all fetuses. In the fetal placental mesenchyme corresponding to PRRSV-positive fetuses, the infected cells were abundant and spread focally. Double staining showed that 100% of the PRRSV-positive cells in the fetal placental mesenchyme were Sn+ and CD163+ (Fig. 1). The amount of TUNEL+ cells was significantly increased in PRRSV-positive endometrium/placentas (Fig. 2). A spatial correlation between the sites of PRRSV replication and TUNEL+ cells was observed (Fig. 3). Double labeling revealed that 9 to 57% of the apoptotic cells in fetal placentas were PRRSV-positive. In conclusion, PRRSV efficiently replicates in endometrium/placentas and causes apoptosis in infected and surrounding cells.

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5. S. Costers, D.J. Lefebvre, P.L. Delputte, H.J. Nauwynck, Porcine reproductive and respiratory syndrome virus modulates apoptosis during replication in alveolar macrophages, *Arch Virol* (2008); pp. 1453-1465.

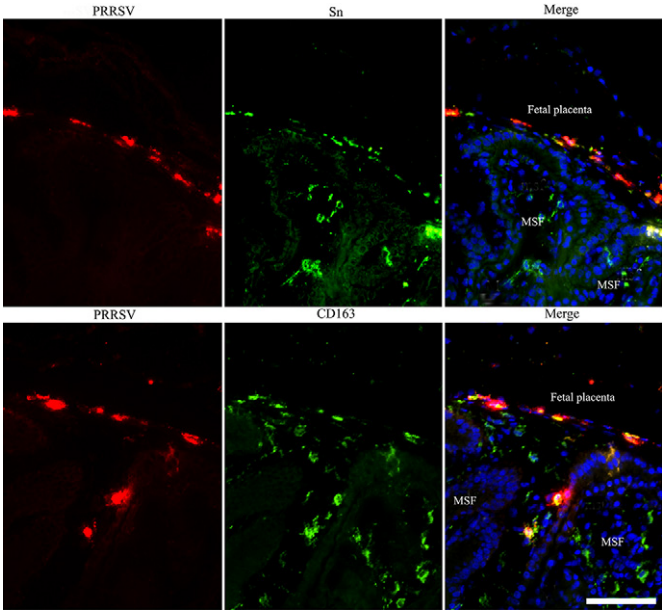

Figure 1. Triple immunofluorescence staining for PRRSV (red), Sn (green) or CD163 (green) and nuclei (blue) in the fetal implantation sites (MSF: maternal secondary fold) of the uterus coming from sows inoculated with PRRSV at 90 days of gestation and sampled at 10 days post-inoculation. Bar, 100 μm. All PRRSV-positive cells in the fetal mesenchyme were Sn+ and CD163+.

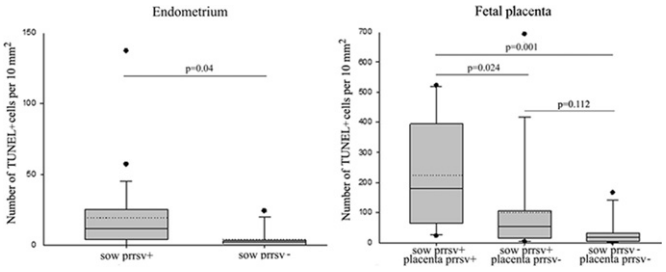

Figure 2. Quantification of TUNEL+ cells in the endometrium and fetal placentas collected from sows inoculated with PRRSV at 90 days of gestation and non-inoculated sows. Animals were sampled at 10 days post-inoculation. Solid and dotted lines are median and mean, respectively. Each box represents 25–75% of observations. Whiskers below and above the box represent the 10th and 90th percentiles. Dots below or above the whiskers on each box represent outliers not included between 10 and 90% of observation. Differences were considered statistically significant at $p \leq 0.05$.

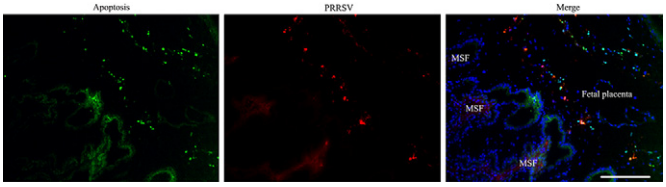

Figure 3. Triple immunofluorescence staining for apoptosis (green), PRRSV (red) and nuclei (blue) in the fetal implantation sites (MSF: maternal secondary fold) of the uterus coming from sows inoculated with PRRSV at 90 days of gestation and sampled at 10 days post-inoculation. Bar, 200 μm. A spatial correlation between the sites of PRRSV replication and TUNEL+ cells was observed.

Keywords: Placenta, PRRSV, Apoptosis, Macrophages

[P3.30]

A LIVE, ATTENUATED CMV VACCINE PROTECTS AT THE PLACENTAL-FETAL INTERFACE IN THE GUINEA PIG MODEL OF CONGENITAL CMV INFECTION

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A vaccine is needed to prevent neurodevelopmental sequelae in newborns caused by congenital human cytomegalovirus infection. Preconception vaccine strategies can be studied in the guinea pig, with a hemomonochorial placenta similar to humans, using guinea pig cytomegalovirus (GPCMV). The objectives of this study were: 1) to assess in guinea pigs the protective efficacy of a recombinant live, attenuated vaccine with a targeted deletion of the GPCMV UL83 homolog, GP83; 2) to examine the role of the placenta in vaccine-mediated protection. Outbred Hartley guinea pigs were vaccinated prior to pregnancy with a two-dose series of 5x10^4^ pfu of attenuated vaccine. After mating, pregnant animals were challenged with salivary gland-adapted (SG) GPCMV (1x10^6^ pfu) in the second trimester, and pregnancy outcomes were compared to unvaccinated controls. Following delivery, placentas were recovered, frozen, cryosectioned, and ISH using a GPCMV GP55 DNA probe was performed. Three sections per placenta were analyzed by a blinded investigator and recorded as GPCMV positive or negative. Vaccination significantly reduced maternal DNAemia following SG challenge, and resulted in significantly decreased pup mortality in litters born to vaccinated dams (3/29; 10%), compared to control (35/50; 70%; $p<0.001$). Recovered placentas from vaccinated and control litters demonstrated placental infection in 6% (2/17) placentas in the vAM409 vaccine group compared to 62% (13/21) in the control group ($p<0.001$). Viral load at the placental level was also reduced. Protection from preconception immunization is mediated at the placental level, with vaccination conferring nearly complete protection against placental GPCMV infection. This is the first report of: 1) efficacy against congenital infection of a live virus vaccine with an attenuating targeted gene deletion; 2) active immunization in an animal model of congenital CMV conferring protection at the placental level. Live attenuated vaccines generated using recombinant mutagenesis techniques may warrant examination in future clinical trials of CMV vaccines.

Keywords: cytomegalovirus, vaccine, congenital infection, viral placental infection

[P3.31]
PEPTIDE INHIBITION OF CYTOMEGALOVIRUS INFECTION

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Human cytomegalovirus (HCMV) is the most prevalent congenital viral infection in the United States and Europe. Treatment regimens for congenital HCMV are controversial given the toxicity associated with pharmacological agents used against the virus. The aim of this study was to develop peptides targeting glycoprotein B (gB), a major glycoprotein of HCMV that is highly conserved across the *Herpesviridae*, which specifically inhibit HCMV-host cell membrane fusion.
Using the Wimley and White Interfacial Hydrophobicity Scale (WWIHS), several regions within gB were identified that display a high potential to interact with the lipid surface of cell membranes. Inhibitory effects of synthetic peptides analogous to WWIHS-positive sequences of HCMV gB were evaluated. Human foreskin fibroblasts (HFF) were infected with the Towne GFP strain of HCMV (0.5 MOI) preincubated with peptides at concentrations ranging from 78 nm to 100 μM, and GFP positive cells were visualized 48 hours post infection by fluorescence microscopy or analyzed quantitatively by flow cytometry. Peptide 174-200 showed 80% inhibition of viral infection at a concentration of 100 μM, and 51% and 62% inhibition at concentrations of 5 μM and 2.5 μM, respectively. Peptide 233-263 displayed 97% and 92% inhibition at concentrations 100 μM and 50 μM, respectively, and 60% inhibition at a concentration of 2.5 μM. While peptides 264-291 and 297-315 separately did not inhibit viral infection, when added together they showed 67% inhibition at a concentration of 0.125 μM each. The ability of peptides to inhibit HCMV infection required the interaction of peptides with virions. Peptides designed to target potential fusogenic domains of gB may prove to be novel therapeutics to thwart herpesvirus infection.
Keywords: HCMV, infection, peptide, therapeutics

[P3.32]
DIFFERENTIAL SUSCEPTIBILITY TO CYTOMEGALOVIRUS INFECTION BY TWO EXTRAVILLOUS TROPHOBLAST-LIKE CELL LINES

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Human cytomegalovirus (HCMV) is the leading cause of congenital viral infection in the United States. It is well established that infection of cytotrophoblast cells (CTBs) by HCMV at the fetal-maternal interface, results in complications during pregnancy. The exact mechanism required for HCMV binding and entry into CTBs is still unclear. Epidermal growth factor receptor (EGFR) and platelet-derived growth factor-α receptor (PDGFR) are two known receptors utilized for binding and entry by HCMV in fibroblast and endothelial cells. The aim of this study is to ascertain the surface receptors required for HCMV binding and internalization in the CTBs cell lines, SGHPL-4 and SGHPL-5. Initially, studies were performed to compare the infection kinetics between the two cell lines using laboratory and clinical strains of HCMV. Interestingly, SGHPL-5 cells displayed minimal viral protein expression, even at a high multiplicity of infection (MOI=50). Further studies suggested that HCMV was internalized in the SGHPL-5 cells, but that viral translocation to the nucleus appeared abrogated and no viral replication occurred. Flow cytometry and western blot assays were undertaken and different levels of surface receptor expression were observed between the two cell lines. Of interest was the marked decrease in EGFR levels and varying expression of numerous integrins co-receptors in the SGHPL-5 cells. These data suggest, that although the two cell lines are currently used interchangeably, there are important phenotypic differences between them. Based on currently accepted surface receptor profiles, our data suggest that the SGHPL-5 cells are further differentiated as opposed to the SGHPL-4 cells. This information provides a strong model to investigate the exact receptors required for HCMV binding and internalization steps in CTBs. With this information, therapy design can be maximized against entry pathways utilized by HCMV during pregnancy.
Keywords: cytotrophoblast cells, cytomegalovirus, tyrosine kinase receptors, integrins

[P3.33]
INFECCIÓN DIFERENCIAL DEL TROFOBLASTO PLACENTARIO POR *TRYPANOSOMA CRUZI* IN VITRO

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Congenital Chagas' disease is caused by *Trypanosoma cruzi*. Chorionic villous does not allow a productive infection.
The Objective was analyze the susceptibility of the syncytiotrophoblast (STB) and of the underlying cytotrophoblast (CTB) of chorionic villi to infection by *T. cruzi* in vitro.
It was employed normal human placentas (n=9) in three models of interaction with 106 T. cruzi trypomastigotes. 1) **Isolated trophoblast**: Employing CTB and differentiated STB for 48h. 2) **Treated placental explants**: denuded of its STB co-cultured for 96h. 3) **Untreated placental explants** (complete placental barrier) co-cultured for 96h. VERO cells as control. **Statistic Analysis** was performed using Student't test and ANOVA. ($p<0.05$).
Cells and tissue were stained with H/E, Giemsa and Cytokeratine-7 for CTB. It was analyzed infection and quantified (Axiovision software), β-hCG and nitrites, and parasite viability.
Level of infection of **isolated STB** and **CTB** were: T. cruzi occupied 1,38% and 12,07% of the total area; 42 and 803 parasites per 100 syncytial or cell nuclei, 12,25% and 41,40% of live parasites in the media culture, respectively. Differences were statistically significant ($p<0,05$).
Level of infection of **untreated explants** and **treated explants** were: T. cruzi occupied 0.019% and 1,33% of the total chorionic villi area, 4,5±2 and 54,75±43,98 amastigotes per nest, 3±0,25 and 31±2,58 total nests, 0,96% and 100% of live parasites in the supernatant media, respectively. Differences were statistically significant ($p<0,05$).
Isolated cytotrophoblast and denuded chorionic villi are very susceptible to infection by *T. cruzi*, contrary to the isolated syncytiotrophoblast and complete chorionic villi. Any alteration in the syncytial layer of the chorionic villi and even the normal renewal of the STB could favor the infection of underlying cells such as cytotrophoblast and other resident cells, which could provide a window for T. cruzi infection of the chorionic villi, leading to the congenital Chagas infection.
Grants: SECyT-UNLaR, SECyT-UNC and MINCyT-Córdoba (PID 48).
Keywords: Chagas disease, Trypanosoma cruzi, trophoblast, infection

[P3.34]

TRYPANOSOMA CRUZI INDUCES APOPTOSIS IN HUMAN CHORIONIC VILLI EXPLANTS

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Chagas' disease, one of the major public health concerns in Latin America, is caused by the haemophlagelated protozoan *Trypanosoma cruzi*. In vector related diseases, it is second to malaria in prevalence and mortality (1). In the past few years congenital transmission of *T. cruzi* has become more important, and partly responsible for the "globalization of Chagas' disease" (2), constituting a public health problem of increasing relevance (3).

Diverse pathogens, including *T. cruzi*, are able to cross the placental barrier and infect both the placenta and fetus (4, 5). Parasite invasion in cell cultures has been studied in some depth. On the other hand, studies that analyze parasite invasion in tissues and organs are scarce.

The activation or prevention of cell death seems to be a critical factor in the outcome of an infection since it can facilitate or difficult the pathogen control and spreading. Apoptosis in the hosts can be managed during the infection with microorganisms, such as parasites (6). *T. cruzi* can induce, delay or inhibit apoptosis in host cells (7).

In order to determine induction of apoptosis of the chorionic villi tissue during parasite invasion into placental villi, we incubate explants of human chorionic villi with 105 and 106 trypomastigotes for 24 hours. Induction of apoptotic cell death was determined by TUNEL analysis, measurement of caspase 3- like activity and immunohistochemical detection of caspase cleaved cytokeratin. Effective infection was tested by inmunohistochemistry (Ac-cruzipain) and PCR. *T. cruzi* induces apoptosis in chorionic villi, evidenced by increase in TUNEL positive cells (Fig 1), caspase 3 like activity (Fig 2) and appearance of cytokeratin 18 neo-epitope (Fig 3). Our results suggest that the induction of apoptosis in chorionic villi helps the parasite to escape from the immune response; alternatively, it could also be a protective mechanism of the placental tissue.

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Keywords: *Trypanosoma cruzi*, mechanism of tissue invasion

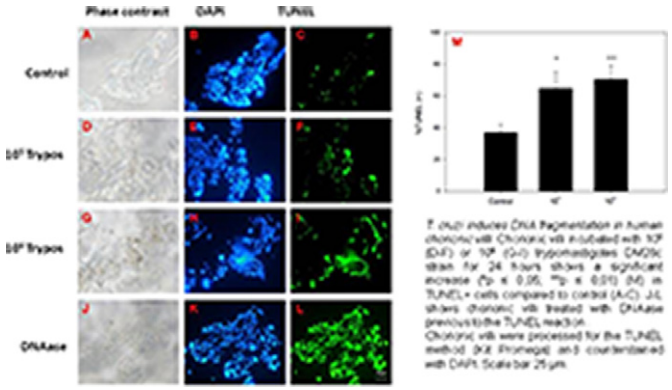
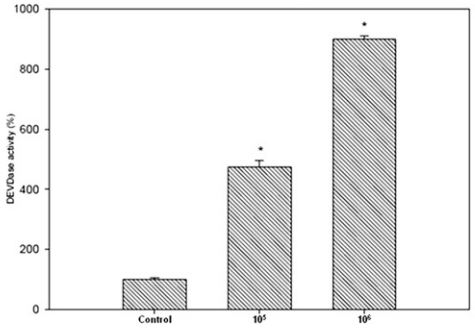
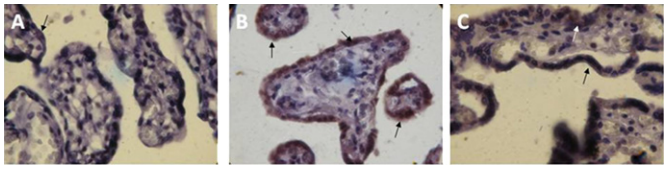


Figure 1.



T. cruzi induces caspase-3-like activity in human chorionic villi. Chorionic villi incubated with 10^5 or 10^6 trypomastigotes DM28c strain for 24 hours shows a significant increase in caspase-3-like activity (DEVDase). Results are expressed as a percentage relative to the control chorionic villi caspase-3-like activity. Data are means ± S.E.M. P<0.01.

Figure 2.



T. cruzi induces caspase-mediated cleavage of cytokeratin 18: Chorionic villi incubated with 10^5 (B) or 10^6 (C) trypomastigotes DM28c strain for 24 hours were immunostained for cytokeratin 18 neo-epitope, an apoptosis marker for epithelial cells. Chorionic villi incubated with the parasites (B and C) shows a strong immunostaining for cytokeratin 18 neo-epitope (arrows) compared to the control chorionic villi (A). Bar scale: 25 µm.

Figure 3.

Keywords: Trypanosoma cruzi, chorionic villi, mechanism, infection

[P3.35]

TRYPANOSOMA CRUZI PRODUCE PLACENTAL STRUCTURAL ALTERATIONS ASSOCIATED TO INCREASE OF OXIDATIVE STRESS IN VITRO

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Human placenta would participate actively in the control of congenital Chagas infection, which would explain, partially, the low transmission rate. The production of pro-inflamatory citokynes such as TNFα among others, promotes nitric oxide (NO) production by the endothelial placental nitric oxide synthase (eNOS) that could be an important part of this control. Nitric oxide reacts with oxygen species and produce peroxynitrites are both trypanosides and potentially deleterious for the placental tissue.
Objetive: Analyze the structural alterations of the first placental barrier, the syncytiotrophoblast (STB), induced by two populations of *T. cruzi* through increased TNFα, NOS and nitrosylated proteins (oxidative stress).
Methods: Placental villi explants co-cultured for 24 hs with 1x106 trypomastigotes of Tulahuen and Lucky strains (isolated from a congenital case); controls without parasites, and with addition of crescent concentrations of H_2O_2 (50 μM, 100 μM, 200 μM and 500 μM). Histological and Immunohistochemical analysis: of eNOS and Nitrotyrosine (NT) (SigmaScan) and detachment area of syncitiotrophoblast. In culture media: quantification of NO (Griess), TNFα (ELISA). NOSe RNA expression by RT-PCR.
Results: Placental explants in presence of both strains of *T. cruzi* showed a significant rise of TNFα production, tyrosin nitrosilation and eNOS expression, but a non significant increase of NO production. The structural alterations (STB detachment) were significantly higher in presence of *T. cruzi* and with H2O2 in a concentration dependent manner.
Discussion: The presence of *T. cruzi* in an in vitro model produced detachment of the STB, associated with the increased production of TNFα, expression of eNOS and oxidative stress, which could facilitate the infection of the chorionic villi. These results could explain the association of some clinical aspects of the congenital Chagas disease, such as prematurely and miscarriages, with the alterations associated with *T. cruzi* infection showed and a new possible route of placental infection via chorionic villi.
Grants: SECyT-UNLaR; SECyT-UNC and MINCyT-Córdoba (PID 48)
Keywords: Congenital Chagas disease, Placenta, Oxidative stress, Trypanosoma cruzi

[P3.36]

REPRODUCTIVE PERFORMANCE AND PLACENTAL HISTOPATHOLOGY OF RATS INFECTED WITH *TRYPANOSOMA BRUCEI BRUCEI* DURING PRE-MATING PERIOD

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Introduction: Animal trypanosomosis, caused by some species of trypanosomes, is an economically important disease of the livestock industry, especially in Africa. It has many clinical manifestations (Taylor and Authie, 2004) with the subspecie *Trypanosoma brucei brucei* reportedly causing severe reproductive disorders in infected animals (Ikede et al., 1988; Sekoni, 1994). The present study examined the reproductive performance of female rats infected with *T. brucei brucei* and their placental pathology.
Methods: Ninety-eight mature wistar rats comprising 70 females and 28 males were used for the study. The females were divided into 4 groups: 25 infected 6 days prior to mating (I6BM), 10 infected non-pregnant (INPC), 25 uninfected but pregnant (UC) and 10 uninfected but sacrificed immediately post delivery (UCSD). Each infected rat received 2.50 x 105 trypanosomes in normal saline intraperitoneally. Packed cell volume (PCV), level of parasitemia (LOP), and pregnancy-related parameters were evaluated. Sections of the placentae routinely processed for histopathology were stained with hematoxylin and eosin while selected sections were stained by Price's Giemsa method.
Results: The rats of I6BM group had more matings before conception ($P<0.05$), longer length of gestation ($P<0.05$) and reduced litter size ($P<0.05$) than the UC group. The I6BM group also had more resorbed fetuses ($P<0.05$) than the UCSD group. In addition, longer survival time and comparatively lower ($P<0.05$) LOP were observed in this group (I6BM) than in the INPC group. The mean PCV of the I6BM and INPC groups were similar but significantly ($P<0.05$) lower than that of the UC group. Placental histology of the infected rats revealed infiltration of inflammatory cells indicative of placentitis.
Discussion: We attribute the observed prolonged gestation among the infected pregnant rats to placental insufficiency and to possible production of interleukin-4 (IL-4) and interferon-gamma (IFN-gamma), which have been reported to promote antibody production and control the level of parasitemia in infected animals that are pregnant.
Keywords: trypanosomes, placenta, infection, histopathology

[P3.37]

EMBRYONIC DEATH DUE TO *TRITRICHOMONAS FOETUS* IS ASSOCIATED WITH INCREASED TH17 AND DIMINISHED HO-1 LEVELS IN A MURINE MODEL

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Bovine genital tritrichomonosis is a venereal disease produced by the flagellate **Tritrichomonas foetus.** The disease is characterized by the repetition of estrus and infertility due to embryonic death. We reproduced genital tritrichomonosis in pregnant *Balb/c* mice, where embryonic death occurs between days 5 and 8 of pregnancy. The pathogenic mechanism of the embryonic death in tritrichomonosis is unclear. We hypothesized that embryonic death may be due to an exacerbated maternal immune response to the pathogen which may lead to increased levels of Th1 cytokines and to down-regulation of pregnancy-important mechanisms as regulatory T cells or TGF-β production. Heme oxygenase-1 is an important molecule whose up-regulation is related to tolerance to paternal alloantigens. Here, we analyzed the expression of pro- and anti-inflammatory cytokines as well as of HO-1 in uterine tissue of infected mice by *real time* RT-PCR in infected and non-infected control pregnant animals. TNFα, a Th1 cytokine, was augmented in infected mice. However, IL-10 and IL-4, Th2 cytokines, were also up-regulated at augmented at mRNA level in infected vs. non-infected mice. Accordingly, foxp3, a Treg-associated- gene was more expressed in the uterus of infected mice compared to controls. In mice which have lost their conceptus, HO1 mRNA was decreased. The RORγt mRNA which is a reliable marker for Th17 cells is augmented in the uterus of infected mice. In the light of our data, we hypothesized that a T effector response of type 1 may be responsible of the embryonic death during tritrichomonosis, which alters protective mechanisms of the embryo as e.g. HO-1 expression are altered. Increased Treg levels may further facilitate embryonic death by promoting the persistence of infection due to its immunosuppressive effects. In conclusion, our model reveals immunological mechanisms behind **Tritrichomonas foetus**-mediated embryo death and may be of help in targeting this disease in the future.

Keywords: embryonic death, Tritrichomonas foetus, Heme oxygenase 1, T effector response

[P3.38]

THE MALARIAL PARASITE TOXIN, HEMOZOIN, ACTIVATES MAP KINASES AND PROMOTES A CHEMOTACTIC AND IMMUNOSTIMULATORY SECRETORY RESPONSE IN PRIMARY HUMAN SYNCYTIOTROPHOBLAST

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Malarial infection during pregnancy is characterized by sequestration of parasitized erythrocytes and accumulation of the parasite hemoglobin metabolite, hemozoin, and maternal mononuclear cells in the intervillous space. We have shown that primary syncytiotrophoblast cells respond immunologically to cytoadherent *Plasmodium falciparum*, in an in vitro culture system, but their responsiveness to hemozoin, a potent pro-inflammatory stimulator of monocytes, macrophages and dendritic cells, has not been reported. Immunoblotting of primary syncytiotrophoblast lysates revealed that exposure to hemozoin induced ERK1/2 and JNK mitogen-activated protein kinase phosphorylation, the former response being sensitive to inhibition of the upstream MEK1/2 kinase. These cells subsequently secreted the chemokines CXCL8, CCL3, and CCL4 and released soluble intercellular adhesion molecule-1 and soluble TNF receptors I and II as detected by ELISA. The stimulated cells also elicited the specific migration of peripheral blood mononuclear cells in a two-chamber assay. Finally, exposure of primary monocytes to conditioned medium from hemozoin-stimulated syncytiotrophoblast resulted in the upregulation of intercellular adhesion molecule-1 on the monocytes. All of these responses were specific to hemozoin, since synthetic hemozoin was relatively inert. Furthermore, the dependence of the hemozoin responses on mitogen-activated protein kinase stimulation was confirmed by inhibition of chemokine release in syncytiotrophoblast pre-treated with MEK1/2 inhibitor. These findings confirm an immunostimulatory role for native hemozoin and expand the cell types known to be responsive to this molecule to include fetal syncytiotrophoblast. Finally, the results provide further evidence that syncytiotrophoblast cells can influence the local maternal immune response to placental malaria.

Keywords: syncytiotrophoblast, chemokines, infection, malaria

[P3.39]

ADHERENCE OF PLASMODIUM FALCIPARUM INFECTED RED CELLS TO THE TROPHOBLAST AND PLACENTAL INFLAMMATORY RESPONSE STUDIED BY DUAL EX VIVO PERFUSION OF AN ISOLATED COTYLEDON

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Introduction: Pregnancy-associated malaria (PAM) results in maternal anemia and poor birth outcome. Pathogenesis of PAM is associated with accumulation of malaria-infected erythrocytes (irbc) in the intervillous space of the placenta. The *Plasmodium falciparum* (Pf) erythrocyte membrane protein-1(PfEMP-1) variant Var2CSA mediates the adherence of irbc only in the schizont stage to syncytiotrophoblastic membrane via binding to chondroitin A sulphate. Here we investigate the adherence of malaria infected erythrocytes and placental inflammatory response of the trophoblast using the dual ex vivo perfusion of an isolated cotyledon.
Method: Following a 60 min closed loop perfusion of both circuits the experiment consists of three phases, each lasting 120 min. In phase I the perfusate consists of medium alone, in phase II uninfected red cells (urbc) and in phase III again urbcs or red blood cells infected with either CS2 (placental adherent) or 3D7 (placental non-adherent) strain of Pf are suspended in perfusate at a hematocrit of 10%. Samples were collected from the maternal and fetal perfusate at 15 or 30 min intervals and tissue biopsies were taken before and after perfusion.
Results: Schizont stage of the placental-adherent strain CS2 showed a marked decline within 45 minutes of adding parasites indicating sequestration of the parasite in the placenta, whereas the non-adherent ring and early trophozoite stages did not (n=1). By contrast the non-placental adherent 3D7 failed to show any sequestration (n=2). The release of the macrophage chemokine MIF into perfusate without red cells (n=5) or with urcbs (n=7) increased with time. When malaria parasites are added in phase III, MIF release almost doubles (n=3). Microarray based gene expression analysis data from perfused tissue showed substantial upregulation of a number of early pro-inflammatory mediators such as the cytokines *IL-1ß, TNFα, IL-6*, the chemokines *IL-8, MIP-1α, CCL3*, etc and their receptors. Addition of Pf irbcs induced further expression changes notably an increase in c-fos (n=3).
Summary: The preferential sequestration of the CS2 strain in the IVS is seen ex vivo in dually perfused placental tissue. The release of the macrophage chemokine MIF and upregulation of the gene encoding the transcription factor c-fos, both of which are associated with immunoregulatory responses seem to be malaria induced, whereas the appearance of other chemokines and proinflammatory cytokines is an unspecific response to oxidative and hypoxic stress related to ex vivo perfusion. The release of the chemokines and cytokines is directed towards the maternal compartment with only trace amounts detectable in the fetal circuit.
Keywords: placental malaria, trophoblast adherence, inflammatory response, gene profile

[P3.40]

ACTIVATION OF PPARGAMMA BY HUMAN CYTOMEGALOVIRUS FOR DE NOVO REPLICATION IMPAIRS MIGRATION AND INVASIVENESS OF CYTOTROPHOBLAST FROM EARLY PLACENTA

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Human cytomegalovirus (HCMV) contributes to pathogenic processes in immuno-suppressed individuals, in fetuses and in neonates and infection during the first trimester of pregnancy is known to cause miscarriages and low-birth weight newborns. It is known that infection of the placenta precedes the transmission to the fetus. HCMV takes advantage of the host response to facilitate and enhance its replication and use the cyclo-oxygenase-2 (Cox-2)-dependent prostaglandin pathway for transcription of the essential immediate-early gene IE2. However, no molecular mechanism was reported so far. The fact that Cox-2 activation could serve as a source of ligand for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPARg) and that PPARg plays a pivotal role in controlling human trophoblast invasion led us to hypothesize that HCMV could impair early placentation through activation of PPARg.
Methods and findings: By using reporter gene activation assays and confocal microscopy in the presence of specific antagonist, we show for the first time that HCMV infection induced PPARg transcriptional activity in infected cells. We demonstrated that PPARg antagonist dramatically impaired IE2 mRNA expression and virus production and that the major immediate-early promoter (MIEP) contained PPAR response elements (PPRE) able to bind PPARg as assessed by EMSA and ChIP assays. We then provided evidence that by activating PPARg, HCMV dramatically impaired early human trophoblast migration and invasiveness, as assessed by using well-established *in vitro* models of invasive trophoblast *i.e.* primary cultures of extravillous cytotrophoblasts isolated from first trimester placentas and the extravillous cytotrophoblast-derived cell line HIPEC.
Conclusions: Our data provide the first evidence that HCMV use the transcriptional activity of PPARg for its replication and as a consequence inhibit trophoblastic cell invasion. This novel finding provides new clues to explain how infection during the first trimester of pregnancy could impair implantation, placentation and therefore embryonic development.
Keywords: Trophoblast invasion, CMV infection, PPARgamma, human placentation

[P3.41]

THE ROLE OF HOFBAUER CELLS AND DECIDUAL MACROPHAGES IN MOTHER-TO-CHILD TRANSMISSION OF HIV-1

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During pregnancy, *in utero* HIV-1 infection is a relatively rare occurrence so that most transmission events occur at delivery or postpartum through breastfeeding. Even in the absence of antiretroviral therapy, 90% of fetuses evade *in utero* transmission, which occurs most commonly during the third trimester suggesting that the placenta plays an on-going role in controlling HIV-1 transmission. Cells within the placenta consist of decidual cells whose lineages including natural killer, regulatory T and antigen presenting cells. Intriguingly these cells interdigitate with the endometrial blood supply, while expressing DCSIGN, CD4 and CCR5 on their cell surface. In a pregnant female with HIV-1 infection, virus-exposed decidual cells expressing these ligands should be readily infectable targets with migratory properties to disseminate virus and facilitate *in utero* HIV-1 transmission. The reason why *in utero* transmission occurs only rarely is not entirely clear but could reflect the limiting nature or permissivity of decidual antigen presenting cells (dAPCs) to cell-free HIV-1 infection and/or the absence of cellular activation in the placental milieu. These properties may contrast with other APC subpopulations in mucosa and blood in respect of uptake and transport of HIV-1, and/or the unique migratory properties of dAPCs. In this study, we examined evidence of infection and immunity in dAPCs within the placenta, specifically CD68+ HLA-II+ CD14low S100+/- CD83- CD86- cmrf-44- cells in chorionic villi, consistent with Hofbauer cells [HCs] and also CD68+ HLA-II+CD14high S100- CD83- CD86- cmrf-44- decidual macrophages (DMs). Here we show by viral infectivity assays and flow cytometry that HCs and DMs are capable of supporting HIV-1 replication. Upon activation, these cells have limited capacity to activate, migrate and infect peripheral and cord blood mononuclear cells which may account for reduced MTCT of HIV-1. Potential restriction factors include TRIM5α, APOBEC-3G, and tetherin.

Keywords: HIV, Hofbauer, Macrophage, Decidua

[P3.42]

ROLE OF PROSTAGLANDINS IN THE PRETERM DELIVERY INDUCED BY SHIGA TOXIN IN RATS IN THE LATE STAGE OF PREGNANCY

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Infection associated with Shiga toxin-producing Escherichia coli (STEC) and subsequent Hemolytic-Uremic Syndrome (HUS) became relevant in public health since it was considered as one of the most important emergent pathogens. The major virulence factor of STEC is Shiga toxin type 1 or 2 (Stx1, Stx2). Shiga toxin-producing *Escherichia coli* (STEC) infections could be one of the causes of fetal morbimortality in pregnant women. We had reported that rats in the late stage of pregnancy treated with Stx2 induced premature delivery of dead fetuses.

Our previous results suggest that nitric oxide (NO) is partially involved in these mechanisms, since aminoguanidine, an iNOS specific inhibitor, blocks the effects of Stx2 on rat pregnancy.

Objective: Our aim was to evaluate the role of prostaglandins (PGs) in the mechanisms by which Stx2 induced preterm delivery.

Materials and methods: Pregnant rats on days 14-16 of gestation were i.p. injected with culture supernatant from recombinant *E. coli* containing 0.4 μg/ml Stx2 and 30 ng/ml LPS (sStx2).

PGE and PGF2α levels were evaluated by radioimmunoassay analyses in deciduas and placentas from treated rats killed 12 h post sStx2 injection.

Results: Injection of sStx2 induced preterm delivery of live fetuses and dead fetuses depending on the toxin concentration.

PGs production was detectable in deciduas and placenta from control animals. Stx2 increased the PGs levels in both tissues of treated rats in a dose dependent manner 12 h post-injection ($p<0.05$).

Conclusions: Our results suggest that the increased of PGs could be modulated by the increased of iNOS activity observed in these experimental conditions. Further experiments could be necessary to clarify if PGs play a central role in Stx2-induced preterm delivery.

Keywords: Shiga toxin, rat pregnancy, infection, prostaglandins

[P3.43]

VITAMIN D REGULATES CATHELICIDIN ANTIMICROBIAL PROTEIN, IL1 AND TNF, BUT NOT GROWTH, IL12A OR IL27 IN CULTURED HTR-8/SVNEO HUMAN PLACENTAL TROPHOBLASTS

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The placenta synthesizes active 1, 25-dihydroxyvitamin D_3 [1, 25(OH)2D3], which may suppress the maternal immune system by regulating placental immunomodulatory peptides and hormones. Whether it also regulates placental growth is unknown. To determine the activation of vitamin D by trophoblasts in vitro and its regulation of placental immunomodulatory peptides and growth, HTR-8/SVneo human trophoblasts were cultured with 1, $25(OH)_2D_3$ or 25-hydroxy-vitamin D_3 [$25OHD_3$] (100-1000 nM) or 0.1% ethanol (C) for 24 h. To determine its temporal regulation, trophoblasts were cultured with C or 1, $25(OH)_2D_3$ (0.1- 100 nM) for 48-96 h. Interleukins (IL1β, IL12A and IL27), tumor necrosis factor α (TNFα), cathelicidin antimicrobial peptide (CAMP), Ki-67, and human chorionic gonadotropin (hCG) mRNA levels were measured by RT-PCR. Growth and media hCG levels were also measured. 1, $25(OH)_2D_3$ and $25OHD_3$ both increased CAMP mRNA levels 1 fold ($p<0.001$), but did not affect IL-27 and IL-12 A mRNA levels at 24 h. 1, $25(OH)_2D_3$ increased CAMP mRNA levels 48-96 h (10 and 100 nM) or 72-96 h (1 nM) 1-2.5 fold ($p<0.001$). 1, 25(OH)2D3 (0.1-100 nM) also slightly increased TNFα mRNA levels (0.5-0.75 fold) at 96 h ($p< 0.001$), but only 10 nM 1, $25(OH)_2D_3$ increased IL1β slightly (0.5 fold) at 96 h ($p<0.001$). Vitamin D did not affect growth of the cells from 24-96 h. 1, $25(OH)D_3$ (1-100 nM) decreased hCG mRNA levels approximately 0.4 fold only at 48 h, but did not alter secreted hCG levels. HTR-8/SVneo human trophoblasts do activate $25OHD_3$, which does regulate immunomodulatory peptide expression in these cells. Vitamin D, thus, may modulate inflammation at the maternal-fetal interface and trophoblast differentiation through its regulation of TNF-α and IL-1β and may play a role in protecting the maternal-fetal interface and placental tissue from infection through its regulation of innate immunity and CAMP.

Keywords: vitamin D, trophoblasts, cytokines, innate immunity

[P3.44]

INFLAMMATION-MEDIATED INTRAUTERINE GROWTH RESTRICTION IN RATS IS LINKED TO DEFICIENT UTERO-PLACENTAL PERFUSION

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A maternal inflammatory response during pregnancy is causally linked to the development of intrauterine growth restriction (IUGR). Although the mechanism underlying the development of inflammation-associated IUGR has not been fully characterized, there is evidence that impaired placental perfusion plays a key role. In this study, we used a model in which pregnant rats were given chronic injections of *E. coli* lipopolysaccharide (LPS) to determine whether aberrant maternal immune activation has a detrimental effect on the structure of the utero-placental vasculature resulting in IUGR. Compared with pups from saline-injected dams, pups from rats receiving daily intraperitoneal injections of LPS (40μg/kg) on gestational days 14.5, 15.5, 16.5 and 17.5 were significantly growth restricted ($p<0.001$). Utero-placental vascular corrosion casts made three hours after the final LPS injection revealed abnormalities in the structure of spiral arterioles and maternal arterial channels (MAC). Immunohistochemistry revealed decreased endovascular trophoblast infiltration in the spiral arterioles of LPS-treated rats as compared to saline-treated controls; distally (away from the MAC), many of these vessels lacked trophoblasts. LPS-mediated IUGR was also associated with fibrin deposition in the lumen of the utero-placental vasculature. Together, these results reveal that abnormal vascular structure is linked to the development of inflammation-associated IUGR. Our findings support a rationale for investigating a potential use of immunomodulatory agents in the prevention of IUGR.

(Supported by the Heart and Stroke Foundation of Ontario).

Keywords: Lipopolysaccharide, Inflammation, Utero-placental perfusion, Endovascular trophoblast invasion

[P3.45]

OXYGEN SENSITIVE POTASSIUM CHANNELS MODULATE HUMAN CHORIONIC GONADOTROPIN (HCG) SECRETION IN HUMAN TERM SYNCYTIOTROPHOBLAST

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Introduction: Potassium (K+) channels regulate cellular proliferation, differentiation, apoptosis and hormone secretion in many tissues. These processes maintain syncytiotrophoblast and are influenced by oxygen tension (O2). Here we test the hypothesis that O2-sensitive K+ channels modulate hCG secretion from term placental syncytiotrophoblast.

Methods: Placental explants from normal pregnancy were cultured at 21% (n=8), 6% (n=8) and 1% (n=3) O2 for 6 days. Medium was collected to measure hCG secretion and lactate dehydrogenase (LDH) release, a marker of necrosis. On days 3-5, explants were treated with 5mM 4-aminopyridine (4-AP) or 5mM tetraethylammonium (TEA), blockers of O2-sensitive voltage-gated (KV) and calcium-activated (KCa) K+ channels. At day 6, tissue was lysed in H2O and hCG measured to indicate cellular levels. To assess the effect of O2 on K^+ permeability, 86Rb efflux was determined (day 6) in untreated explants. Data are median +/- IQR.

Results: At culture day 6, hCG secretion (mIU/ml/hr/mg protein) was significantly lower at 6% than 21%O2 (0.6 +0.3/-0.8 vs 1.2 +0.8/-2.1; $p<0.02$) and further reduced at 1%O2 (0.3 n=3). At 21%O2, 4-AP and TEA significantly inhibited hCG secretion (day 6) (33% +30/-36 and 58% +51/-78 of control; $p<0.03$) but had no effect at 6% or 1%O2. LDH release was similar in all conditions. 4-AP/TEA did not alter cellular hCG at 21% or 6% O2 (n=4). 86Rb efflux from explants maintained at 6%O2 was half that at 21%O2 (n=2).

Discussion: We propose that low O_2 inhibits hCG secretion from syncytiotrophoblast by inactivating K+ channels. KV/KCa channels may mediate this effect as 4-AP/TEA inhibited hCG secretion at 21%O2 but not at lower O2 when the channels may already be inactivated. Reduced O2 is a feature of specific pregnancy pathologies and inactivation of KV/KCa could contribute to abnormal maintenance of syncytiotrophoblast in these conditions.

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Keywords: human placenta, ion channels, hypoxia

[P3.46]

SPREADING ENDOTHELIAL CELL DYSFUNCTION: SOLUBLE FACTORS RELEASED FROM ENDOTHELIAL CELLS THAT HAVE PHAGOCYTOSED NECROTIC SYNCYTIAL KNOTS CAUSE DYSFUNCTION OF ADDITIONAL ENDOTHELIAL CELLS

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Introduction: Preeclampsia is characterised by hypertension and proteinurea and these maternal symptoms are thought to be dependent upon endothelial cell dysfunction. The cause of preeclampsia is unclear but it is known to be triggered by a factor from the placenta. One of the possible triggering factors is dead fragments of the placenta, called syncytial knots, which become trapped in the maternal lungs where they are phagocytosed by the endothelium of the pulmonary capillaries. We have previously shown phagocytosis of necrotic syncytial knots induced endothelial cell activation involving release of inflammatory cytokines whereas, phagocytosis of apoptotic syncytial knots did not affect endothelial cells. We hypothesised that factors released from endothelial cells after they have phagocytosed necrotic syncytial knots would adversely affect multiple functions of endothelial cells from remote sites.
Methods: Conditioned medium from endothelial cell monolayers that had phagocytosed either apoptotic or necrotic syncytial knots that had been shed from an in vitro placental explant model were centrifuged then exposed to fresh subconfluent endothelial cell monolayers. The proliferation of endothelial cells was measured by Alamar Blue assay and endothelial cell surface endoglin was quantified by ELISA.
Results: The proliferation of endothelial cells was significantly ($p = 0.004$) reduced after treatment with conditioned medium from endothelial cells that had phagocytosed necrotic but not apoptotic syncytial knots. In addition, endothelial cell surface endoglin levels were significantly ($p = 0.001$) reduced after treatment with conditioned medium from endothelial cells that had phagocytosed necrotic but not apoptotic syncytial knots.
Discussion: Our results suggest a mechanism to explain how necrotic syncytial knots phagocytosed by endothelial cells in the maternal pulmonary capillaries could lead to the systemic endothelial cell activation that is characteristic of preeclampsia.
Keywords: trophoblast deportation, endothelium dysfunction, cytokines

[P3.47]

PLACENTA-SPECIFIC MIRNAS DERIVED FROM THE MIRNA CLUSTER IN THE CHROMOSOME 19 WERE UPREGULATED IN PRE-ECLAMPSIA PLACENTAS

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Objective: Pre-eclampsia (PE) increases not only maternal but also infantile morbidity/mortality. Early detection of PE is one of the most important issues in Perinatology. Micro RNAs (miRNAs) are single-stranded non-coding RNAs of approximately 22 nucleotides in length, and accumulating evidence indicates that they serve as promising biomarkers for various diseases. In this study, with an aim to discover miRNAs as promising biomarkers for PE, we quantitatively determined differential expression of miRNAs in placentas from normal and PE pregnancy using a real-time PCR-based miRNA array.
Materials and Methods: Human placentas from normal and severe PE were obtained according to protocols approved by the Nippon Medical School Hospital Ethics Committee and the Jichi Medical School Ethics Committee. Total RNAs from normal and PE placentas (n=10 for each) were isolated. Subsequently miRNA-derived cDNAs were synthesized using Megaplex Primer Pools (Applied Biosystems, Foster City, CA, USA), and subjected to real-time PCR analyses using TaqMan Array microRNA Cards (Applied Biosystems). MiRNAs with significantly differential expression were identified by statistical analyses using Mann-Whitney U Test. MiRNAs upregulated significantly in PE placentas were further analyzed by the laser microdissection (LMD) of human placenta sections to examine which cell types express them.
Results: We identified 44 miRNAs upregulated significantly ($p < 0.05$) in placentas from PE pregnancy, 9 of which were placenta-specific miRNAs derived from the primate-specific miRNA cluster in the Chromosome 19. LMD analyses revealed that most of the upregulated placenta-specific miRNAs were predominantly expressed in villous trophoblasts..
Conclusion: This study revealed for the first time that placenta-specific miRNAs were deregulated in PE placentas. Thus, these miRNAs could be promising biomarkers for the diagnosis, especially early diagnosis, of PE.
Keywords: microRNA, preeclampsia, laser microdissection, miRNA array

[P3.48]

INJECTION OF NECROTIC TROPHOBLASTIC CELLS INCREASED BLOOD PRESSURE IN THE FINAL THIRD OF GESTATION IN RATS

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During pregnancy large numbers of dead trophoblastic cells are extruded from the placenta into the maternal blood. In normal pregnancy these deported trophoblasts are thought to die via programmed cell death whereas, in preeclampsia they may die by a more necrotic mechanism. Many deported trophoblasts become lodged against the capillary endothelium of the maternal lungs. We have shown, in vitro, that phagocytosis of necrotic trophoblasts leads to activation of endothelial cells, which is a hallmark of preeclampsia. We postulate that necrotic deported trophoblasts are one of the placental factors that trigger the maternal endothelial cell activation and vascular dysfunction of preeclampsia. This preliminary study was undertaken in order to investigate whether necrotic trophoblastic cells would induce hypertension in vivo.

Groups of 3 female Wistar rats were chronically instrumented with a continuously recharging blood pressure telemetry device (Telemetry Research) and an injection port secured into the ascending Jugular. After recovery, the animals were date-mated and on day 6 of pregnancy were injected daily for the remaining two weeks of gestation with either, Jeg-3 cells (5x106 /body weight) that had been rendered necrotic by freeze-thawing, or with vehicle.

The mean arterial blood pressure (MAP) the two groups of rats was not significantly different during the second week of gestation but the MAP of the control rats declined in the last week of gestation in accordance with the literature. In contrast, the MAP of the group of rats injected with necrotic Jeg-3 cells did not decline and was significantly higher ($p=0.0001$) than the control group.

Our results suggest that necrotic trophoblastic cells can adversely affect blood pressure, increasing it significantly above the normal levels that would be expected in late gestation as occurs in preeclampsia. This work provides preliminary evidence that necrotic trophoblasts may be one of the placental triggers of preeclampsia.

Keywords: blood pressure, deported trophoblasts, necrotic, endothelium

[P3.49]

THE PROFORM OF EOSINOPHIL MAJOR BASIC PROTEIN (PROMBP) IS REDUCED IN FIRST TRIMESTER MATERNAL SERUM IN WOMEN DEVELOPING SEVERE PRE-ECLAMPSIA AND HELLP SYNDROME

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Background: Preeclampsia (PE) is a major contributor to fetal and maternal morbidity and mortality. Recently, the concentration of the pregnancy-associated proform of eosinophil major basic protein (ProMBP), the endogenous inhibitor of IGFBP-2 – and -4 protease and ligand of angiotensinogen, was found reduced in first trimester in pregnancies that developed PE. We examined the level of ProMBP in first trimester serum in PE pregnancies and the relation between clinical characteristics of PE and the ProMBP level.

Methods: The ProMBP concentration was determined in first trimester (gestational week 10^{+3} – 13^{+6}) serum samples from 123 PE pregnancies and 285 control pregnancies.

Results: ProMBP was reduced by 16% ($p = 0.001$) in PE pregnancies, and by 25 % in patients developing severe PE ($n = 21$) and HELLP syndrome ($n = 6$). ProMBP increased significantly with gestational age ($p = 0.001$) and maternal BMI ($p < 0.001$) in controls. PAPP-A and ProMBP correlated significantly in both controls and PE pregnancies. ProMBP as a screening marker for severe PE and HELLP syndrome had a detection rate of 45 % for a false positive rate of 20 %.

Conclusions ProMBP is reduced in first trimester maternal serum in pregnancies that develop severe PE and HELLP syndrome. The reduction in ProMBP could influence the bioavailability of IGF-I and –II at the maternal-fetal interface and thus affect trophoblast invasion or could interfere with the local renin-angiotensin system. The clinical usefulness of ProMBP as a marker for severe PE and HELLP syndrome remains to be clarified, particularly when compared to other markers.

Keywords: eosinophil major basic protein, first trimester, screening

[P3.50]

LEPTIN AND SOLUBLE LEPTIN RECEPTOR IN FIRST TRIMESTER PREGNANCY SERUM: A METABOLIC MARKER OF PREECLAMPSIA

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Background: The maternal serum concentration of the adipocytokine leptin (Lp) is increased in second and third trimester of pregnancies that develop pre-eclampsia (PE). We wanted to assess the significance of Lp and its soluble receptor (sLR) as well as the free leptin index (fLI) in first trimester sera from women developing preeclampsia (PE) and controls.
Design and Patients: Retrospective case control study using first trimester serum samples, 123 from singleton pregnancies that developed PE and 285 control pregnancies matched for gestational age, parity and maternal age, from the Copenhagen First Trimester Screening Study.
Results: Maternal serum Lp was significantly increased, median 22.7 ng/mL (range: 5.0 – 70.1 ng/mL) in PE pregnancies compared to median 13.9 ng/mL (range: 2.0 – 54.3 ng/mL) in controls ($p < 0.001$). On the contrary, sLR was significantly reduced, median 27.7 ng/mL (range: 14.3 – 100.9 ng/mL) in PE pregnancies compared to controls, median 36.9 ng/mL (range: 15.1 – 71.8 ng/mL) ($p < 0.001$). There was no significant correlation between neither the Lp nor sLR level and clinical severity of PE. In patients developing severe PE there was a significant negative correlation (Spearman's rho = -0.501, $p = 0.03$) between the level of TNFα and fLI, whereas in patients developing mild PE, this correlation was positive (Spearman's rho = 0.403, $p < 0.008$). In controls no correlation was found between TNFα and fLI.
Conclusions: The constantly increased Lp and fLI in PE pregnancies in first trimester suggests that either may be very early and easily used markers of PE. The relation between TNFα and fLI differs between severe and mild PE suggesting that fLI plays a prognostic role in PE.
Keywords: leptin, metabolism, first trimester

[P3.51]

PLACENTAL PROTEIN-13 (PP13) IN COMBINATION WITH PAPP-A AND FREE LEPTIN INDEX (FLI) IN FIRST TRIMESTER MATERNAL SERUM SCREENING FOR SEVERE AND EARLY PREECLAMPSIA

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Background: Placental protein-13 is synthesised by the placenta and is belived to play a role in placental invasion. It has been described as a first and second trimester maternal serum marker for preeclampsia (PE). However, the discriminatory ability of PP13 has not been completely clarified.
Materials and Methods: PP13 was determined in maternal serum from 120 pregnancies that later developed PE and 267 controls. All serum samples were from gestational week 10^{+3} – 13^{+6}. Clinical and biochemical parameters as well as pregnancy outcome were studied as a function of PP13. The population performance of PP13 in combination with the PE markers PAPP-A and fLI was assessed by Monte Carlo simulation.
Results: PP13 was decreased in PE cases, with a median of 51.8 pg/mL (range: 13.1 – 534 pg/mL), compared to controls with a median of 54.8 pg/mL (range: 15.4 – 142.6 pg/mL) ($p = 0.037$). In severe PE and HELLP cases ($n = 26$) the median was 35.8 pg/mL (range: 17.8 – 85.5 pg/mL). In PE cases resulting in birth prior to week 34, the median was 30.6 pg/mL (13.1 – 50.1 pg/mL). In mild PE cases PP13 was not significantly different from the level in controls. PAPP-A and PP13 correlated significantly in controls (Spearman's rho = 0.233, $p < 0.001$), but not in PE pregnancies. fLI did not correlate in neither controls nor PE pregnancies. The population screening detection rate for a false positive rate of 10% for severe PE and HELLP was 26% for PP13, 28% for PP13+PAPP-A and 40% for PP13+PAPP-A+fLI.
Conclusion: PP13 is a moderately effective first trimester marker of severe PE and HELLP syndrome, but not for mild PE. The discriminatory ability of PP13 can be markedly improved by adding fLI.
Keywords: PP13, PAPP-A, screening, first trimester

[P3.52]

SERUM URIC ACID MAY BE A DIFFERENTIAL BIOMARKER OF RISK OF PREECLAMPSIA, ECLAMPSIA AND GESTATIONAL HYPERTENSION DEVELOPMENT

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Preeclampsia is a hypertensive disorder unique to human pregnancy which etiology is still unknown. It is responsible for approximately 12% of the world maternal deaths. Clinically, it is diagnosed primarily by the onset of hypertension and proteinuria in the later half of gestation. Eclampsia is the final and most severe phase of preeclampsia and can cause coma, seizures and even death of the mother and baby.

Gestational hypertension is defined as the development of new arterial hypertension in a pregnant woman after 20 weeks of gestation without proteinuria.

Up to now, predicting which women is at risk of developing preeclampsia -eclampsia is difficult.

Here, we evaluate uric acid concentrations during the course of pregnancy to differentiate women who are at risk of preeclampsia-eclampsia development from women who have gestational hypertension.

We conducted a case-control study of 138 women (49 with uncomplicated pregnancies, 62 with preeclampsia, 17 with eclampsia and 10 with gestational hypertension). Serum uric acid, proteinuria, urea and creatinine were measured both before and after the 20th week of gestation.

We compared all groups and found no significant differences in serum uric acid levels before the 20th week of gestation. However, after the 20th weeks of gestation serum uric acid levels from preeclamptic-eclamptic women were higher than levels from women with gestational hypertension ($P<0,05$). Serum urea and creatinine profiles in all groups showed no differences and the proteins in urine were also negative until the hypertension appeared in the preeclamptic-elamptic group.

In conclusion, we suggest that an increase in the levels of uric acid, documented by two sequential measurements between the 20th and 30th weeks of gestation, may be useful to define and differentiate a risk group with requirements of more frequent controls to detect the clinical changes involved in the appearance of hypertensive gestational disorders.

Keywords: Preeclampsia, Eclampsia, Gestational Hypertension, Uric Acid

[P3.53]

SEASONAL VARIATION IN PRE-ECLAMPSIA IN AFRICAN AMERICANS LIVING IN NEW YORK CITY A PILOT STUDY

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Objective: To evaluate the seasonal incidence of pre-eclampsia and the associated severity of placental inflammation in the African American population.

Methods: 350 African American and 67 Caucasian women with singleton pregnancies delivering from 1/2006-12/ 2009 were included. Medical records were reviewed and the following parameters were evaluated: maternal age, BMI, last menstrual period, parity, gestational age at delivery, 24 hour urine protein, blood pressure, liver function tests, birth weight, placental weight and placental chronic inflammation (chronic villitis), and maternal medical history. The incidence of conception was assumed to be uniform across calendar months. Kolmogorov-Smirnov tests were performed to test whether the distribution of months of conception differed from uniform in the population, and in African-Americans compared to Caucasians. Contingency tables compared distributions of categorical variables. Placental weight was analyzed as the residual after adjustment for gestational age in Mann-Whitney U tests.

Results: The distribution of months of conception differed significantly from a normal distribution in the population; this effect was confined to African-Americans (K-S $Z=3.01$, $p<0.0001$), with the difference in Caucasians being of borderline significance (K-S $z=1.78$, $p=0.059$). Gestational age at delivery and placental weights did not differ by month of conception, nor did the rate of severe pre-eclampsia. Consistent with a lack of effect on placental weight, no placental pathologies differed between races; chronic villitis was present in 15% of placentas of African American mothers, compared with 11% of Caucasians.

Conclusion: Our data support an effect of seasonality on the incidence of preeclampsia in African-American but not in Caucasian mothers, consistent with the hypothesis that Vitamin D deficiency moderates maternal inflammatory responses and may predispose to preeclampsia. We did not find evidence that systemic maternal inflammatory pathology has a direct effect on the placenta, as measured by the diagnosis of chronic non-specific villitis.

[P3.54]

PREVALENCE OF HYPERTENSIVE DISORDERS OF PREGNANCY (2004-2008) IN WESTERN RIO GRANDE DO NORTE, BRAZIL

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Among the most common clinical complications in the pregnancy cycle is the hypertension, classified as chronic hypertension, pre-eclampsia, pre-eclampsia on chronic hypertension, eclampsia and gestational hypertension. This work aims to investigate the prevalence of hypertensive disorders treated at emergency of the public health system in Mossoró, West Rio Grande do Norte. It is a retrospective documental and epidemiological research with a descriptive, qualitative and quantitative approach, and using the technique of analysis of handbooks which have not received an analytical treatment yet. The maternity hospital attended 19,120 women who gave birth from January 2004 to December 2008. 6.5% of them (1,238 women) suffered from a hypertensive disorder, High Pressure Syndrome, and 112 women (9%) presented eclampsia. 4.8% of neonates and only 3 parturients have died in 5 years. Among the parturients who presented hypertension, 239 women (19%) gave birth to preterms, in which 60 stillbirths were confirmed; 15 of these refer to pregnant teens and only 3 were over 40 years old. Fetal mortality rates in these age groups are not consistent with the literature. In this casuistry, we highlight that 37% of pregnant women were younger than 19 years old, in which 72% of the women who suffered from hypertensive disorders were teenagers and 53% presented eclamptic syndrome. Regarding the pregnant women with admission to labor, the hospital documented that it was possible to observe the high number of pregnant teenagers with hypertensive disorders. However, 78.2% of the pregnant teenagers in the hospital had their gestation complete. 43.5% of all hypertensive parturient attended in the hospital had less than 7 years of schooling. The results show that there is a good quality service rendered to parturients during prenatal and a concern to promote maternal and fetal health resulting in many quality medical assistances, the establishment of the maternal immunization program and the prevention, diagnosis and treatment of diseases intercurrent of pregnancy. The quality assistance allows the early perception of risks. Moreover, we observed that most pregnant teenagers attended during this period presented hypertensive disorder or exclusively eclamptic one. Thus, it is clear the necessity of rendering greater assistance to pregnant teenagers and search for the reasons why they are especially affected by hypertensive disorders.

Keywords: pregnancy, preeclampsia, hypertensive disorders, prevalence

[P3.55]

ADRP IS ASSOCIATED WITH FOAM CELLS AND ACUTE ATHEROSIS IN DECIDUAL TISSUE

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Acute atherosis of the maternal spiral arteries resembles the early stages of atherosclerotic lesions, with areas of lipid deposition in the vessel walls, characterized by subendothelial lipid-filled foam cells and fibrinoid necrosis. These foam cells are thought to derive from degenerated smooth muscle cells and infiltrating CD68 positive macrophages. Oxidative stress is regarded as a key factor in atherogenesis, and closely associated with vessel wall inflammation and formation of bioactive lipids. In preeclampsia, placental oxidative stress is associated with generalized systemic inflammation and endothelial dysfunction and development of maternal hypertension and proteinuria. Lipid droplets are intracellular storage depots of triglycerides and cholesterol esters. ADRP is a lipid droplet associated protein thought to be involved in the formation and function of these droplets. ADRP is expressed in foam cells *in vitro* and increase during foam cell formation, and ADRP mRNA is increased in symptomatic carotid atherosclerosis. We hypothesized that ADRP and other lipid droplet proteins could be involved in acute atherosis.

Decidual tissue was obtained from women with normal pregnancy (n=30) or preeclampsia (n=27) during cesarean section and analyzed with real-time PCR. Decidual sections of 10 normal and 6 preeclamptic patients were analyzed with immunohistochemistry.

ADRP was strongly expressed and localized to macrophages in areas of lipid deposition within non-transformed spiral arteries. On the gene expression level we did not find any dysregulation of ADRP expression in decidua in women with preeclampsia compared to normal pregnancies.

We propose that there are more macrophages and lipids in the non-transformed vessels in preeclampsia due to tissue injury because of the alterations in the blood flow to the placenta and subsequent oxidative stress. The acute atherosis reduces vessel lumen and contribute to placental infarcts and dysfunction and therefore we suggest that ADRP could play a role in the clinical development of preeclampsia.

Keywords: Spiral arteries, Acute atherosis, Foam cells, Adipose differentiation related protein (ADRP)

[P3.56]

LIPID PEROXIDATION AND CA-ATPASE ACTIVITY IN SEVERAL ORGANS FROM PREGNANT RATS CHRONICALLY DRINKING 1.8% NACL DURING THE SECOND HALF OF THEIR PREGNANCY

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Pregnant rats, chronically drinking 1.8% NaCl during the second half of their pregnancy, develop hypertension, proteinuria, placental oxidative stress and reduction in fetal size and weight. In a previous study, it was found a rise in the level of lipid peroxidation (TBARS) and a diminution in the activity of the Ca-ATPase of red cell ghosts and placenta homogenates in the experimental pregnant rats (drinking 1.8% NaCl) as compared to control rats (drinking tap water).

Objectives: To determine the Ca-ATPase activity and TBARS in homogenates of placenta, liver, brain, kidney, small intestine and heart from pregnant rats drinking a solution of 1.8% NaCl during the second half of their pregnancy.

Methods: Rats with 14 days of pregnancy were divided: a control group had free access to tap water and a experimental group had access to a drinking solution of 1.8% NaCl. After 21 days of pregnancy, the animals were killed under anesthesia and several organs were removed and homogenized. The homogenates were assayed for TBARS and Ca-ATPase activity.

Results: The homogenates of placenta, liver, brain, kidney, small intestine and heart from the experimental pregnant rats show a rise in TBARS and a disminution of the Ca-ATPase activity when compared with those from control rats.

Conclusions: The pregnant rats drinking a solution of 1.8%NaCl during the second half of their pregnancy show a rise in the level of lipid peroxidation and a disminution of the Ca-ATPase activity for several organs: placenta, liver, brain, kidney, intestine and heart.

Keywords: prengant rats, lipid peroxidation, homogenates placenta, several organs

[P3.57]

EFFECT OF MAGNESIUM SULFATE ON THE THE NA,K-ATPASE ACTIVITY IN PLASMA MEMBRANES OF HUMAN PLACENTAL EXPLANTS UNDER HYPOXIA

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Since the beginning of the century, magnesium sulfate has been used, along with antihypertensive drugs and rest, as a treatment for preeclampsia. This clinical syndrome is detected after the 20th week of gestation by the appearance of hypertension, proteinuria and edema. The pathogenesis of preeclampsia indicates an abnormal invasion of the spiral arteries, which causes a decrease in uteroplacental perfusion, and a generalized endothelial dysfunction. Placental hypoxia promotes a lipid peroxidation process which changes the composition of the plasma membrane and consequently, affects the activity of membrane proteins, such as the Na,K-ATPase. It has been reported a decrease in the activity of this protein in microvillous and basal membranes of human placental explants incubated under hypoxia conditions, and an increase in the levels of their lipid peroxidation . On the other hand, the Na,K-ATPase activity and the level of lipid peroxidation of plasma membranes from explants incubated under hypoxia in the presence of magnesium sulfate, show similar values to those reported for membranes from explants incubated under normoxia.

Objective: To determine the effect of the incubation with magnesium sulfate on the activity of the Na,K-ATPase and the levels of membrane lipid peroxidation of plasma membranes from human placental explants incubated under hypoxia conditions.

Results: The experimental membranes presented values of Na,K-ATPase activity and lipid peroxidation levels similar to those reported for explants incubated under normoxia.

Conclusions: Magnesium sulfate appears to be able to modify the lipid-protein interactions in the placental plasma membranes, recovering in this way their values of Na,K- ATPase activity and levels of lipid peroxidation.

Keywords: Hypoxia, Placental Explants, Magnesium Sulfate, Na,K ATPase

[P3.58]

PERFUSION OF HUMAN PLACENTA WITH HEMOGLOBIN, INTRODUCES PREECLAMPSIA-LIKE INJURIES THAT ARE PREVENTED BY α1-MICROGLOBULIN

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Objectives: Preeclampsia (PE) is a major cause of fetal and maternal morbidity and mortality. Preeclamptic women have increased plasma fetal hemoglobin (HbF), increased placental HbF-expression and accumulation of free HbF in the placental vascular lumen. Free hemoglobin (Hb) is pro-inflammatory and vasoconstrictive and its metabolites, heme and iron, generates reactive oxygen species which cause cell and tissue damage. To show the impact of free Hb in PE we used the dual *ex vivo* placental perfusion model. The placentas were perfused with Hb and investigated for physical parameters, Hb leakage, gene expression and morphology. Furthermore, we investigated the protective effects, of a1-microglobulin (A1M), a human heme- and radical-scavenger and antioxidant.

Findings: Hb-addition into the fetal circulation led to a significant increase of the perfusion pressure, feto-maternal leakage of free Hb, similar morphological damages of as observed in PE placentas and up-regulation of immune response- apoptosis- and oxidation-related genes. Simultaneous addition of A1M to the maternal circulation clearly inhibited the Hb leakage, morphological damage and gene up-regulation. In addition, A1M-addition up-regulated extracellular matrix genes.

Conclusions: The *ex vivo* Hb-perfusion of human placenta resulted in physiological and morphological changes and a gene expression profile similar to what is observed in PE placentas. This underlines the important role of free Hb in PE etiology. The damaging effects were counteracted by A1M, which we suggest as a new potential PE therapeutic agent.

Keywords: oxidativ stress, electron microscopy, microarray, therapeutics

[P3.59]

PRESENCE OF NON-FUNCTIONAL HCG IN PREECLAMPSIA AND RESCUE OF NORMAL PREGNANCY BY RECOMBINANT HCG

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Objective: Pre-eclampsia (PE) is one of the most common complications of pregnancy with an elusive etiology. Dysregulation in hormonal activity, angiogenesis and immunity is considered to contribute to the onset of PE. Although hCG is an early pregnancy hormone, we hypothesize that dysregulated hCG can lead to PE-like symptoms by affecting the trophoblast functions, angiogenesis and uterine regulatory T cells. The objective of our study was to establish a serum-based causal link between non-functional hCG and PE using *in vivo* and *in vitro* models.
Method: hCG levels in serum from normal and PE patients were evaluated. Glycosylation of hCG from normal and PE serum was determined by a sandwich-ELISA. *In vitro* functional assays included serum-based MAPK signalling and angiogenic cross-talk between the endothelial cells and trophoblasts. Pregnant IL-$10^{-/-}$ mice were injected (gd 10, i.p) with either normal pregnancy serum (NPS) or PE serum (PES) with or without recombinant hCG (rhCG). On gd 16/17, blood pressure and pregnancy outcome were recorded. Urinary albumin, creatinine, serum sFlt-1, sEng were measured and renal pathology was monitored. On gd 12/13, splenic and uterine Treg cells (CD4+CD25+FoxP3+) cells were monitored by FACS.
Results: Higher levels of hCG were observed in PES by ELISA. Glycosylation pattern showed excessive presence of Sialyl-Lewis a (SLea), Sialyl-Lewis X (SLeX) and Lewis Y (LeY) on PES hCG. Dysregulation of hCG signalling in PES was demonstrated by defective ERK phosphorylation and disruption in the dual cell angiogenesis which was reversible with rhCG. *In vivo* treatment with PES resulted in significant reduction in Treg cells at the maternal-fetal interface accompanied by IUGR, hypertension, proteinuria and renal pathology, typical features of preeclampsia. This was reversed by rhCG.
Conclusions: *In vivo* and *in vitro* assays indicate that dysregulated hCG contributes to angiogenic defects, altered Treg cell density and PE-like symptoms in mice.
Keywords: hCG, angiogenesis, regulatory T cells, preeclampsia

[P3.60]

LPS INDUCES REMODELING OF TROPHOBLAST CELLS MEDIATED BY MIR-155

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Background: *Ultra*-low dose of LPS injection has induced preeclampsia model in rats. We investigated the alterations of trophoblast cells stimulated with different doses of LPS and explored the underlying mechanisms.
Methods: We detected two major subunits of AP-1, JunB and FosB and micro RNA-155 in 20 severe preeclampsic and normal placentas as well as the trophoblast cells treated *in vitro* with low/high dosage of LPS. We used DNA precipitation assay and luciferase reporter analysis to evaluate the regulation of miR-155 by AP-1 through recruitment to its binding sites upstream of BIC/miR-155 gene promoter.
Results: Expression levels of miR-155, along with JunB and FosB showed a significant increase in severe preeclamptic placentas. Low dose of LPS induced an upregulation of AP-1, which in turn enhanced the expression of miR-155. Trophoblast cells enforced expression of miR-155 showed a markedly reduced ability of migration and an increase of syncytiotrophoblast-like multinuclear cells. Anti-miR-155 potentially reversed the above effects.
Conclusion: *We identified an Ap-1 dependent upregulation of miR-155 following low dose* LPS treatment of trophoblast cells. We also demonstrated roles of miR-155 in promoting trophoblastic synchronization and inhibiting migration.
Key Words inflammation, AP-1, preeclampsia, migration, synchronization

[P3.61]

MICRO-RNA-PROFILES IN RESPONSE TO LIF IN TROPHOBLASTIC CELLS

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Background: Micro-RNAs are recently detected key regulators of the immune system and of carcinogenic processes, but their function in pregnancy is poorly investigated yet. Leukemia Inhibitory Factor (LIF) is a cytokine which plays a crucial role in trophoblastic cells and which triggers their invasion via Signal Transducer and Activator of Transcription 3 (STAT3). Therefore, we aimed to further analyze LIF induced signal transduction and LIF-regulated micro-RNAs in two trophoblastic cell lines.
Material and Methods: JEG-3 choriocarcinoma cells were stimulated with LIF for 1 to 24 hours, and total mRNA was isolated. Phosphorylation of signal transducers ERK1/2 (thr 702 and tyr 704) and STAT3 (ser 727 and tyr 705) was assessed by gel electrophoresis and Western blotting. STAT3 gene expression was analyzed as control of induction by RT-PCR. Expression of five different micro-RNAs, which are either known to be expressed in placental tissue or involved in tumor invasion, was quantified at six time points by qRT-PCR. Finally, by using a 7900HT Fast Real-Time PCR System combined with TaqMan micro-RNA assay, the expression profile of 760 micro-RNA was screened and compared in JEG-3 cells and HTR8/SVneo immortalized first trimester trophoblast cells stimulated or not with LIF for 4 hours.
Results: ERK1/2 and STAT3 were phosphorylated from 10 to 30 minutes after LIF stimulation. Three out of five micro-RNA (miR-9, miR-141 and let-7g) were time-dependently down-regulated by LIF with a peak after 4 hours of treatment, which correlates with an increase of STAT3 expression. The micro-RNA profiling showed that expression of approximately 65% of all micro-RNA was at least 10fold different in JEG-3 and HTR8/SVneo cells. LIF influenced around 10% of all micro-RNA, but with little coincidence between both cell lines.
Discusion: LIF modulates micro-RNA expression in trophoblastic cells, but different cell lines behave diversely.
Keywords: trophoblast, choriocarcinoma, micro-RNA, LIF

[P3.62]

CHANGES IN OXYGEN TENSION MODIFY THE EXPRESSION OF NA+/H+ EXCHANGER TYPE 3 IN HUMAN PLACENTA

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Transport of water, solutes and electrolytes between mother and fetus is one of the most important functions of the human placenta. The syncytiotrophoblast is the site of exchange and since its syncytial nature the fetal and maternal transport should take place primarily via transcellular routes.

It is well known that in some pathological conditions such as preeclampsia, the shallow trophoblast invasion and the poor remodelling of the maternal spiral arteries result in an insufficient uteroplacental oxygenation. However, the assumption that the placental changes are induced only by hypoxia may be simplistic. It has been proposed that intermittent placental perfusion secondary to deficient trophoblast invasion of the endometrial arteries leads to an ischemia-reperfusion [hypoxia-reoxygenation] type insult. This insult may modify the expression and/or the function of transport proteins and the placental pH homeostasis.

It was reported that the Na+/H+ exchanger (NHE) is expressed in human placenta and may play a role in the maintenance of intercellular pH.

Recently, we described that isoform 3 of NHE (NHE-3) was reduced in preeclamptic placentas. However, up to now placental NHE regulation remains unclear.

The aim of our work was to establish if changes in oxygen concentration may alter NHE-3 expression in human placenta.

Explants from normal placenta were cultured in normoxia, hypoxia, and in hypoxia/reoxygenation. Western blot analysis and immunohistochemistry were performed to study NHE-3 expression.

We found that the expression of NHE-3 decreased when explants were cultivated under hypoxic conditions and the posterior reoxygenation treatment partially restored NHE-3 expression. In all cases, NHE-3 was located in the apical membranes and in citoplasmatic regions.

These results suggest that changes in oxygen tension may modify NHE-3 expression in human placenta. Further studies are needed to evaluate if this changes correlate with NHE-3 activity.

Keywords: hypoxia, syncytiotrophoblast, human placenta, Na+/H+ exchanger type 3

[P3.63]

POTASSIUM CHANNELS IN HUMAN PLACENTA FROM PREECLAMPTIC AND IUGR PREGNANCIES: EXPRESSION, FUNCTION AND ASSOCIATION TO LIPID RAFTS

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Human placental syncytiotrophoblast (hSTB) is an epithelium responsible for materno-fetal exchange. Potassium conductances in placental membranes have functions similar to other epithelial channels, such as the resting potential of the hSTB microvillous membrane and are involved in volume regulation processes. In pregnancy diseases as preeclampsia (PE) and intrauterine growth restriction (IUGR), the potassium channel activity and consequently their influence on placental function could be modulated by their localization on specialized membrane domains and microdomains. Currently our aim is to identify the potassium channel channels in apical membrane from IUGR and PE and to establish the targeting of potassium channel subtypes (TREK, Kir, TASK and Kv) to raft and non raft domains. METHODS: Purified microvillous membrane from PE and IUGR hSTB was reconstituted into giant liposomes suitable for electrophysiological studies by the patch-clamp method. Microdomains (DRMs, detergent resistant membranes) were extracted from theses purified membranes using 1%Triton X-100 and sucrose flotation. Afterwards, we collected the samples and probed them by Western blot. RESULTS: Our data show an uneven expression of potassium channel subtypes in the membrane fractions. All of them are expressed mainly (70%) in apical both from normal and IUGR placentas. In contrast, the distribution in membranes from PE showed a 60% of expression in the basal membrane. Single channel recordings show the functional presence of K+ channels in the hSTB microvillous membrane from PE and IUGR. However these channels show a different sensitivity to blockers such as BaCl, MgCl2, TEA and pH. Additionally, the single channels conductances are altered compared with potassium channel reported previously in normal placentas. Furthermore some of them have a different localization within the membrane microdomains. Kir and Task-1 but not TREK and Kv, target a specialized cholesterol rich lipid raft domains. Conclusion: The membrane compartmentalization of potassium channels and their biophysical characteristics may be critical to understand the molecular mechanism of transport processes that occur in the placental hSTB from normal and pathological pregnancies. Supported by FONDECYT 1070695 (Chile).

Keywords: POTASSIUM CHANNELS, PREECLAMPTIC, IUGR

[P3.64]

CHLORIDE CHANNELS IN HUMAN SYNCYTIOTROPHOBLAST APICAL AND BASAL MEMBRANE FROM PREECLAMPTIC AND IUGR PREGNANCIES

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Chloride transport involving conductive pathways is associated to numerous epithelial functions such as maintenance of membrane voltage, solute transport and cell volume regulation. There is evidence that transepithelial chloride transport in placental syncytiotrophoblast may also be involved in such functions. Alterations in the activity of these channels associated with pathologies of pregnancy as preeclampsia and IUGR could involve alterations in these functions. We have previously studied a Maxi chloride channel in apical membranes from normal and Preeclamptic placentas. This channel has changes in its conductance and kinetics in this pathology. Our current aims are to characterize the Maxi chloride channel in apical membrane from IUGR and determine the presence of functional chloride ion channels in the human placental STB

Methods: Purified basal membranes from human placental STB were reconstituted into giant liposomes suitable for single-channel recordings by the patch-clamp technique, or were transplanted into Xenopus laevis oocytes for voltage-clamp total current recordings.

Results: Exogenous currents of chloride were detected in oocytes transplanted with STB apical membrane from IUGR placentas. Additionally in the last, single-channel experiments show two frequent conductance states of approximately 160 and 90 pS in contrast to the 240pS obtained for the conductance of normal placenta. Similar experiments with normal basal membrane show the presence of three types of chloride conductances: a high conductance of 220 pS, a medium conductance of 170 pS, and a low conductance of 50 pS with different sensitivities to two classical chloride channels blockers: DIDS (4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid) and DPC (diphenylamine-2-carboxylate). In the same way, in basal membrane from PE and IUGR we have obtained three populations of chloride channels, however, the conductances are smaller than the values for normal placentas.

Conclusion: To identify the functional differences of chloride channels in these pathologies is important to understand the role of these channels in health and disease-. Supported by FONDECYT 1070695 (Chile).

Keywords: CHLORIDE CHANNELS, HUMAN SYNCYTIOTROPHOBLAST, PREECLAMPTIC, IUGR

[P3.65]

CHRONIC BUT NOT ACUTE EXPOSURE TO EPIDERMAL GROWTH FACTOR INCREASES SYSTEM A AMINO ACID TRANSPORT IN PLACENTAL EXPLANTS

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Introduction: Fetal growth restriction (FGR) is associated with altered cell turnover and decreased activity of the system A amino acid transporter in villous trophoblast. Growth factors represent a means of manipulating trophoblast renewal and function, and may represent a novel therapeutic approach to FGR.

Objective: To investigate whether treatment with epidermal growth factor (EGF) enhances placental function following acute (2h) or chronic (96h) exposure.

Methods: Villous explants were taken from normal term placentas (n=8) and cultured with or without 100ng/ml EGF for 2h or 96h at 6% O2. Following treatment, the activity of system A was assessed by Na^+-dependent uptake of ^{14}C-methyl-aminoisobutyric acid. After 96 hours, culture media was assayed for human chorionic gonadotrophin (hCG) and cell turnover was quantified by immunostaining for cytokeratin M30 (apoptosis) and Ki67 (proliferation). Morphology was assessed on semi-thin sections.

Results: Treatment with EGF for 2h did not alter system A activity (median at 90 minutes [IQR], 297.5 [88.6-437.5] vs. 263 [165-372.5] pmol/mg protein/min). However, system A activity was increased by EGF treatment in 96h cultured explants (median at 90 minutes 221.5 [183.1-232.5] vs. 177.0 [146.5-232.8] pmol/mg protein/min, $p<0.05$). There was no significant change in apoptosis following EGF treatment (median apoptotic index EGF 0.48 [0.28-1.60] vs. control 0.35 [0.12-1.53]). Trophoblast proliferation also was not altered by EGF (median proliferative index EGF 1.59 [0.95-2.33] vs. control 1.37 [0.74-1.97]). Morphological differentiation of the trophoblast layer was more pronounced following treatment with EGF, but hCG secretion was not altered.

Conclusion: Chronic exposure to EGF increases activity of the system A transporter. At 6% oxygen, this does not appear to be directly related to altered cell turnover. Further work is necessary to determine the mode of action of EGF and whether system A transport can be restored in pregnancies complicated by FGR.

Funded by the Peel Medical Research Trust

Keywords: Epidermal Growth Factor, System A transport, Placental explants

[P3.66]

ACTIVITY AND EXPRESSION OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 IS REGULATED BY D-GLUCOSE UPTAKE AND METABOLISM IN UMBILICAL VEIN ENDOTHELIUM

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In human umbilical vein endothelial cells (HUVEC) adenosine uptake is mainly mediated by human equilibrative nucleoside transporters 1 (hENT1). High extracellular D-glucose reduces hENT1 expression and activity in this cell type. HUVEC express functional facilitative D-glucose transporters (GLUTs), but the role of D-glucose transport and metabolism on the modulation of hENT1 is unknown. We studied whether D-glucose transport and metabolism modulates hENT1 expression and activity in HUVEC.

Methods: Confluent HUVEC were exposed (24 hours) to 5 mM (normal) or 25 mM (high) D-glucose in absence or presence (3-24 hours) of phloretin (GLUTs inhibitor, 100 μM) or sodium iodoacetate [glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inhibitor, 1 μM]. Adenosine uptake (10 μM, 2 μCi/ml, 20 s, 22°C) was then measured in absence or presence of nitrobenziltioinosine (NBTI, 1 μM, ENT1 inhibitor). 2-Deoxi-D-[3H]glucose (2DG) uptake (1.6 mM 2-Deoxi-D-glucose, 3 μCi/ml, 5-40 s, 22°C) was measured in absence or presence of phloretin. hENT1 mRNA level and protein abundance were evaluated by RT-PCR and western blot, respectively.

Results: hENT1-adenosine uptake, hENT1 protein abundance and mRNA level were reduced (40 ± 12%, 43 ± 12% and 29 ± 3%, respectively; $P<0.05$, two way ANOVA test) in cells exposed to 25 mM compared with 5 mM D-glucose. Coincubation of cells with phloretin or sodium iodoacetate blocked the effects of 25 mM D-glucose on hENT1-mediated transport, but these inhibitors did not alter these parameters in cells in 5 mM D-glucose. Parallel results show inhibition of 2DG uptake by phloretin in 5 and 25 mM D-glucose.

Conclusion: The reduced activity and expression of hENT1 by high D-glucose is a phenomenon that will depend on D-glucose transport and may require D-glucose metabolism via GAPDH in HUVEC.

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Keywords: High Glucose, Nucleoside Transporter, Endothelial cells, Glucose Metabolism

[P3.67]

ADENOVIRAL-MEDIATED PLACENTAL GENE TRANSFER OF IGF-1 CORRECTS PLACENTAL INSUFFICIENCY VIA ENHANCED PLACENTAL NUTRIENT TRANSPORT MECHANISMS

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Previous work in our laboratory demonstrated that over-expression of human insulin-like growth factor -1 (hIGF-1) in the placenta corrects fetal weight deficits in mouse, rat, and rabbit models of intrauterine growth restriction without changes in placental weight. The underlying mechanisms of this effect have not been elucidated. To investigate the effect of intra-placental IGF-1 over-expression on placental function we examined native murine placental IGF-1 levels and nutrient transporter expression in a mouse model of IUGR.

Methods: At gestational day 18, animals were divided into four groups; sham-operated controls, mesenteric uterine artery ligation (MUAL), MUAL + Ad-hIGF-1 (MUAL+IGF,10^8 PFU), MUAL + Ad-LacZ (10^8 PFU). At gestational day 20, pups and placentas were harvested by C-section and maternal blood samples collected by cardiac puncture. Human IGF-1 and mouse IGF-1 levels were analyzed by ELISA. The RNA expression of amino acid transporters from System A (SNAT 1,2,4) and System L (LAT1,2 and 4F2hc) and glucose transporters GLUT1 and GLUT3 were analyzed by qPCR.

Results: Only the MUAL+ hIGF-1 placentas showed expression of human IGF-1 by ELISA (27.4ng/mg,$n=5$), no human IGF-1 was detectable in maternal serum. Endogenous mouse IGF-1 levels were not different in placenta or maternal serum between treatments. SNAT2 RNA expression was reduced by 30% ($p<0.01$,$n>3$) in the MUAL group compared to sham, but returned to sham levels with Ad-hIGF-1 treatment. Ad-LacZ treatment did not lead to a recovery in SNAT2 RNA expression. GLUT1 expression was reduced by 30% ($p=0.01$,$n\geq3$) in all treatment groups compared to sham. There were no other differences in transporter expression between groups.

Conclusion: These changes demonstrate that mesenteric uterine artery ligation results in significant reduction in SNAT2 and GLUT1 expression. IGF-1 over-expression enhances placental amino acid but not glucose transport. This enhanced transport gene expression may be an important mechanism in IGF-1mediated correction of placental insufficiency.

Keywords: IUGR, Mouse, amino acid transport

[P3.68]

LITHIUM INHIBITS CATION TRANSPORT IN THE TRP CHANNEL POLYCYSTIN-2 (TRPP2)

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Introduction: Lithium (Li^+) is a potent pharmaceutical agent that has a profound effect in the treatment of bipolar affective disorder and mania. Although Li+ is not devoid of prenatal safety concerns, and its use during lactation has been widely discouraged, it continues to be favoured by many clinicians for use during pregnancy. Nevertheless, Li^+ use during late pregnancy is associated with particular clinical concerns that have not been investigated. Numerous reports of neonatal complications have been associated with Li^+ treatment during late pregnancy, including cardiac dysfunction, diabetes insipidus, hypothyroidism and low muscle tone. Polycystin–2 (PC2) is a TRP-type, non-selective cation channel that permeates $Ca2^+$ and is abundantly expressed in term human syncytiotrophoblast. We have postulated that based on its biophysical properties and regulatory mechanisms, PC2 may be an important contributor to Ca^{2+} delivery from the mother to the foetus. Herein, we explored the effect of Li^+ on PC2 channel function.
Methods: Both *in vitro* translated and human syncytiotrophoblast PC2 were assessed for permeability to Li^+. Lipid bilayer reconstituted PC2 in the presence of either a K^+ chemical gradient (150/15 mM) and increasing concentrations of Li^+, or different symmetrical concentrations of either ion. RESULTS: A decreased in the single channel conductance, and changes in reversal potential of the current-to-voltage relationships in the presence of Li^+, consistent with both, permeability to, and a blocking effect of, Li^+ on PC2 channel function was observed. The single channel conductance in asymmetrical K^+ was 160 pS, and 115 pS in asymmetrical Li^+. Calculations of the reversal potentials of the fitted data with the Goldman-Hodgkin-Katz equation indicated a K^+/Li^+ perm-selectivity ratio of 1.5-2.0.
Conclusions: The data were in agreement with anomalous mole-fraction properties of PC2, and the possibility that Li^+ blockage of PC2 may impair cation transport, particularly Ca^{2+} in the human placenta.
Keywords: Polycystin-2, lithium, term human syncytiotrophoblast

[P3.69]

PHOSPHOLIPID TRANSFER PROTEIN IS EXPRESSED AND ACTIVE IN HUMAN PLACENTAL ENDOTHELIAL CELLS

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Objectives: Phospholipid transfer protein (PLTP) plays a crucial role in lipoprotein metabolism. It is regulated by liver X (LXR) nuclear receptors which can be activated by oxysterols. Reports on high PLTP mRNA expression levels in human placenta strongly imply a role of PLTP in the placenta. This together with the important role of HDL and LXR regulation in maternal-fetal cholesterol transfer across placental endothelial cells prompted us to investigate PLTP at the feto-placental endothelial barrier.
Methods: PLTP was localised by immunohistochemistry. Its mRNA and protein expression levels in isolated human arterial and venous fetal term placental endothelial cells (HPEC) were analyzed by real-time PCR and immunoblotting, respectively. PLTP activity in supernatants of cultured HPEC and fetal human hepatic (WRL-68) cells was determined using a radiometric assay. PLTP expression and activity were measured after treatment with the LXR agonists 24*(S)*- and 27-hydroxycholesterol.
Results: In placenta, PLTP was located in endothelial cells. Both, arterial and venous HPEC express and secrete active PLTP but mRNA expression (3.4 x, $p < 0.05$) and phospholipid transfer activity in supernatants of arterial HPEC was greater (96 %, $p < 0.001$) as compared to venous HPEC. LXR activation by 24*(S)*OH cholesterol, which represents the major enzymatic produced oxysterol in the fetal circulation, upregulated PLTP mRNA expression (77 %, $p < 0.01$) in arterial HPEC concomitantly with increased PL transfer activity in cellular supernatants (40 %, $p < 0.05$). Even though mRNA expression in fetal human liver cells was significantly higher, PLTP secreted by arterial HPEC showed a greater phospholipid transfer activity.
Conclusion: Placental endothelial cells, arterial more than venous, contribute to the release of active PLTP into the fetal circulation which can be influenced by LXR activation. PLTP may mediate PL transfer and participate in reverse cholesterol transport pathways at the feto-placental barrier.
Keywords: phospholipid transfer protein, human placental endothelial cells, 24(S)-hydroxycholesterol

[P3.70]

TRAFFICKING OF MMP-9 THROUGH AMNIOCHORION IS ASSOCIATED TO TERM HUMAN GESTATION

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Rupture of the fetal membranes (FM) during human labor is associated with a highly controlled local inflammatory response. Several leukocytes subsets are recruited to the choriodecidua, once delivery is approaching these cells participate in the conditioning of the local microenvironment through the secretion of inflammatory signals and mediators of membrane rupture. Leukocytes are the main source of MMP-9, which is involved in the degradation of connective tissue, the major structural component of the FM and responsible of their tensile strength during gestation. Since the major substrate of MMP-9 is in the amnion side, it is expected that a trafficking mechanism that allows MMP-9 to transit across amniochorion must exist.

The aim of this study was to analyze the transport of proMMP-9 through human amniochorion.

Methods: FM were collected from caesarean sections and mounted in an *in vitro* model, which allowed us to maintain their anatomical relationships. Labelled human recombinant proMMP-9 was added to the choriodecidua side and incubated at different times. Frozen sections were obtained and analyzed by confocal laser scanning microscopy for location of pro-MMP-9. Labelled BSA was used as a control.

Results: proMMP-9 was localized in the amniotic epithelium after 3 hours and until 24 hours of incubation. An unexpected finding was that proMMP-9 was found as intracellular dense granular deposits. We demonstrated the presence of entire, functional MMP-9 in the interior of these cells. There was no evidence of transport when albumin was added.

Conclusions: MMP-9 can be specifically trafficked trough the chorioamnion when added to the choriodecidual side in FM obtained from women near to term gestation. MMP-9 is stored as intracitoplasmic granules, which may be related to formation of macromolecular complexes that can be unassembled at the moment of labor, liberating massive amounts of MMP-9, provoking the rupture of the FM during late phases of normal labor.

Keywords: MMP-9, Fetal Membranes, Transport, Human gestation

[P3.71]

INDUCTION OF P53 PROMOTES APOPTOSIS IN THE VILLOUS TROPHOBLAST

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Introduction: The villous placenta in preeclampsia and IUGR is characterised by exaggerated trophoblast turnover, apoptosis and exaggerated expression of p53. Previously we have demonstrated that both apoptosis and p53 expression is exaggerated *in vitro* by treatment with the p53 activator Nutlin-3. In cell lines we have demonstrated that Nutlin-3 induces apoptosis, which is reduced by co-treatment with the p53 inhibitor Pifithrin-α. We present studies of the effect of p53 modulation in the human villous explant model.

Methods: Villous fragments from term placentas were cultured in 6% oxygen for 48hrs before exposure to 30μM Nutlin-3 or 30μM Nutlin-3 and 10μM Pifithrin-α in combination for a further 48hrs. Explants were wax embedded for immunohistochemistry or homogenised for protein. Trophoblast apoptosis was assessed by M30 and TUNEL staining and by activity of caspase-3/7. Proliferation was assessed by Ki67 staining.

Results: Treatment with Nutlin-3 resulted in an increase in M30 staining ($p<0.0079$, Mann-Whitney, $n=5$) and TUNEL positivity ($p<0.0079$, Mann-Whitney, $n=5$). This increase in M30 was reduced by co-treatment with Pifithrin-α ($p<0.0079$, Mann-Whitney, $n=5$) and TUNEL ($p<0.0317$, Mann-Whitney, $n=5$). However, there was no difference in caspase-3/7 activity. Proliferation was reduced between fresh villous tissue and Nutlin-3 treated tissue but not control or combination treatment ($p<0.0317$, Mann-Whitney, $n=5$).

Discussion: Using the villous explant model we demonstrate that treatment with Nutlin-3 induces apoptosis in trophoblast, an effect which is reduced by co-treatment with Pifthrin-α. Our data suggest that the main effect of Nutlin-3 treatment may be upon the syncytiotrophoblast with protection of the stroma, as suggested by the lack of effect upon caspase-3/7 from whole villous tissue. These studies suggest that the p53 pathway may be a suitable target for future therapies in placental pathologies.

Keywords: p53, apoptosis, trophoblast

[P3.72]
AUTOPHAGY IN NORMAL HUMAN TERM PLACENTAS

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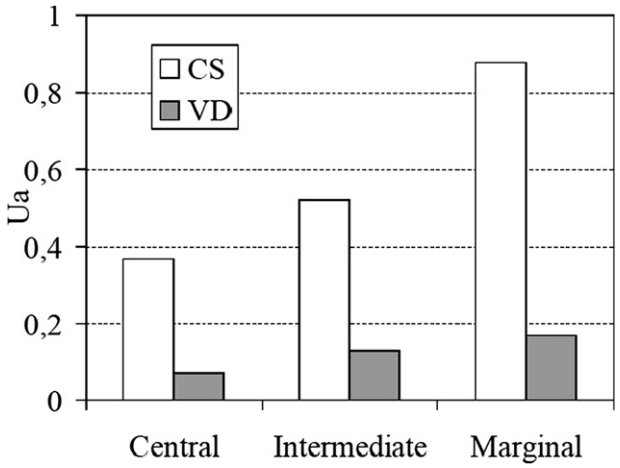
Introduction: Cell strategy under stress is to slow down metabolism and decrease proliferation rate by triggering autophagy, which represents the autodigestion of redundant proteins and organels, limiting cell demand and prolonging survival. Autophagy has been detected in the human placenta but it is not known whether it is triggered to sustain cell survival or it is switched on in severe stress conditions leading to cell death. The aim of our study was to study the presence of autophagy in normal term placentas.
Methods: 14 placentas were collected at the time of term uncomplicated vaginal delivery (VD; n=7) and elective caesarean section (CS; n=7) after uneventful pregnancies with singleton liveborn healthy infants with appropriate birthweight.
Autophagy detection: LC3 (autophagy marker) was evaluated by immunoblotting from frozen tissues and by immunoistochemistry of formalin-fixed paraffin-embedded tissue section (antibody from Novus, Biologicals, Littleton, CO, USA).
Biochemical analyses Umbilical blood gases, pH, glucose and hemoglobin concentrations were measured with a Radiometer ABL 330 Analyzer.
Results: *Western Blotting*
LC3II (cleaved product of LC3, associated to late autophagosomes) was significantly higher [$p<0.05$] in CS than VD placentas [Figure 1].



Immunohistochemistry
LC3 immunostaining was observed in all cases, in the villous trophoblast, with no differences between VD and CS.

Biochemical analyses
No difference was present with the exception of glucose concentration that was lower in CS than in VD.
Conclusion: Autophagy is present in normal term placentas. Whereas uncomplicated labor is well sustained by the healthy placenta which tolerates the intermittent stress of contractions, the placenta at surgery exhibits a higher level of autophagy in association with lower blood glucose concentrations. We speculate that mechanisms alternating muscle and vasal constriction, such as in labor, may actually cause a burst in cell metabolism whereas autophagy represents a response to prolonged stress such as low glucose supply or surgery.
Keywords: Autophagy, Placenta, Human pregnancy

[P3.73]
ASSOCIATION OF APOPTOTIC GENETIC POLYMORPHISMS AND PREECLAMPSIA

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Introduction: Alterations in apoptosis mechanisms in trophoblastic cells are involved in the physiopathology of preeclampsia (PE) and the FAS/FASL complex seems to play an important role. Polymorphisms in *FAS* and *FASL* gene promoter regions may alter the expression of these molecules. Our aim was to investigate a possible association between *FAS* (-670) and *FASL* (-844) gene polymorphisms and PE.
Methods: This case-control study included 130 preeclamptic patients and 260 normotensive women without any obstetric or systemic pathology as controls. Genomic DNA was extracted from whole blood, and polymorphism genotyping were obtained by digesting PCR products with the following restriction endonucleases: *Bst*NI (*FAS*) and *Bsr*DI (*FASL*). Results were analyzed by chi-square and Fischer exact tests, with significance set at $p<0.05$.
Results: Genotypic frequencies of *FAS* polymorphism were 34.9% GG and 65% GA+AA in the PE group; and 24.5% GG and 75.5% GA+AA in controls ($p=0.03$). Genotypic frequencies of *FASL* were 33.8% CC and 66.1% TC+TT in the PE group; and 20% CC and 80% TC+CC in controls ($p=0.004$).
Conclusion: This study suggests that *FAS* (-670) and *FASL* (-844) gene polymorphisms are associated with the risk of developing PE in a Brazilian population.
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Keywords: preeclampsia, apoptosis, gene polymorphism, Fas-Fas-L complex

[P3.74]

THE EFFECT OF HOMOCYSTEINE ON TRANSSULFURATION PATHWAY, PROLIFERATION AND APOPTOSIS IN HUMAN PLACENTA

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Introduction: Homocysteine (Hcy), a thiol-containing amino acid, is situated at the cross point of two metabolic processes from where it follows either remethylation or inverse catabolism while synthesis of cysteine (Cys). Impairment of Hcy metabolism provokes hyperhomocysteinemia with characteristic pathologies particularly developmental defects of the fetus, and other complications of pregnancy, e.g. preeclampsia, still birth etc.

Taking into account the substantial role of placental metabolism in the initiation of obstetrical pathology the goal of our study was to explore the effect of HCy on transsulfuration pathway, proliferation and apoptosis in human placenta.

Methods: The placental samples and explants from the I and the III trimesters of gestation were used in the study. The expression of key enzymes of transsulfuration pathway, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), was detected by RT-PCR, Western blot analysis; CBS localization – by immunochistochemical approach; CBS catalytic activity – by radioactive assay; Cys concentration - by HPLC conjugated with coulochmical detector and proliferation and apoptosis - by immunochemical procedure with antibodies to Ki67 and M30 antigens, respectively.

Results: The expression of CBS and CSE in placental samples from the I and the III trimesters of gestation were confirmed at RNA and protein levels, CBS catalytic activity - by positive conversion of serine and Hcy to cystathionine, and CBS protein was localized in syncythio- and cytotrophoblast. Treatment of explants from both terms of gestation by 20, 40 and 80 μM of Hcy induced the concentration dependent decrease of proliferation index and increase of apoptotic index. The rate of changes in apoptotic index in the explants from both terms of gestation exceeds that of proliferation index. The apoptotic index in term explants is 5-6 times higher than in explants from the I trimester of gestation. Elevated concentrations of Hcy stimulate the expression of CBS and Cys synthesis.

Conclusion: Elevated concentrations of Hcy infringe the vital processes (proliferationa and apoptosis) ensuring normal placental function and promote accumulation of CBS protein and Cys.

Keywords: Homocysteine, Transsulfuration, Proliferation, Apoptosis

[P3.75]

FETAL ACTIVATION OF PPAR: METABOLIC EFFECTS IN THE FETAL LIVER AND THE PLACENTA

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Introduction: Maternal diabetes induces alterations in lipid and nitric oxide (NO) metabolism and affects feto-placental development and growth. PPARα is a nuclear receptor involved in lipid homeostasis in different tissues.

Objective: To analyze whether fetal PPARα activation regulates lipid and NO metabolism in the placenta and fetal liver from diabetic rats, and to study the involvement of this activation in the feto-placental growth.

Methods: Diabetes was induced by neonatal streptozotocin administration. Fetuses from control and diabetic rats were injected through the uterine wall with the PPARα agonist leukotriene B4 (LTB4, 0.1μM) on days 19, 20, and 21 of gestation. On day 21 of gestation, placental and fetal liver concentrations of lipids, lipoperoxides and NO were evaluated.

Results: Placentas, fetuses and fetal livers from diabetic rats showed increased weight ($p<0.05$). In diabetic animals, overaccumulation of triglycerides and cholesteryl esters was observed in the fetal liver ($p<0.05$), an increase in lipid peroxidation was observed in both the placenta and the fetal liver ($p<0.01$), and an increase in NO production was observed in the fetal liver.

In diabetic animals, fetal treatment with LTB4 reduced the placental, fetal and fetal liver weight ($p<0.05$). This fetal treatment reduced the concentrations of triglycerides, cholesteryl esters and phospholipids ($p<0.05$), as well as lipid peroxidation, and NO production in the fetal liver ($p<0.01$). In addition, it reduced NO production ($p<0.05$) but did not alter lipid concentrations in the placenta.

Conclusions: Our results provide evidence of a fetal role of PPARα activation in the regulation of both lipid homeostasis in the fetal liver and NO production in the fetal liver and the placenta, with an overall impact on fetal and placental growth.

Keywords: Diabetes, Fetal Liver, Placenta, lipids

[P3.76]

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5 (STAT5) SIGNALING IN TROPHOBLASTIC CELLS IS INDUCED BY EPIDERMAL GROWTH FACTOR (EGF)

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Background: Epidermal Growth Factor (EGF) displays mannifold functions in the placenta including tuning of invasion. Recent reports have demonstrated that activation of Signal Transducer and Activator of Transcription (STAT) proteins, including STAT3 and STAT5 actively participate in tumor development and progression. STAT5 is an intracellular protein downstream tyrosine-kinase receptors, which stimulates proliferation and cell cycle progression. Several cytokines involved in the regulation of trophoblast behaviour have been shown to mediate their effects though STAT3, but less is known about STAT5 activation in pregnancy. The aim of this study was to assess correlation of STAT5 and STAT3 phosphorylation.
Materials and Methods: JAR, BeWo and JEG3 choriocarcinoma cells as well as HTR-8/SVneo immortalized trophoblast cells were stimulated with IL-2, IL-11, Leukemia Inhibitory Factor (LIF), Epidermal Growth Factor (EGF) or Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) by applying diverse concentrations and times. The effect of the cytokines and growth factors on the expression, phosphorylation and localization of STATs were determined by Western blotting and immunocytochemistry.
Results: Only EGF induces STAT5 phosphorylation (Tyr 694) in all analyzed cell lines, while the other cytokines, except IL2, induce different intensity of STAT3 phosphorylation (tyr 705) depending on cytokine and cell line.
Discussion: It can be concluded that cytokines that activate STAT3 and STAT5 in trophoblastic cells are different. The activating cytokines are highly expressed in the placenta. Therefore, it can be assumed that simultaneous activation occurs frequently and may further influence functions and behaviour of cells.
Keywords: trophoblast, STAT5, EGF, STAT3

[P3.77]

PLACENTAL *IGF2* SIGNALLING PLAYS A MAJOR ROLE IN THE FETO-PLACENTAL RESPONSE TO UNDERNUTRITION IN MICE

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During maternal undernutrition, the placenta adapts morphologically and functionally to maintain fetal growth, despite its small size. In part, these adaptations may be due to reduced expression of the placental-specific P0 transcript of the paternally expressed *Igf2* gene and alterations in its growth promoting signalling pathway.
Aim: To determine the role of placental *Igf2* signalling in the fetal and placental response to maternal calorie-restriction.
Methods: Wildtype (WT) females were mated with WT or *Igf2P0* -/+ males to generate homozygous WT and *Igf2P0* -/+ litters. Females were fed either control diet *ad libitium* (CT) or 80% of the control intake (UN) during pregnancy. After euthanasia on D19, the placenta next closest to the mean per litter was frozen for analysis of protein abundance by western blotting (n=4-5 litters per group). Differences between groups were considered significant if $P<0.05$.
Results: In WT placentas, UN increased the abundance of IGF type 1 receptor (IGF1R) and reduced the p85α subunit of PI3K, active AKT (phosphorylated at S473 and T308) and the activity of AKT's targets, p70S6K and glycogen synthase kinase (GSK)-3β ($P<0.05$, all cases). The placental expression profile of IGF2 downstream signalling proteins was similar in CT*Igf2P0* and UNWT. In *Igf2P0* placentas, UN had no effect on expression of IGF1R or p85α but increased phosphorylation of AKT (S473) and GSK3β. Fetal and placental weights were reduced in UNWT and CT*Igf2P0* relative to CTWT. UN reduced fetal but not placental weight in *Igf2P0* litters compared with CT*Igf2P0*.
Conclusions: Dietary restriction produced a different signalling phenotype in WT and *Igf2P0* placentas. Signalling in the CT*Igf2P0* placenta resembled that of the UNWT, whilst UN*Igf2P0* was similar to CTWT. This suggests that the *Igf2P0* placenta maybe receiving appropriate nutrition for its small size in the UN state. Furthermore, the placental-specific *Igf2P0* transcript plays a major role in the placental response to maternal undernutrition.
Keywords: insulin-like growth factor, nutrition, AKT (protein kinase B)

[P3.78]

LEPTIN INCREASES PROTEIN SYNTHESIS IN TROPHOBLASTIC CELLS BY ACTIVATING MAPK AND PI3K PATHWAYS

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Leptin, the Ob gene product is produced in placenta where it may work as an autocrine hormone, mediating growth, angiogenesis and immune modulation. In fact, leptin receptor (LEPR, also known as Ob-R) is expressed in placenta, and leptin seems to be be an important autocrine signal for trophoblastic growth during pregnancy. Thus, we have recently described the antiapoptotic and trophic effect of leptin on choriocarcinoma cell line JEG-3, stimulating DNA and protein synthesis. We have also demonstrated the presence of leptin receptor and leptin signaling in normal human trophoblastic cells, activating JAK-STAT, PI3K and MAPK pathways.

In the present work we have employed dominant negative forms of MAPK and PKB constructs, as well as pharmacological inhibitors of MEK and PI3K to find out the signaling pathways that specifically mediates the effect of leptin on protein synthesis. As previously shown, leptin stimulates protein synthesis as assessed by 3H-leucine incorporation. However, the inhibition of MAPK and PI3K pathways by using both dominant negative forms of MAPK and PKB, or by blocking PI3K and MEK activation by using pharmacological inhibitors prevented the leptin stimulation of protein synthesis in JEG-3 choriocarcinoma cells. The inhibition of both pathways also prevented the leptin stimulation of p70 S6K, which is known to be an important kinase in the regulation of protein synthesis. Moreover, leptin stimulation of phosphorylation of EIF4EBP1 and EIF4E phosphorylation, which allows the initiation of translation was also prevented by blocking the activation of MAPK and PI3K pathways.

Therefore, these results demonstrate that both PI3K and MAPK are necessary to observe the effect of leptin stimulating protein synthesis in choriocarcinoma cells JEG-3, and this effect is mediated at least in part by activating the translation machinery signalling.

Keywords: Leptin receptor, Signal transduction, Protein synthesis, Trophoblast

[P3.79]

INVESTIGATING TRANSPLACENTAL SIGNALLING MECHANISMS PRODUCED FROM TROPHOBLASTIC TISSUE INVOLVED IN DNA DAMAGE

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Background: Many drugs and substances are known to be teratogenic in pregnancy, however much remains to be learned about the mechanisms of cell damage across the placenta. Recent research has investigated DNA damage across an artificial barrier consisting of a confluent layer of Be Wo cells, a tissue culture model of the human placenta. Bhabra et al showed DNA damage in fibroblasts when the barrier was subjected to nanoparticle exposure [1]. Damage in the fibroblasts was caused by a signalling process as the metal nanoparticles did not cross the barrier. The process of intercellular signalling involved gap junctions and purinergic (ATP) transmission. The research has shown that the barrier increases cell and DNA damage instead of being protective. Blocking cell to cell communication processes reduced this damage.

Objectives: Investigate if the real human placenta releases signals that could cause DNA damage in human embryonic stem cells (or fibroblasts as control) if the placenta is exposed to nanoparticles or hypoxia

Design: Laboratory Study

Main outcomes: Level of DNA damage in two types of human cells; fibroblast cells and human embryonic stem cells. Ability of stem cells to differentiate into germ cell layers after the exposure to signals from the placenta.

Methods: We plan to recruit women over a 3 month period (May- August 2010) to sample first and third trimester trophoblastic tissue. The extracted placental tissue will be dissected and either nanoparticles or medium without nanoparticles will be added. This medium will then either be analysed for the messaging chemicals which it contains, or transfered onto other human cells and these cells analysed for DNA damage. Assessment of DNA damage in fibroblast cell lines will be done by Comet assay and Gamma-H2AX. FISH will be used to assess aneuploidy or tetraploidy.

References:

(1) Bhabra G, Sood A, Fisher B, Cartwright L, Saunders M, Evans, WH, Surprenant A, Lopez-Castejon G, Mann S, Davis S, Halis L, Ingham E, Verkade O, Lane J, Heesom K, Newson R, Case CP. Nanoparticles can cause DNA damage across a cellular barrier. Nature Nanotechnology Vol 4 December 2009

Keywords: Signalling mechanisms, Teratogenesis, Trophoblastic tissue

[P3.80]

ERK1/2 AND JAK2-STAT3 INTRACELLULAR SIGNALING PATHWAYS ARE INVOLVED IN SEROTONIN-5HT2A RECEPTOR-INDUCED JEG-3 CELL PROLIFERATION

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Introduction: Serotonin has a mitogenic effect on various cell types. In a previous study, we have demonstrated that serotonin through its 5-HT_{2A} receptor induces proliferation of trophoblast-like JEG-3 cells and activates the MEK-ERK1/2 and JAK2-STAT3 signaling pathways.To better understand the signaling pathway involved in the 5HT2A-induced JEG-3 cell proliferation, in this study we investigated the intracellular signaling cascade involved in the $5HT_{2A}$-induced MEK-ERK1/2 and JAK2-STAT3 activation.
Methods: The effect of specific inhibitors of phospholipase C (PLC)-β (U73122), PKC-β (Gö6976), PKC-ζ (GF109203X) and Ras (farnesylthiosalicylic acid) on DOI-induced ERK1/2 phosphorylation was determined by western blot. The effect of U0126 and AG490, specific inhibitors of ERK1/2 and JAK2 respectively, on DOI-induced JEG-3 cell proliferation, was analyzed by WST-1 proliferation assay.
Results: Western blot analysis showed that U73122 and farnesylthiosalicylic acid dose-dependently abolish, and Gö6976 decreases, the $5HT_{2A}$-induced ERK1/2 activation whereas GF109203X has no effect. The JEG-3 cell proliferation induced by DOI (25μM) was fully inhibited by both U0126 (25μM) and AG490 (30μM), as well as by U73122 (560nM) and chelerythrine (500nM, specific inhibitor of PKCs).
Discussion: Our data demonstrates that both ERK1/2 and JAK2/STAT3 are required for 5-HT_{2A}-induced JEG-3 cell proliferation. Moreover, this study indicates that $5HT_{2A}$-activates ERK1/2 phosphorylation through activation of the PLCβ-PKC-β-Ras pathway. These findings indicate that serotonin through the activation of the 5-HT_{2A} receptor is a key regulator of trophoblast-like cell proliferation and may play a role in placental, and by consequence in fetal development., Supported by a Natural Sciences and Engineering Research Council of Canada (NSERC)
Keywords: serotonin 5-HT2A receptor, cell proliferation, JAK-STAT, MAPKs

[P3.81]

AROMATASE (CYP19) ACTIVITY AND EXPRESSION ARE INDUCED BY SEROTONIN AND 5-HT2A-MEDIATED SIGNALLING IN PLACENTAL TROPHOBLAST CELL MODELS

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Introduction: Estrogens regulate placental development and function and are critical for maintenance of pregnancy and fetal health. It is known that serotonin can affect the production and function of sex hormones, such as estrogens. The key enzyme controlling estrogen synthesis during pregnancy is placental aromatase (CYP19). To better understand the regulation of placental aromatase, this study was designed to determine whether serotonin and 5-HT_{2A}-signalling can regulate the expression and catalytic function of this enzyme.
Methods: Aromatase activity and expression of placental I.1 and exon II-derived CYP19 mRNA were determined in BeWo and JEG-3 cell lines following exposure to serotonin and selective 5-HT_{2A} agonists, or activators of PKA and PKC signalling.
Results: Serotonin and 5-HT_{2A} agonists increased aromatase activity and expression in both cell lines. Serotonin caused a maximal, statistically significant increase in aromatase activity of 1.5-fold in BeWo cells following 24h exposure, while the selective 5-HT_{2A} agonists DOI and TCB-2 increased activity by 1.8-fold and 2-fold, respectively. The expression of CYP19 mRNA was also increased by serotonin, particularly through the placental promoter I.1 (+ 1.5-fold in BeWo cells). Phorbol ester-mediated PKC activation increased aromatase activity (+ 3.4-fold) and mRNA expression (+ 1.9-fold), while activation of PKA by forskolin elevated activity and expression by over 2.3-fold. Responses in JEG-3 line were similar, but less pronounced, particularly for PKC-mediated activation of aromatase. Effects on aromatase activity were blocked by 5-HT_{2A}- and PKC-selective antagonists in both cell lines.
Discussion: This study shows for the first time that serotonergic signalling modulates aromatase expression in placental trophoblast cells. More detailed studies of serotonin-estrogen interactions through the 5-HT_{2A} receptor are vital to better understand their role during pregnancy, and may help to explain gender-specific differences in symptoms of disorders related to serotonin dysfunction, such as depression, and response to selective serotonin-reuptake inhibitors (SSRIs).
Keywords: CYP19 (aromatase), serotonin, trophoblast-like cells, 5-HT2A receptor

[P3.82]

PRIMARY DECIDUAL STROMAL CELLS: A MODEL SYSTEM TO STUDY WINGLESS (WNT) SIGNALLING IN HUMAN DECIDUALIZATION

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Objective: Recent investigation indicated that various components of the Wnt signalling pathway are regulated in a menstrual cycle-dependent manner suggesting a role in implantation and endometrial function. However, activity of canonical Wnt signalling during human decidualization still remains to be elucidated. To study differentiation-dependent expression of Wnt genes we sought to evaluate an in vitro decidualization model system using isolated stromal fibroblasts of the placental bed.
Methods: Primary human fibroblast of the decidual part of legal abortions were isolated by enzymatic digestion and stimulated with 8-Bromo-cAMP or progesterone (P)/17β-estradiol (E) for 3, 6, 9, and 12 days. To analyse decidualization-dependent gene expression two well described marker genes, IGFBP1 and prolactin, were evaluated by quantitative real-time PCR. Subsequently, mRNA expressions of selected Wnt ligands, Frizzled receptors as well as inhibitors of canonical Wnt signalling such as Dickkopf-1 (DKK1) were analyzed by semiquantitative RT-PCR or real-time analyses, respectively. Furthermore, canonical Wnt reporter plasmids were transfected into decidualizing cultures to gain more insight into the temporal regulation of Wnt signalling.
Results: Real time analyses revealed cAMP-dependent mRNA expression of prolactin and IGFBP1 peaking on day 3, while mRNA levels steadily increased from 3 to 12 days upon addition of E/P. Secreted IGFBP1 protein detected in supernatants of cultures by Western blotting mirrored mRNA expression. DKK1 mRNA was induced on day 3 in the presence of E/P, but not by cAMP, remaining at a high levels during the whole stimulation period. Furthermore, decidualization-dependent induction of Wnt4 and Wnt6 could be observed. Measurement of luciferase activity showed enhanced transcription of Wnt reporter plasmids in the presence of E/P.
Conclusion: Primary human fibroblasts isolated from the placental bed provide a valuable tool to study decidualization-dependent processes when stimulated with E/P. Differentially regulated Wnt components at early and late stages of differentiation and E/P-dependent stimulation of reporter plasmids suggest a critical role of canonical Wnt signalling in decidualization.
Keywords: human decidua, stromal cells, Wnt signalling, differentiation

[P3.83]

THE RELATIONSHIP BETWEEN PLACENTAL VOLUME IN EARLY PREGNANCY, MEASURED USING A NOVEL SEMI-AUTOMATED TECHNIQUE, AND THE SMALL FOR GESTATIONAL AGE (SGA) FETUS

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Introduction: Evidence is growing that fetal growth restriction secondary to placental insufficiency may be predicted early in pregnancy by placental volume. However, until now, 3-D ultrasound measurement of the placenta has had to be undertaken by hand using manual delineation or VOCAL (GE Medical Systems). This is time consuming and relegates the calculation of placental volume to a research tool. We sought to investigate whether our novel, semi-automated technique for estimating placental volume in the first trimester could be used to predict the small for gestational age (SGA) fetus.
Methods: With ethical approval, we prospectively recruited women with a singleton pregnancy and a BMI of $\leq$35. A 3-D ultrasound scan was performed using a Voluson E8 (GE Medical systems) at between 12 and 15 weeks gestation. The placental volume was calculated using our previously validated technique. Uterine artery Doppler screening was undertaken at 23 weeks gestation. Delivery data including birth-weight was collected and customised centiles calculated using the 'Grow' package (West Midlands Perinatal Institute); SGA was defined as $<$10th centile. The placental volumes of SGA fetuses were compared with those with a birth-weight $\geq$10th centile by linear regression analysis using SPSS (SPSS inc).
Results: 65 women were recruited to the study, all had an ultrasound scan between 12+5 weeks and 15+0 weeks gestation. Seven women had SGA babies (birth-weights between $<$1st and 8th centile); only one had raised uterine artery Doppler indices at 23 weeks (birth weight $<$1st centile). Linear regression analysis with correction for gestation showed that SGA babies had significantly smaller placentas in early pregnancy ($p=0.028$).
Discussion: Low placental volume in early pregnancy appears to correlate with low birth-weight. Our semi-automated technique is robust enough to detect these differences and may aid in the development of an ultrasound-based screening test for growth restriction