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Abstracts

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Plenary Lectures

THE PLACENTA AND FOETAL WELL-BEING: WHAT IS THE ROLE OF AMINO ACID TRANSPORTERS?

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It is self-evident that the conceptus must obtain nutrition from the mother via the placental (uterine) circulation in placental mammals. For amino nitrogen, the delivery of amino acids may be rate limiting for growth, particularly for those essential amino acids that are not synthesized at a rate sufficient for optimal foetal and placental growth. The elucidation of physiological transport systems in the (human) placenta and their molecular identification over the last 10 years has opened a new chapter with respect to the patho-physiology of these processes. In will: a) review what is known (highlighting obvious deficiencies in knowledge), b) describe recent important studies on the roles of imprinted genes in foetal growth and c) indicate the possible importance of novel roles of amino acid transporters in the placenta, specifically in trphoblast cell biology and nutrient sensing. Supported by the Wellcome Trust. References: *Reproduction* 124:593-600, 2002; *Ped Res*.

PROTEIN PROFILING – THE APPLICATION OF PROTEOMIC TECHNOLOGIES IN REPRODUCTIVE BIOLOGY

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The aim of this review is to consider the application of protein profiling platforms within the discipline of reproductive biology. The challenge of contemporary proteomics is how to apply multivariate data in a clinical context - this is the aim of the burgeoning field of translational proteomics. Two solid-phase affinity capture approaches that seek to leverage the output of proteomic platforms are (i) protein solution array and (ii) direct mass spectrometry protein profiling. The former has its technological roots well founded in traditional immunoassay and is a hybrid technology that combines the sensitivity and specificity of immunoassay with the liquid handling and particle identification attributes of flow cytometry. The latter combines affinity-based sample fractionation with MALDI-ToF mass spectrometry (MS) (e.g. ClinProt MALDI ToF-ToF MS). The putative benefits afforded by antibody-based solution array are: (i) quantitation of analytes in multiplex format (up to 100 analytes per array); (ii) good reproducibility (with CV <10%); (iii) sensitivity in the pmol-fmol range; and (iv) specificity that minimises the effect of the dynamic range of protein concentrations in the sample to be assayed. While direct mass spectrometry offers the opportunity for diagnosis based upon changes in the m/z protein profile. The application of these approaches has identified biomarker profiles that are of utility in identifying women at risk of complications of pregnancy.

Lectures

IMPLANTATION IN THE PRIMATE: CELLULAR AND MOLECULAR RESPONSES

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Embryo-derived factors directly or indirectly influence endometrial receptivity and implantation in primates. Our studies in the baboon demonstrated that chorionic gonadotropin (CG), has physiological effects on the three major cell types in the uterine endometrium. The glandular response to CG infusion is characterized by a marked increase in glycodelin. The primary effect of CG on stromal fibroblasts is the induction of α-smooth muscle actin [αSMA]. Following the initial signaling cascade activated by CG, the implanting embryo enhances the process of decidualization within the endometrium. Since IL-1 was identified as one modulator of the communication between the maternal endometrium and embryo we investigated the possible involvement of IL-1β in decidualization. IL-1β induces COX-2 expression, prostaglandin E_2 (PGE_2) synthesis and in the presence of steroid hormones also IGFBP-1 expression in stromal fibroblasts. IL-1β induced mRNA expression and synthesis of proMMP-3 protein in baboon stromal fibroblasts. We propose that the regulation of MMP's and specifically MMP-3 activity by progesterone is necessary for transformation of stromal cells into fully differentiated decidual cells during pregnancy. During pregnancy, decidualized endometrial stromal cells express high levels of IGFBP-1 which act either as an insulin-like growth factor (IGF)-binding protein or via insulin-like growth factor (IGF)-independent effects at the maternal-fetal interface. We asked whether the two transcription factors FOXO1A and HOXA10 might contribute to the regulation of IGFBP-1 in uterine cells. In summary, our studies suggest that the interaction between the embryo and the maternal endometrium initiates a complex sequence of events that results in the transformation of stromal cells to decidual cells.

DECIDUA IN RODENTS

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Decidua has been studied since Krebiel (1937) published his observations on the rat. Although a transitory structure that develops during pregnancy in the endometrium of some animals, has a relevant role for the successful of pregnancy. The decidua is formed by endometrial fibroblasts that, following hormonal stimulation, acquire new phenotype transforming in decidual cells. Contrary to the fibroblasts from which they originate, decidual cells are round or polygonal cells. In rodents, the decidual cells are compactly arranged as an endocrine gland. The first decidual cells are seen in the antimesometrial side of the uterus under the uterine epithelial lining that surrounds the implantation crypt, during the stage of apposition of the trophoblast to the epithelium. Differently from humans, the decidua of rodents is discontinuous along the uterus, being restricted to the stroma surrounding the embryos (implantation sites). So, the interimplantation site of the uterus is almost unchanged. Moreover, in rodents an initial stimulus is necessary, as spontaneous decidualization does not occur. Signal molecules including prostaglandins, cyclooxigenase 1 and 2, interleukin-11, leukemia inhibitor factor are believed to participate in the decidual transformation in rodents. Decidualization also comprises a remarkable remodeling of the extracellular matrix. In mice proteoglycans are temporospatially expressed in the endometrium indicating a role of these molecules in the embryo implantation and development. Despite many unanswered questions regarding the functions of its cells and molecular products and their interaction with the embryo, there are evidences that decidual cells secrete several hormones and growth factors and probably help to control the migration of the embryo within the endometrium.

ALTERATION OF THE PLASMA MEMBRANE CALCIUM TRANSPORT AND ITS IMPORTANCE FOR THE PATHOGENESIS OF PREECLAMPSIA

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Preeclampsia affects 7% to 10% of pregnant women and appears after the twentieth week of pregnancy. This disorder is characterized by vascular endothelial damage, abnormalities in plasma volume, hypertension, proteinuria, edema and generalized arteriolar vasospasm. A modification in the calcium homeostasis seems to be involved in the pathogenesis of preeclampsia. The plasma membrane Ca-ATPase (PMCA) is an enzyme that participates in the fine control of the cytosolic free calcium concentration. We showed that PMCA activity of red cell ghosts from pregnant women with preeclampsia, is reduced ~50% as compared to the ATPase activity of red cell ghosts from normotensive pregnant women. This appears to be associated with a rise in membrane lipid peroxidation. As soon as hypertension, proteinuria and edema recede in the puerperium of the preeclamptic women, both PMCA activity and lipid peroxidation of the plasma membranes return to normal values. There is a close relationship between the level of lipid peroxidation, the PMCA activity and preeclampsia. The diminution in PMCA activity and the increase in the level of lipid peroxidation with preeclampsia are not restricted to red cell ghosts from preeclamptic women, since similar findings have been found for red cell ghosts of their neonates as well as myometrium plasma membranes and basal plasma membranes (fetal side) of syncytiotrophoblast from preeclamptic women. A reduced PMCA activity, caused by an increased level of lipid peroxidation, may result in an increase in the cytosolic calcium concentration in the vascular smooth muscle cells of preeclamptic women and this partially could explain the high blood pressure developed by these patients. Support in part: H9/181/R427, Project 96350 (World Health Organization).

AQUAPORINS IN DEVELOPMENT

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Fluid balance in the fetus is essential for normal growth and development. For most of the early life of the fetus water is 85% of the body composition, decreasing to 75% at birth. The mother is the only source of all water and electrolyte, via the placenta. Amniotic fluid surrounds the fetus, and input by lung liquid and fetal urine are essential; transmembrane transport (via amnion/fetal skin) is important for normal volume maintenance. The continuous production of lung liquid is crucial to the normal growth of the fetal lung, but this fluid must be reabsorbed at birth to allow for gas exchange.. The fetal kidney normally produces a high volume of dilute urine, but must be ready, at birth to conserve water. Of the 11 mammalian aquaporins (AQP) known, seven (AQPs 0,1,2,4,5,8,10) function primarily as water channels, whilst others are channels for water and urea, glycerol (AQPs 3,7,9) or nitrate(AQP6). In long-gestation species, such as the human and the sheep, the lungs and kidneys are much better developed before birth than these organs are in the mouse. Thus the ontogeny and function of aquaporins are much better studied in organs in the sheep than in the mouse. Our studies of the ontogeny, regulation, and function of AQPs 1, 2, 3, 4, 5 and 8 in the placenta, fetal membranes, heart, lung and kidney of the sheep will be discussed. It is suggested that studies of mice with a particular aquaporin gene deletion (AQP -KO) do not, necessarily, provide adequate evidence for the function of various AQPs in the pre-natal, intrauterine period (Liu and Wintour, *Reprod Biol Endocrinol* 3:18, 2005).

THE HUMAN PLACENTA IN DIABETES MELLITUS

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The human placenta is exposed to metabolic and hormonal diabetes-associated derangements of both mother and fetus, and one can expect some impact of diabetes on the placenta. Studies have produced divergent results, likely because of the variety of confounding factors, for which better control is needed in future investigations. Apart from the quality of metabolic control, the most important factor appears to be the time point of the metabolic or endocrine insult. In the first trimester, hyperglycemia reduces trophoblast proliferation, which may account for the early fetal growth delay in diabetes. Poor glycemic control later in gestation results in placental oversupply of the fetus with maternal nutrients, because of the steeper maternal-to-fetal concentration gradient, leading to the well-known fetal phenotype. Increased levels of insulin secretagogues result in fetal hyperinsulinemia, which stimulates aerobic glucose metabolism and increases fetal oxygen demand. In case of inadequate oxygen supply across the placenta, fetal hypoxemia will result, which in turn will stimulate placental angiogenesis to increase the surface area of exchange by hypervascularization. Fetal hyperinsulinemia along with fetal hyperglycemia stimulates placental glycogen synthesis resulting in higher placental glycogen deposition, a common feature of diabetic placentas suggesting a storage function for excess fetal glucose. The glycogen stores may be utilized for the fetus in a situation of fetal emergency energy demand, e.g. transient hypoglycemia, when lactate rather than glucose would be the outflowing product because of low-level expression of glucose-6-phosphatase. Collectively, a variety of changes has been found in the diabetic placenta, which may affect fetal outcome, but studies are still in their infant stage. More data are needed in order to fully understand whether and how the placenta protects the fetus from the maternal derangements in a diabetic pregnancy.

FETAL PROGRAMMING: WHAT IS THE RELATIONSHIP WITH LOW BIRTH WEIGHT?

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The universally accepted concept of Fetal Programming refers to the long term consequences of fetal exposure to a given stimulus or insult. These consequences stem from changes in tissue structure, physiology and/or gene expression and express themselves as a predisposition of the individual to develop a “disease” in adult life. Although several reports have confirmed the original observation by David Barker of an increased risk for developing hypertension in individuals born with low birth weight, it is not clear if the trigger is in fact low birth weight. An association between low birth weight and disease risk has been found predominantly in studies with relatively small sample size. An important contributor to the increased risk seems to be the weight gain rate during childhood and early adolescence. Studies by Barker and others have shown that a disproportionate weight gain relatively to height is a very good predictor for developing coronary heart disease. Particularly intriguing is the lack of association between high blood pressure and birth weight among Dutch adults who were exposed as fetuses to extreme under nutrition at different stages of gestation.

METABOLIC DIFFERENTIATION, INTERACTION AND SIGNALING IN THE SEMINIFEROUS EPITHELIUM: LESSONS FROM BELOW

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Micro anatomical cell localization and compartmentalization in the brain and testicle set the conditions for metabolic interdependence between some cells in these organs. The metabolic interdependence between neurons and astrocytes is similar to the interdependence between spermatogenic and Sertoli cells in the testis. Sertoli cells have a large glycolytic capacity and secrete lactate in the presence of glucose. Spermatocytes and spermatids, developing surrounded by Sertoli cells, take up and aerobically metabolize lactate. Glucose can also reach spermatogenic cells, which can transport and metabolize this substrate. A peculiar aspect of glucose metabolism in spermatids is its induction of a decrease in ATP and increase in AMP. Correlating with the intracellular adenine nucleotide changes, intracellular Ca^{2+} raises and pH decreases in these cells upon glucose addition. The opposite is observed when these cells metabolize lactate. Lactate and glucose concentrations in the microenvironment of the spermatogenic cells are expected to fluctuate with endocrine and paracrine signals (FSH, IL-1) sensed by Sertoli cells, and a similar pattern of changes is expected in intracellular Ca^{2+} and pH in spermatogenic cells, linking the endo-paracrine status sensed by Sertoli cells to physiological changes in spermatogenic cells. This aspect of metabolic interdependence has not been described in other cell systems where metabolic interactions occur. The molecular entities that allow these metabolic interactions in the seminiferous tubule appear transiently during the spermatogenesis, strongly suggesting that they are related to differentiation control rather than to epiphenomena in cell differentiation to spermatozoa. Supported by FONDECYT, DI-PUCV.

A SCIENTIST AND THREE OF HIS SUBJECTS: PLACENTA, DARWIN AND HUMBOLDT

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I graduated as a medical doctor but my intention was to be a scientist. My thesis and my first researches were on the regulation of red cells production and with my mentor, George Hodgson, we contributed to the discovery of erythropoyetine, a hormone which today cures anemic patients. My interest turned into capillary permeability and the endothelium, in various organs, and my last twenty years were dedicated to the placenta and the umbilical vein. Many of those subjects continue to be investigated today by my disciples with the same dedication and creativity that helped me when they were students. From human normal and “diabetic” placentas we prepared basal and brush-border membranes from the syncitiotrophoblast and from the umbilical vein we cultivated endothelial cells. First in England and after my return, in Chile we studied transport of glucose, amino acid and nucleosides. My farewell to science was an article in *Physiological Reviews* (83:183-252, 2003) by G.E. Mann, D.L. Yudilevich and L. Sobrevia: *Regulation of Amino Acid and Glucose Transporters in Endothelial and Smooth Muscle Cells*. The title of my conference relates to my interest on two great naturalists, which in there youth explored, our Spanish America: Charles Darwin and Alexander von Humboldt. I have followed there steps and this has helped me to stay young and I hope that this geniuses will create among young people a love for nature and travel as good for a spirit as science. They will benefit of my two books, 1996 and 2004 (Editorial Universitaria, Santiago de Chile): *Darwin en Chile* and *Alexander von Humboldt: Mi viaje por el Camino del Inca 1801-1082.*

UTEROPLACENTAL CYTOKINES AND THE TH1/TH2 PARADIGM
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It has been put forward that there is a TH2-type bias in local cytokine production in the uteroplacental environment that favours the immunological survival and growth of the feto-placental unit in pregnancy. Experimental support has come predominantly from murine rather than human pregnancy. More recent studies have identified a vast range of cytokines that are locally produced, with the notable exception of IL-2, as well as complex local temporal and cellular control of cytokine expression. A significant proportion of uteroplacental cytokines are released from non-immune cells. It has now become established that many non-Th1/Th2 cytokines have important roles in implantation and placental development (eg. LIF) as well as some Th1-type cytokines (eg. γ-IFN). Also, there is no increased fetal loss in quadruple Th2 cytokine-knockout mice lacking IL-4, IL-5, IL-9 and IL-13, even in allogeneic pregnancy. It is becoming increasingly clear that uteroplacental cytokines are regulated by hormonal controls, suppressors of cytokine signalling (SOCS) and local production of diverse molecular variants acting as receptor antagonists or 'decoys' and allowing the uteroplacental environment itself to self-regulate local cytokine functional activities. These cytokine regulatory networks need to be further elucidated both in normal pregnancy and in unexplained recurrent miscarriage.

OXIDATIVE AND NITRATIVE STRESS IN THE PLACENTA
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The placenta exists in a state of oxidative stress which is further increased in pregnancies complicated by preeclampsia or diabetes mellitus. Increased production of both reactive oxygen and nitrogen species is seen under these conditions. Interaction of superoxide and nitric oxide produces peroxynitrite a powerful oxidant that nitrates tyrosine residues on proteins causing a loss or gain of function. We have previously shown p38MAP kinase to be a nitrated protein in the preeclamptic placenta and that this is associated with loss of catalytic activity. We have subsequently determined the nitrated tyrosine residues and effect on catalytic activity upon peroxynitrite treatment of p38MAP kinase in vitro together with studying the effect of peroxynitrite on expression and activity of p38MAP kinase in BeWo cells. **Methods:** Serum starved BeWo cells were treated with 1mM peroxynitrite or vehicle for 15-20 minutes. Cell lysates were prepared and centrifuged at 20,000xg and supernatant used for all studies. Proteins were seperated by SDS-PAGE and western blotted for proteins of interest. The ability of p38MAP kinase to phosphorylate ATF2 was used as a measure of catalytic activity. Data were compared by one way ANOVA and post hoc analysis. **Results:** Peroxynitrite treatment gave an immediate reduction in p38MAP kinase catalytic activity in BeWo followed by a reproducible and significant increase in activity by 2 hrs following treatment. By 4hr activity was decreasing towards control. Expression of p38MAP kinase followed a similar trend to activity. With mass spectrometry we identified 3 tyrosine residues nitrated in vitro which were associated with loss of catalytic activity. **Conclusions:** Peroxynitite treatment in vitro nitrates p38 MAP kinase and reduces catalytic activity. This is similar to the situation seen in the placenta in preeclampsia. Nitration of certain tyrosine residues is associated with loss of catalytic activity.

NUCLEOSIDE TRANSPORTERS: FROM PHYSIOLOGY TO PHARMACOLOGY
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The uptake of nucleosides into cells is mediated by at least, two unrelated protein families, CNT (Concentrative Nucleoside Transporter) and ENT (Equilibrative Nucleoside Transporter), encoded by SLC28 and SLC29 gene families, respectively. CNT1, CNT2, and CNT3 are high-affinity, Na-coupled, nucleoside transporters that show pyrimidine, purine and broad substrate selectivity, respectively. Their expression was initially thought to be restricted to absorptive epithelia but it is now known to be much broader than expected, being its occurrence a typical feature of differentiated specialized cells, including neurons, immune system and selected endothelial cell types, as well as most epithelia. ENT1 and ENT2 are plasma membrane, low affinity, facilitative nucleoside transporters, showing relatively broad substrate selectivity, ENT2 being a nucleobase transporter also. ENT1 expression is ubiquitous. Two other members of the ENT protein family, ENT3 and ENT4 are less well characterized. Both CNTs and ENTs mediate the uptake of most nucleoside-derived drugs used in anticancer therapy, although their ability to recognize antiviral nucleosides is compromised by particular structural determinants that appear to be essential for substrate recognition. The following aspects of CNT and ENT physiology and pharmacology will be reviewed: a) How CNT and ENT polarized insertion might contribute to nucleoside and nucleoside-derived drug vectorial flux across epithelia; b) How CNT expression and insertion into the membrane is regulated in selected epithelial cell types; c) How CNT and ENT expression contribute to the pharmacogenomic response associated with nucleoside-derived drug therapy in tumors. Support: Ministerio de Sanidad y Consumo, Ministerio de Educación y Ciencia, Generalitat de Catalunya, Universitat de Barcelona, Fundación Ramón Areces, Fundación "la Caixa" y FIPSE.

SCIENCE FOR ALL CHILDREN: A NEW DEAL BETWEEN SCIENCE AND SCHOOL
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In spite of the extraordinary advances of science and technology in the last decades and the increase of their influence, science continues to be a privileged knowledge. The achievement of a more equitable access requires improving the quality of science education in the schools and this demands an international effort. Following the Transition to Sustainability Conference held in Tokyo (2000), the Science Academies called for a stronger involvement of scientists to work as active partners with their local educational systems to ensure effective science education. In Chile, this encouraged the "Inquiry based Science Education Program (ECBI)" a joint initiative of the Chilean Academy of Sciences, the Ministry of Education and the Faculty of Medicine of the University of Chile. The program is inspired by the belief that high quality science education is important for all children. Effective science education is expected not only to expand the children´s understanding of the natural and material world, but also to stimulate their curiosity, introduce them to the practice of scientific inquiry and prepare them for life long learning. It is anticipated that these will contribute to their full expression of their creative potential, improving their quality of life and that of their community. The principles, strategies and results of the ECBI Program will be presented in the context of other international initiatives having the same aim. Funded by Ministry of Education and Fundación Andes.

REGULATION OF TROPHOBLAST INVASION DURING EMBRYO IMPLANTATION AND PLACENTATION

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Tumour invasion and trophoblastic invasion share the same biochemical mediators: the matrix metalloproteinases (MMPs) and their inhibitors. MMPs are a family of enzymes capable of digesting the extra-cellular matrices of the host tissues. Human cytotrophoblastic cells (CTB) are constitutively invasive and produce MMPs. That MMPs are causally related to trophoblast invasion in the endometrium is shown by the fact that tissue inhibitor of metalloproteinases inhibits cytotrophoblastic invasion in vitro. In contrast to tumour invasion of a host tissue, trophoblastic invasion during implantation and placentation is stringently controlled in both space and time. The factors responsible for these important regulatory processes are unknown but in-vitro studies point to autocrine (trophoblastic) and paracrine (endometrial) controls by cytokines, growth factors and hormones. These regulators exert their effects directly or indirectly by activating nuclear transcription factors. Transcription factors are proteins or protein complexes (often the products of oncogenes) that activate genes by binding to specific sites of the DNA located in the regulatory (5' flanking) region of genes. This presentation describes the different regulators of trophoblast invasion and summarises our knowledge about the signalling cascade and the transcription factors involved. We will also speculate about a potential role of onco-suppressor genes (particularly p53) in regulating trophoblast invasion.

LOCAL REGULATORS OF ENDOMETRIAL RECEPTIVITY

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Failed implantation is an important cause of unexplained infertility and the most common cause of failed *in-vitro* fertilisation. Endometrium becomes receptive to embryo implantation, under the influence of ovarian steroids, but the mechanisms by which this occurs are little understood. It is likely that a multi-step process involving many different adhesions and signalling molecules is involved in embryo-attachment. Through two complimentary approaches, we have shown the synergistic regulation of uterine gene expression by cytokine and steroid pathways. Firstly, exposure of human secretory phase endometrium to RU486 results in the rapid down-regulation of Jak1 mRNA levels. Jak 1 and Stat3 are part of the intracellular signalling pathway for Leukaemia Inhibitory Factor (LIF), a cytokine that is essential for blastocyst attachment in rodents. We have also shown that the introduction of a cell permeable Stat3 peptide inhibitor into the mouse uterine lumen on day 3 of pregnancy dramatically reduces the implantation rate. Secondly, we have shown that physiological expression in mouse luminal epithelium, of immune response gene 1 (IRG1), insulin like growth factor binding protein 3 (IGFBP3) and amphiregulin, requires both progesterone and LIF. However, of these molecules only IRG1, whose function is unknown, has been shown to be essential for implantation. It has previously been speculated that blastocyst derived factors are necessary for the acquisition of complete, functional uterine receptivity. Using an in-vivo primate model, we have studied the direct actions of hCG on endometrium, during the pre-implantation period. We have shown that hCG induces alterations in the endometrial expression of genes that regulate embryo attachment, extracellular matrix remodelling and the modulation of the immune response around the implanting blastocyst.

ROLE OF A CYSTEINE PROTEASE IN MALE CHROMATIN REMODELING AFTER FERTILIZATION AND IMPACT OF ITS INHIBITION ON THE INITIAL CELL EMBRYONIC DIVISION

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In sea urchins male chromatin remodeling after fertilization involves the replacement of sperm specific histones (SpH) by CS histone variants. We have found a nuclear cysteine-protease that displays a fundamental role in male chromatin remodeling by degrading the SpH leaving the maternal CS variants unaffected. Based on the N-terminal sequence of this enzyme we have cloned the gene encoding this enzyme and found that this protease belongs to the catepsin L family. By using antibodies against the N-terminal peptide of this enzyme we have immuno-localized this enzyme after fertilization and found that it is present in unfertilized eggs. After fertilization it localizes into female pronucleus and tranlocates to male pronucleus, persists in the nucleus of the zygotes during S phase of the initial cell cycle, at the first mitosis co-localizes with α-tubulin that is organizing the mitotic furrow and segregates into the nucleus of the initial two blastomers afterwards. The inhibition of this enzyme blocks SpH degradation after fertilization *in vivo* but does not affect the fusion of both pronucleus. The micro-inyection of antibodies against this protease provokes the arrest of the initial S phase of the zygotes, followed by a severe alteration of the formation of the initial mitotic furrow. In addition, E 64-d an inhibitor of cysteine-proteases mimics perfectly the effects observed in microinjected zygotes. Taken together our results, we postulate that the inhibition of male chromatin remodeling blocks the initial cell cycle and aborts embryonic development. Grants: FONDECYT 1050100, PICS–CNRS France /CONICYT Chile and DIUC 204-037.001-1.0.

CELLULAR AND MOLECULAR DYNAMICS OF EPITHELIAL SUGAR TRANSPORT

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The transport of D-glucose in the small intestine and in the kidney involves at the luminal cell surface a sodium-D-glucose contransport system (SGLT), which is a prime example of ion gradient-driven secondary active transport systems. In recent years our knowledge about the transporter and its regulation has progressed considerably. Generation of specific antibodies revealed that the transporter not only resides in the brush border membrane but that a large intracellular pool exists located in tubulin-associated late endosomes. Using GFP-labelled transporter, time laps video imaging, and total internal reflection fluorescence the movement of these SGLT-loaded vesicles and their fusion with the plasma membrane could be demonstrated. SGLT thereby is detected in vesicles different from those involved in the endo/exocytosis of transferrin. The recycling of the transporters seems to be one of the ways to regulate transepithelial sugar transport. At the molecular level tryptophan scanning fluorescence studies on subdomains and molecular recognition AFM studies on intact cells have identified an extracellular loop between transmembrane domains 13 and 14 as potential inhibitor binding site and other extracellular loops as initial interaction sites for the substrate D-glucose. The binding involves considerable conformational changes in the transporter, that also can be detected on the isolated transporter in solution or reconstituted in proteoliposomes. Thus a very dynamic picture at different levels of complexity and with different spatial and temporal dimensions arises for epithelial transport- a prerequisite for the existence of individual compartments and environments.

SLIMP Brazil 2003 Award Presentation

EPITHELIAL SODIUM CHANNEL IN THE HUMAN SYNCYTIOTROPHOBLAST

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The syncytiotrophoblast (SCT) of placenta regulates the transport of solutes and water between maternal and fetal blood. This transport involves movement of Na^+ and its contribution to the osmotic pressure is an important determinant of the extracellular fluid volume. The aim of this work was to detect and characterize the Epithelial Sodium Channel (ENaC) in human SCT from normal and preeclamptic placentas. ENaC is associated with hypertensive disorders when its expression is deregulated in absorptive epithelia. **Methods:** We carried out studies using RT-PCR, western blot and immunohistochemistry in normal and preeclamptic placenta. We also detected ENaC in the BeWo cell line (a human SCT model) and we studied the sodium currents generated in these cells with patch clamp. **Results:** α, β and γ-ENaC subunits are present in the apical membrane of the human SCT although α-ENaC subunit has a reduced expression in preeclamptic placentas. In BeWo cells, ENaC is modulated by aldosterone, vasopressin, estradiol and progesterone. Aldosterone (100 nM, 12 hs) induces a 20-fold increment in an AMPc-activated amiloride-sensitive current ($p < 0.05$) with a reversal potential close to 3 mV and an inward conductance of 127 ± 26 pS/pF, values similar to those described in other human tissues with a variable expression of ENaC subunits. **Conclusions:** Although the role of ENaC in SCT is still poorly understood, the reduction in α-ENaC expression in preeclamptic placentas may have consequences for ion and water transport. Our data could be of help for future studies on the mechanism involved in the physiopathology of preeclampsia. Support: UBACYT ME044 & PRODIM (Argentina).

Symposia

Symposium 1: Diabetes and placenta

Coordinator: M. Rudge (Brazil)

UMBILICAL ARTERY DOPPLER VELOCIMETRY AND PLACENTAL MORPHOMETRIC CHANGES IN DIABETES AND PREGNANCY

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Diagnostic accuracy of Doppler velocimetry of umbilical arteries is not defined in pregnancies complicated by diabetes. This study was carried out with 143 pregnant women with minimum gestational age of 34 weeks, and Doppler velocimetry in maximum intervals of 10 days from delivery, which were classified in normal glycemia and PI group (n = 26), hyperglycemia/normal PI group (n = 102), hyperglycemia/altered PI group (n = 15). Placental samples were randomized for morphometric study in image analyzer. Villous areas and numbers as well as their respective vessels were assessed. Qui-square and Fisher tests, variance analysis and stepwise were used for statistical analysis, with $p \leq 0.05$. Maternal glycemic levels were different, with average of 85.8, 103.0 and 116.8mg/dL, respectively, in the normal glycemia and PI, hyperglycemia/normal PI and hyperglycemia/altered PI groups. The placentas of pregnant women with hyperglycemia and normal PI had smaller terminal villi and higher number, with similar villous area to those of the normal glycemia and PI group. The villous vessels defined smaller vascular area and lower capillarization index in this group. The terminal villi and their respective vessel characteristics in the hyperglycemia/altered PI group were similar to those of the normal glycemia and PI group. The results showed that maternal hyperglycemia with varied origin and intensity determines placental morphometric changes, responsible for the normal or altered PI values of umbilical artery and for the resistance in the uteroplacental flow.

MATERNAL DAILY HYPERGLYCEMIA DIAGNOSED BY GLYCEMIC PROFILE (IB GROUP RUDGE, 1983): A MATERNAL AND PERINATAL PUBLIC HEALTH PROBLEM

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The aim of the obstetric researcher staff of School of Medicine of Botucatu is to invest in the recognition of the Group IB of Rudge (1983), pregnant women with daily hyperglycemia, despite a normal response to 100g GTT. In order to clarify why 100g GTT is not a good predictor of fetal macrosomia, the Database of the Diabetes and Pregnancy Service (grants FAPESP-INFRA IV) was analyzed and the pregnant women were re-ranked according to new GTT and GP threshold values. By this way, investigations were carried out to include a comparative study from pregnant women placentas in group IB and non-diabetic one (group IA) and diabetic pregnant women (group IIA and IIB). In the group IB placentas, absolute density values and morphological abnormalities were similar to those in diabetic women, and showed a higher incidence of endarteritis. Morphometrically, these placentas revealed a larger number of vilosities and vilous vessels that, despite having a smaller individual area, granted a larger maternal-fetal interface. Umbilical artery doppler revealed that intravilous vascularization (maternal-fetal interface extension) is directly related to pulsatility index (PI). In the pregnancies complicated by diabetes or daily hyperglycemia, an abnormal PI value related to morphometric abnormalities in placental vessels and vilosities, standing out as a predictor of intrauterine hypoxia. Thus, the utilization of GP as a predictor test of maternal hyperglycemia is very important to prevent maternal and perinatal complications because pregnancy women (group IB) have been considered low risk by Technical Manual from Brazilian Health Ministry.

PLACENTAL STUDIES IN DIABETES AND PREGNANCY: ASPECTS IN LABORATORY ANIMALS

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In early stages of pregnancy, placental development may be affected by any metabolic or endocrine insult. The rat placenta presents morphological structures very different to human placenta. In diabetes, oxygen free radicals are thought to be produced because of prolonged periods of exposure to hyperglycemia, which is known to cause nonenzymatic glycation of plasma proteins. Diabetes may be associated with increased advanced glycosylation end product formations on the fetuses and placenta. Many studies are been carried out to evaluate the effects of diabetes on the maternal-placental-fetal organisms in laboratory animals. These agents are used to induce experimental diabetes (corticoids, alloxan and streptozotocin). In our research laboratory, the diabetes of the Wistar rats is induced by streptozotocin (STZ), which presents antibiotic and diabetogenic properties. STZ per se does not induce DNA damage, oxidative stress and other alterations on the maternal environment, but its hyperglycemic effect is a causative factor. The aim of this presentation is to show and discuss the streptozotocin induced-diabetes effects on placental morphometry and DNA oxidative damage, associated or not to exhibition to tobacco cigarette smoking in Wistar rat pregnancy.

Symposium 2: Cytokines and trophoblast: a cellular and molecular approach

Coordinator: E. Bevilacqua (Brazil)

CYTOKINES AT THE MATERNAL-FETAL INTERFACE IN NON-MAMMALS

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Interleukin-1 (IL-1) is a pro-inflammatory cytokine with many functions in the immune system and in defence against infections. There have been many studies on the presence and role of IL-1 in human and murine reproduction. Previous studies in our laboratory showed that IL-1 is also expressed at the maternal-foetal interface of placental viviparous squamates and elasmobranch fishes. More recently, using a model of *Lacerta vivipara*, a species which exhibits reproductive bimodality, i.e. the coexistence of oviparous and viviparous populations, we showed that IL-1 system, including IL-1α, IL-1β and the functional IL-1 receptor, is expressed by the uterine tissues of both, viviparous and oviparous animals. In *L. vivipara*, as in most oviparous squamates, an important phase of embryonic development takes place in the mother's oviduct, before egg-laying. This may explain why immune mechanisms, including IL-1, are expressed in maternal tissues in both oviparous and viviparous squamates. Studies are in progress to investigate whether IL-1 system is expressed in oviduct tissues of amphibians, a vertebrate class poorly known for the maternal-foetal relationships. Supported by Research Grants from the University of Siena (PAR Projects).

IFN-γ–INDUCED EXPRESSION IN TROPHOBLAST CELLS

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Interferon (IFN)-γ is considered an abortion-inducing factor even though this cytokine has been localized at the maternal-fetal interface in the first half of the gestation during normal pregnancy in mice and other species. Experimental evidences also point to specific functions of this cytokine in stimulating proliferation and phagocytosis and, inducing indoleamine 2,3-dioxygenase (IDO) protection mechanisms in trophoblast cells. To better understanding the behavior of IFN-γ-treated trophoblast cells, in this study, special attention has been done to the induced-gene expression of mice ectoplacental cones obtained at the day 7.5 of gestation and treated with this cytokine at the physiological dose of 100 U/mL. The presence of IFN-γ strongly modified the expression of 42 genes when determined through semi quantitative RT-PCR and DNA macro-arrangements. Among these genes, particularly interesting were those related to IFN-γ-induced transcription factors such as IRF-1 and SGF3 and, the expression of genes that encode the main proteins of the IFN-γ-signaling pathway, which include JAK1, JAK2 and STAT1. Phosphorilated STAT 1 protein at the trophoblast cells nuclei was also dependent of IFN-γ induction. Since this cytokine is able to induce a specific gene response, unrelated to cell death it deserves to be deeper studied on the framework of the roles played by the placenta for adaptation to the maternal environment and immunological adaptations of pregnancy. Supported by FAPESP.

LEUKEMIA INHIBITORY FACTOR (LIF) AND INTERLEUKIN-6 INDUCED INTRACELLULAR SIGNALLING IN JEG-3 CHORIOCARCINOMA CELLS

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Leukemia Inhibitory Factor (LIF) is major regulator of the natural course of pregnancy. Invasion of trophoblast cells requires a fine-tuning, which is fundamental for correct placentation. This tuning is regulated on intracellular level and influenced by LIF. Several signal transducers and their suppressors, known from former studies and tumor invasion, are expected to be involved. **Methods:** RNAinterference (RNAi) was applied to Jeg-3 cells to knock down STAT3, SOCS3 and STAT6. Small interfering RNA (siRNA) and scrambled oligonucleotide controls were self-designed. Jeg-3 cells were stimulated with LIF, IL-4 and IL-6. Expression and phosphorylation of factors were analyzed by Western blots. Proliferation was measured by using a colorimetric assay, invasion by matrigel assays. **Results:** Knock down of STAT3 reduced LIF induced proliferation and invasion, whereas SOCS3 knock down increased IL-6 induced Tyr705 phosphorylation of STAT3 simultaneously with proliferation. STAT6 RNAi had no influence on invasion, but IL-4 induced proliferation was inhibited. **Conclusions:** LIF and IL-6 use STAT3, SOCS3 and STAT6 to regulate proliferative and invasive capacities of Jeg-3 choriocarcinoma cells, which may be regarded as a model for trophoblast cells.

Symposium 3: Molecular bases of endometrial function

Coordinator: L. Velásquez (Chile)

SEARCHING GENES INVOLVED IN HUMAN ENDOMETRIAL INFERTILITY

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High-density oligonucleotide DNA microarrays comprising around 22,000 gene targets, were used to determine gene expression in endometrial biopsies obtained during the implantation window from two groups of volunteer women who were previously recipients in oocyte donation cycles: Group A = women, who failed repeatedly to have implantation of transferred embryos, Group B = women who succeeded in having implantation. The same oocyte pool that provided their embryos also provided embryos that implanted in the uteri of the donors. A third Group C, were women with history of high fertility in natural cycles. All women were subjected to a standard protocol to induce an endometrial cycle with exogenous estradiol and progesterone. The expression level of 65 transcripts showed a 2≥ fold difference between groups A and B whereas there were no significant differences between group B and C. Twelve of these 65 transcripts were listed among transcripts previously reported to exhibit significant changes at the opening of the implantation window, including chemokine receptor, matrix metalloproteinase 7 and progestagen-associated endometrial protein, believed to play critical roles during implantation. We conclude that repeated failure of implantation in some oocyte recipients is associated with an intrinsic defect in the expression of multiple genes in their endometrium.

EFFECT OF LEVONORGESTREL ON mRNA TRANSCRIPT LEVELS IN THE HUMAN ENDOMETRIUM DURING THE WINDOW OF IMPLANTATION

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It is debated whether Levonorgestrel (LNG) used as emergency contraceptive interferes with embryo implantation. The objective of this investigation was to compare endometrial level of mRNAs during the receptive period after administering a single dose of LNG 1.5mg or placebo on day 1 of the luteal phase. **Methods:** Eight healthy, surgically sterilized volunteers with regular menstrual cycles participated after they signed informed consent. Each woman contributed with a cycle treated with placebo and one with LNG separated by two resting cycles. An endometrial biopsy was taken on day LH+7 or LH+8 in a cross-over, placebo controlled, double blind, randomized design. The protocol was approved by the institutional Ethics Committee. The tissue level of transcripts corresponding to 20.383 genes was determined using DNA microarrays, (DMA). Real time RT PCR (RT) was used: 1) to confirm the differences found in DMA; 2) to determine the effect of LNG on genes which are upregulated during receptivity and on genes that respond to mifepristone. **Results:** The level of PAEP, TGM2, CLU, IGF2 and IL6ST5 mRNAs increased by DMA and RT analyses after administering LNG while 9 others decreased. mRNAs consistently upregulated during receptivity were slightly increased or unchanged after LNG, and none of the ones responding to mifepristone changed in response to LNG. **Conclusion:** Postovulatory administration of LNG causes minimal changes in gene expression profiling of the human endometrium in the receptive period. Neither the magnitude nor the nature of the changes endorses the hypothesis that LNG interferes with implantation. Partially funded by DICYT, USACH and ICMER.

Symposium 4: Sincytiothrophoblast: membrane, transport and pathology

Coordinator: G. Riquelme (Chile)

VDAC-1 AS THE MOLECULAR CORRELATE OF THE MAXI CHLORIDE CHANNEL FROM HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

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Chloride transport involving conductive pathways is associated to functions such as maintenance of membrane voltage, solute transport and cell volume regulation in several types of cells including the placental syncytiotrophoblast. The Maxi Chloride channel is responsible for part of the conductive chloride transport in the apical syncytiotrophoblast plasma membrane. Using electrophysiological methods, we have studied the biophysical characteristics of the Maxi Cl^- channel from human placental syncytiotrophoblast, of unknown molecular nature. This channel has a large conductance, voltage dependent bell-shaped Po (open probability) and permeability to anionic amino acids. Its electrophysiological properties resemble those of voltage-dependent anion channel (VDAC-1) of mitochondria. An extramitochondrial localization of VDAC-1 seems likely, but the hypothesis that VDAC-1 could be the molecular correlate of the plasma membrane Maxi Cl^- channel continues being debated. Regarding this matter, we have demonstrated an extramitochondrial localization of VDAC-1 in syncytiotrophoblast. Confocal microscopy shows its presence in the apical membrane and Western blot analysis reveals that apical purified membrane fractions contain VDAC-1. From a functional point of view, the unitary current of the Maxi Cl^- channel from purified membranes is blocked by anti VDAC-1 antibodies. This molecular and functional evidence allows us to raise the hypothesis that VDAC is a possible candidate for the molecular identity of the Maxi Chloride channel from human placental syncytiotrophoblast. Supported by FONDECYT 1040546, Beca AT-403031 CONICYT and Beca PG/48/2004 (Chile).

DOES TRYPTOPHAN TRANSPORT REGULATE IDO-MEDIATED IMMUNE SUPPRESSION BY THE PLACENTA?

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The enzyme indoleamine 2, 3-dioxygenase (IDO) has been the focus of great interest over the last seven years because of the discovery of its role in regulation of T lymphocyte function. By utilization as a substrate (and hence localized depletion) of the amino acid L-tryptophan, cells expressing IDO are able to produce a microenvironment around them in which there is profound T cell suppression. The function of this enzyme producing such 'immunosupression by starvation' can be explored by two classical enzymological manoeuvres: either by inhibition of the enzyme's active site using a competitive inhibitor (such as 1-methyl D,L-tryptophan), or by over-riding of the effect of the enzyme's activity by addition of excess substrate (tryptophan supplementation). Both of these have been used to examine IDO biology in distinct areas of immunobiology (for example in exploring how tumour cells avoid immune attack, for example in studying how specific populations of dendritic cells regulate the immune response). Much attention, following seminal findings in the pregnant mouse by DH Munn and A Mellor, has additionally recently been focussed on the role of IDO in materno-fetal interactions. I will discuss, in the context of human placental biology, the evidence for a role of IDO in pregnancy in health and disease; and will focus in particular on the role of L-tryptophan transporters in the biology of this mechanism. Supported by the Wellcome Trust.

CHLORIDE CONDUCTIVE PATHWAY AND ITS ASSOCIATION TO NUTRIENTS TRANSPORT: A PATHOLOGICAL ROLE IN PREECLAMPSIA?

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To improve the transepithelial transport model across the syncytiotrophoblast, the major fetal–maternal transport barrier with no paracellular pathways, our group has characterized ion transport through conductive pathways that contribute to the understanding of physiological and pathophysiological roles of ion channels. We have obtained a highly enriched preparation of apical (maternal facing) and basal (fetal facing) membranes from normal and preeclamptic human placenta and reconstituted them into giant liposomes (suitable for patch clamp single channel recordings) or injected them into *Xenopus laevis* oocytes (for total current recordings). Preeclampsia, a high incidence pathology of pregnancy, exerts great impact on fetal morbimortality. This relies, among other factors, on intrauterine growth restriction (IUGR). Taurine, not produced by human fetus, is essential for growth and neurological development and its fetal plasma concentration is reduced in IUGR. We have characterized channels and their regulation from healthy trophoblast and trophoblast from preeclamptic placentas, detecting an anomalous biophysical behavior of the Maxi chloride channel, a channel with permeation to anionic amino acids including taurine. The chloride transport involving conductive pathways is associated to numerous epithelial functions, such as maintenance of membrane potential, cell volume regulation and nutrient transport. The latter could involve taurine diffusion through the maxi Chloride channel. Our results suggest that the altered activity of placental taurine transport across chloride channel could partially explain low fetal taurine levels. Supported by FONDECYT 1040546 (Chile).

FUNCTIONAL EXPRESSION OF AQUAPORINS IN FETAL MEMBRANES FROM HUMAN TERM PLACENTA

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To be absorbed into the chorionic or placental circulation, amniotic fluid water and solutes must cross the avascular amniotic membrane, probably mediated by water channels. The expression of aquaporins (AQPs) 1, 3, 8 and 9 was previously described in fetal membranes. Noteworthy, AQP9 expression is regulated by endocrine factors. We also detected AQP7 by RT-PCR and immunohistochemistry. In this study, we characterize the functional expression of AQPs and its hormonal regulation. **Methods:** Human fetal membrane of term placentas obtained from cesarean were dissected and immediately mounted as a diaphragm in a Ussing chamber. The net trans-epithelial water flux (Jw) was recorded minute by minute by an equipment used currently in our laboratory. **Results:** In amnion and chorion, a similar absortive Jw [(0.35 ± 0.05) and (0.38 ± 0.07) $\mu L.\ min^{-1}.cm^{-2}$] was observed. These Jw were increased when an osmotic gradient was generated by adding (to the maternal side) different concentrations of poly(ethylene gycol) (Mr ~ 8000). The osmotic permeability (Posm) calculated was similar in both fetal membranes [(1.6 ± 0.3) vs (1.3 ± 0.3) 10^{-2} cm s^{-1}] and they were significantly decreased ($P<0.05$, n=6) after preincubation with 0.3 mM $HgCl_2$. Finally Jw increased [(0.61 ± 0.06) $\mu L\ min^{-1}\ cm^{-2}$, $P<0.05$, n=4] when chorion was cultured during 24h with 300ng/mL progesterone (P4).This is the first study that reports the functionality of the AQPs present in the human fetal membranes and its up-regulation by P4. **Conclusions:** These results suggest that AQPs mediate water transport across fetal membranes and the activity of AQPs is promoted by P4. This hormonal mechanism indicates a potential regulation of amniotic fluid homeostasis. ANPCYT PICT 9508/02.

Symposium 5: Neonatal and materno-fetal health

Coordinator: J. Cabrera (Chile)

GLUCOCORTICOIDS TREATMENT DURING PREGNANCY AND HYPERTENSION IN ADULT LIFE

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Antenatal steroid administration for accelerating fetal lung maturation has been in use for over thirty years, however, controversy still exists regarding potential long-term side effects. Following the 1994 NIH consensus conference recommendation, the use of corticosteroid therapy in the United States has increased from less than 15% of individuals threatened to deliver prematurely in 1990 to more than 75% now. Studies in animals have demonstrated that administration of glucocorticoids during pregnancy alters renal expression of several key regulatory molecules at different developmental stages. These alterations are followed in most cases with the development of hypertension in the adult offspring. Clinical and experimental data will be reevaluated.

GROWTH FACTORS IN TERM NEWBORN

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The growth is the result of a complex and continues interaction between genes and ambient media, that begins in the intrauterine life and it stays throughout the life. The aim of this study was to analyze of growth factors in term newborn, small, adequate and bigger for the gestational age. **Methods:** An analytical prospective and randomized study was carry out in the hospital G.Grant B. of Conception between November of 2003 and May of 2004. The studied population was constituted by 62 new born alive, with gestational age between 38 and 41 weeks, classified in SGA (Group A, n = 20), BGA (Group B, n=20) and AGA (Group C, n=22). Insulin, growth hormone, IGF-1 and IGF-BP32 were measured by quimioluminiscense in cord blood.. **Results:** The maternal variables, such as: age, tall, weight and parity does not shows significant differences. The average of gestational age was of 38,8 weeks in the SGA, 39,3 weeks in the AGA and 38 weeks in the BGA $p > 0.05$. The foetal anthropometric variables like weight, tall and cranial circumference were different between the 3 groups ($p<0.05$). The insulin levels show significant difference between groups AEA (5.13mUI/ml) versus SGA (2.83mUI/ml) $p< 0.05$. The mean values of GH were 14,4 ng/ml in SGA, 9.61ng/ml in AEA and 7.88ng/ml in BGA, in spite of the tendency the analysis was not significant ($p > 0.05$). The levels of IGF-1 show to a proportional tendency with anthropometric measures SGA 144 ng/ml versus 207.5 ng/ml in BGA $p<0.05$. The IGF-BP-3 showed 0.87 ug/ml in the SGA groups versus 1.26 ug/ml in the AGA $p< 0,05$ **Conclusions:** Our results suggest that the growth factors related to the insulin, play a roll in the regulation of the newborn growth.

THE ROLE OF PLACENTA IN FETAL, MATERNAL AND NEONATAL HEALTH

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Mechanisms that regulate fetal growth and development are complex, determined genetically and modulated by a set of extra genetic factors. Pregnancy outcome is associated with fetal demand for nutrients and the materno-placental capacity to meet that demand. A failure in the materno-placental satisfaction of fetal requirements results in a range of fetal adaptations and developmental changes that could lead to permanent alterations in the body's structure, weight and metabolism, and thereby lead to cardiovascular and metabolic disease in adult life. We studied the effect of maternal biological and sociodemographic variables on fetal and placental weight. **Methods:** A cross sectional comparative study that included 2500 patients who had their delivery between 28 and 42 weeks of pregnancy at Hospital G. G. Benavente (public hospital) and Clínica Francesa (private maternal clinic) was performed. Social, demographic and biological variables of the mother, new born and placenta were analyzed using multivariate regression. **Results:** There are important differences in fetal and placental weight between patients from private and public maternal wards. There is evidence that maturational changes are taking place throughout gestation within the placenta. Significant differences in social, demographic and biological variables of the mother and newborn were observed when compared to fetal weight. **Conclusions:** Short and long-term effects of fetal weight have been identified, but preventive strategies are still lacking. It is unlikely that a single factor will reduce a multi-causal outcome. Appropriate population-specific interventions should be now a priority. Supported by DIUC 202.84B.010-1.0.

Symposium 6: Calcium channels: function and mechanism

Coordinator: M.A. Delpiano (Chile)

THE DIHYDROPYRIDINE RECEPTOR IN SKELETAL MUSCLE IS INVOLVED IN MORE THAN ONE SIGNALING PATHWAY

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The most classical response to tetanic electrical stimulation in skeletal muscle evokes a ryanodine receptor related fast calcium signal, through the activation of L-type calcium channels (dihydropyridine receptors, DHPr). The voltage sensing mediated by these channels relays the information to the sarcoplasmic reticulum to release calcium for muscle contraction. Once the tetanic stimulation is over, we have found that there is a second rise of cytoplasmic calcium. This slow calcium signal, is also linked to the DHP receptor but not to the release of calcium through ryanodine receptor activation but rather by the activation of PLC and IP3 receptors, it lasts several seconds and is independent of extracellular calcium. So far we know that this slow calcium signal is activated by a rise in inositol trisphosphate (IP3), which is generated via a G protein and a phosphatidylinositol 3-kinase (PI3K) pathway. The fact that the nuclear signal is stronger than the cytoplasmic component, suggests its involvement in gene activity modulation by the repetitive membrane depolarization. We speculate about the likely events in which it may participate considering its time course and intracellular location. Funding for this project has been provided by Fondap 15010006, Conicyt predoctoral fellowship (JME).

EFFECT OF ASTROCYTE-RELEASED FACTORS ON INTRACELLULAR CALCIUM HOMEOSTASIS AND EXOCYTOSIS

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Calcium induces exocytosis in neurons and endocrine cells through a common molecular mechanism. However, hormone exocytosis from endocrine secretory vesicles differs in some physiological aspects from neuronal synaptic vesicle exocytosis, in particular in calcium sensitivity and kinetics. To examine the mechanisms that determine such differences, we have used an astrocyte-conditioned medium (ACM) to promote a neuronal phenotype in bovine chromaffin cells and investigate modifications in the exocytotic process when these cells change their phenotype. **Methods:** We have preformed intracellular calcium imaging experiments, electron microscopy and amperometric detection of exocytosis. **Results:** In the ACM-promoted neuronal phenotype, secretory vesicles and intracellular calcium signals were preferentially distributed in neurite terminals. Electron microscopy revealed the presence of both large dense-core vesicles (LDCV) and small clear vesicles at neurite terminals. Using amperometry, we observed that the kinetics of individual exocytotic events was drastically accelerated in neurite terminals of cells exposed to ACM during 7-9 days. Experiments performed in cells loaded with EGTA, suggest that the distance between secretory vesicles and calcium channels does not account for the changes in the exocytosis kinetics. The distribution analysis of the cubic root of the spike quantal size, a parameter related to the vesicle diameter, indicates that the rapid exocytotic events observed in neurites of cells exposed to ACM correspond to exocytosis of LDCV. Additionally, both the duration and the current amplitude of pre-spike current were significantly smaller, suggesting that ACM changes the stability and the conductance of the fusion pore. **Conclusions:** Astrocyte-released factors elicit a neuronal phenotype in chromaffin cells, wherein the LDCV exocytosis is drastically accelerated.

DETERMINANT FOR TEMPERATURE SENSING IN TRANSIENT RECEPTOR POTENTIAL CHANNELS

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Temperature sensation in mammals is possible due to the presence of temperature dependent transient receptor potential (TRP) channels in our peripheral nervous system and related cells. Six temperature dependent TRP channels have been cloned: four of them heat-activated (TRPV1-V4) and two activated by cold (TRPM8 and ANKTM1)[1]. These channels denominated ThermoTRPs, respond to changes in temperature but are able also to respond to other stimuli like specific agonists and voltage[1]. How these channels are able to be gated by temperature or which are the structural determinants in temperature sensing are at present two fascinating but still unanswered questions. We showed that TRPM8, a cold-(8-28 ºC) voltage- and menthol-activated channel, behaves as an allosteric protein and we proposed the existence of different structural domains involved in the channel voltage- and temperature-dependence. On the other hand, TRPV1, a channel able to bind the ¨hot¨ pepper-derived vanilloid capsaicin, is potentiated by heat and depolarizing voltages (>43 ºC). An interesting possibility is, therefore, that TRP channels are modular and that there might be a common TRP gating mechanism. In this work we assessed the role of the C-terminal domain of thermoTRPs and its relation to thermal activation. Taking advantage of chimerical constructions between TRPV1 and TRPM8, we conclude that the C-terminal domain is modular and determines the channel phenotype regarding temperature sensitivity. [1]Clapham, D.E. *Nature* 426:517-524, 2003. Supported by grant Fondecyt 103-0830.

AUXILIARY SUBUNITS OF VOLTAGE-ACTIVATED CALCIUM CHANNELS: MORE THAN SIMPLY CALCIUM CHANNEL REGULATORS?

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Functional, biochemical and molecular studies have suggested that high-voltage activated (HVA) calcium channels are macromolecular complexes containing one of the pore-forming α1 subunits (α1A, α1B, α1E, α1C, α1S, α1D and α1F) and auxiliary subunits (β, α2-δ and γ), while low voltage-activated (LVA) calcium channels appear to be formed only by one of the corresponding α1 subunits (α1G, α1H and α1I). So far, it has been demonstrated that the function of auxiliary subunits is to modulate the membrane targeting, gating kinetics and voltage dependence of the α1 subunit. In this respect, β subunits (β1, β2, β3 and β4) are recognised as the major regulatory subunits. Using transgenic animals, we study the *in vivo* function of the β3 subunit that is mainly expressed in the nervous system. The β3 deficient animals show basically defects in pain processing due to the reduced functional expression of N-type calcium channels (α1B) in sensory neurones. We analysed this phenotype throughout various levels, including protein expression and calcium channel currents in single dorsal root ganglion neurons, functioning of neuronal circuits in the spinal cord of anesthetised animals, and nociceptive behaviour in awake animals. Since recent evidences suggest that β subunits are scaffolding proteins with also non-α1-related functions, we currently analyse the effects of the β3 deletion on HVA and LVA calcium channels as well as on other ionic channels expressed in sensory neurones.

OXYGEN-SENSING CALCIUM CHANNELS AND THEIR MODULATION DURING HYPOXIA

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Oxygen (O_2), an essential element required for cellular metabolism is crucial for maintaining animal life on earth. As an electron acceptor in the mitochondrial respiratory chain it enables production of energy reach ATP molecules during the oxidative phosphorylation. Disturbances in the metabolic status resulting from reduced oxygen tension (low PO_2 = hypoxia) or ischaemia, triggers regulatory changes in the respiratory and cardiovascular system in order to maintain adequate O_2 supply. Oxygen-sensing K^+ channels modulated by hypoxia were first reported in carotid body type-I cells, later also oxygen-sensing Ca^{2+} channels were found. In myocytes of the systemic arteries it seems that, dihydropyridine-sensitive L-type high-voltage activated (HVA) Ca^{2+} channels are modulated by changes in PO_2. They can be directly and reversible inhibited by threshold hypoxic PO_2 values of about 70 mm Hg (9.3 kPa) in a voltage-dependent manner that results in reduced cytosolic Ca^{2+} and arterial vasorelaxation. In contrast, in pulmonary myocytes HVA Ca^{2+} channels exhibit a more differential sensitivity to hypoxia. In conduit arteries they are inhibited leading to vasodilatation, while in resistance arteries they are activated inducing vasoconstriction of small pulmonary arteries. In endothelial cells of rat brain capillaries we have found a paradoxical response. While short hypoxia (5 min.) inhibited low-voltage activated (LVA) Ca^{2+} channels, 30 min. hypoxia increased cytosolic Ca^{2+}. The modulation of those Ca^{2+} channels by low PO_2 and the suggested molecular mechanism will be discussed.

Symposium 7: Extracellular matrix in the maternal-fetal interphase

Coordinator: S. San Martín (Chile)

EXTRACELLULAR MATRIX AND DECIDUALIZATION IN THE MOUSE

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Preparation for embryo implantation requires extensive adaptation of the uterine microenvironment including the decidualization of the endometrium. Likewise decidualization leads to modifications on the endometrial extracellular matrix (ECM). This is specially so in the mouse where a remarkable remodeling of the fibrilar and non-fibrilar ECM taken place particularly, in the periimplantation period. Very thick collagen fibrils with very irregular profile appear around mouse decidual cells as soon as decidualization is tiggerd. Ultrastructural cytochemistry indicated that glycosaminoglycans (GAGs) and proteoglycans (PGs) modulate in the mouse endometrium during the periimplantation period. This study is to characterize the nature of GAG and PGs in the mouse pregnant endometrium. **Methods:** Uterine horns from 1nd and 7th day of pregnancy were collected, fixed in methacarn and embedded in paraplast. Sections were immunostained against anti-decorin, byglican and versican PGs antibodies using peroxidase method. Hialuronan was detected using biotinilated fragment of PG aggrecan. **Results:** Hialuronan that is present in the whole endometrium before decidualization almost disappear from the decidualized stroma. In parallel, versican increases in the decidualized areas. Moreover, decorin and biglycan also show a differential expression in the endometrium during pregnancy. Before the embryo implantation, decorin is present in the endometrium desapearing as soon as decidua is formed. Contrary, biglycan is highly expressed in the decidualized areas. **Conclusions:** The differential expression of these ECM molecules indicates that they participate in the process of decidualization and/or embryo implantation.

EXTRACELLULAR MATRIX AND PLACENTATION IN THE RAT

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The establishment of the fetal-maternal unit is a critical step for the success of reproductive process in mammals. At the time of implantation, rat trophoblast cells become invasive and mediate the penetration of the embryo into the decidualized stroma. This process result in the formation of a rich network of lacunae connecting the embryo to the maternal vessels, and it is characterized by cell proliferation, cell differentiation and cell migration, and are probably influenced by the composition and organization of the extracellular matrix (ECM) molecules of the endometrial stroma. The goal of this study is to characterize the ECM during placentation in rat. **Methods:** Uteri and placentas of female Wistar rats on 7, 14 and 20 days of pregnancy were removed and fixed in methacarn solution. Collagen, fibronectin, laminin, decorin, biglycan and versican were evaluated by histochemistry and immunocytochemistry. **Results:** The ECM components were decreased and reorganized in decidualized areas of the uteri. Collagens were restricts to the nondecidualized region and myometrium whereas glycoproteins and proteolgycans were distributed mainly in predecidualized and nondecidualized regions, and myometrium of implantation sites. In the placental tissues, collagen, glycoproteins and proteoglycans increased in the umbilical cord and in different regions of 20- day-old placenta compared with 14-day-old samples. **Conclusions:** The embryo implantation and the development of the placenta modify the distribution of specific ECM components of both uterine and placental tissues. Supported by DIPUV (University of Valparaíso, Chile), CNpq and FAPESP (Brazil).

EXPRESSION OF CYTOKINES, GROWTH FACTORS AND THEIR RECEPTORS ON PERIMPLANTATION MOUSE EMBRYOS

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Implantation is a prerequisite for subsequent development and its failure is a cause of pregnancy loss. The molecular dialog between the implanting conceptus and the endometrium involves cell-cell and cell-extracellular matrix (ECM) interactions. The release of the zona pellucida exposes receptors and cell adhesion molecules to ECM components that are likely to be involved in implantation. It is ethically impossible to study implantation in humans; therefore, animal models are necessary to reach a better understanding of both the molecular and mechanical events associated with this process. The different animal models in which implantation have been studied have shown wide mechanistic variation. The study of these differences and similarities allows us to gain insight into cellular and molecular interactions that occur as part of implantation. Little is known about relationship between members of the epidermal growth factor (EGF) family and their receptors, heparansulfate proteoglycans (HSPGs) and human implantation. Showing conservation with the mouse, HB-EGF plays important roles in implantation in several species, and ErbB4 is expressed in the trophoectoderm in mouse and human periimplantation blastocysts. Previous results from our laboratory utilizing a mouse model, demonstrated that interferon-gamma could alter the expression of ErbB4 and HSPGs, perlecan, at the blastocyst stage. We hypothesized that altered expression of these molecules could be related to an altered acquisition of adhesion competence and low implantation rates. New data are also emerging for roles of cytokines and their receptors during early stages of embryo implantation, like initial blastocyst attraction, attachment to the endometrium and trafficking of the trophoblast to invade the blood vessels; we decided to study the effect of these molecules in the same experimental model.

Symposium 8: Intrauterine growth restriction and fetal programming

Coordinator: P. Casanello (Chile)

INSULIN SENSITIVITY DURING EARLY LIFE IN LOW BIRTH WEIGHT CHILDREN

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The correlation between low birth weight and various metabolic abnormalities has been the subject of much research in recent years. Several recent studies examining this topic are reported. A prospective cohort study of small and average for gestational age infants found that insulin sensitivity and secretion showed a marked transition from lower fasting insulin and increased insulin sensitivity (IS) at birth to insulin resistance over the first 3 years of life. This transition was related to rapid postnatal weight gain, which could indicate a propensity to central fat deposition. In addition we observed a reduced compensatory ß-cell secretion, underlining the need for long-term surveillance of glucose homeostasis in all SGA subjects whether or not they show postnatal catch-up growth. In another cohort of premature, very low birth weight children, intrauterine growth restriction (IUGR) rather than low birth weight was associated with reduced sensitivity to insulin. A further study revealed that post-glucose ghrelin levels, but not fasting levels, correlated positively to current length, current weight, and change in weight in small for gestational age children. We next determined whether Adiponectin, an adipocytokine that increases IS, contributed to postnatal catch-up growth and IS changes in these children. Serum adiponectin levels at 1 and 2 years were higher compared with reported levels in adults and older children and significantly decreased from 1 to 2 years and in females compared to males. No differences were seen in adiponectin levels between SGA and AGA infants at 1 or 2 years. Remarkably, changes in serum adiponectin levels during the first 2 years of life were related to patterns of weight gain in SGA infants, but not to early changes in insulin sensitivity. Finally, yet another study revealed an association between the insulin gene polymorphism and insulin sensitivity and secretion in infancy. Identification of those infants born with LBW showing a rapid postnatal weight and/or length gain may help to focus preventive measures aimed at controlling the current increased incidence of metabolic syndrome in these children. Supported by FONDECYT 1000939.

PROGRAMMING HYPERTENSION IN ANIMAL MODELS WITHOUT IUGR

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Although human epidemiological studies have shown a strong link between smallness at birth, for gestational age (IUGR), and increased incidence of diseases such as diabetes and hypertension in the adult, there is no evidence that the IUGR is causal, rather than coincidental. In many animal models a perturbation of the intrauterine environment has resulted in hypertension in the adult offspring, without decreasing birth-weight. **Methods:** Three models to be discussed are 1) sheep exposed, briefly, to early (26-28d, term~150d) excess synthetic (dexamethasone - Dex) or natural (cortisol -F) glucocorticoid; 2) rabbits whose mothers were hypertensive due to clipping of one kidney; 3) rats whose mothers were hypertensive as a result of vitamin D deficiency. **Results:** In (1) the newborn are of normal birth weight (BW), and both sexes become hypertensive at 4 months of age. In (2, 3) the BW is increased. In (2) only the females are hypertensive by 30 weeks, whereas in (3) both sexes are hypertensive at 8 weeks. In (2, 3) maternal renin is increased, which could affect placental function. The glucocorticoid-programmed sheep offspring have a significant decrease in nephron number, whereas the rat offspring of vitamin D deficient mothers have an increased nephron number. There are significant increases in expression of components of the renin-angiotensin system (RAS) in blood pressure control centres in the brains of the Dex-exposed offspring, which are functional. **Conclusions:** These data suggest that exposure to an unfavorable intrauterine environment, even for a brief period, can alter both renal and brain mechanisms controlling blood pressure, and lead to hypertension in offspring without concomitant IUGR. Supported by NHMRC (Australia).

ROLE OF THE PLACENTA IN FETAL PROGRAMMING

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The concept of fetal programming proposes that alteration in fetal development or adaptations of the fetus to alterations in the normal amount or pattern of substrate supply across the placenta leads to cardiovascular, metabolic and other diseases in adult life. There is abundant evidence from human epidemiologic and animal studies that maternal nutrition may program the fetus for adult disease. This effect is likely mediated by placental structure/function that regulates the amount or composition of nutrients. The placenta may therefore play an active or a passive role. Associative data from human studies shows changes in placental structure/function in condition such as diabetes and intrauterine growth restriction where programming occurs. Determinants of substrate supply to the fetus are both utero-placental and fetal-placental blood flows to the placenta and the physical structure and activity and expression of transporter proteins on the placental barrier, the trophoblast. The role of each of these components is being investigated using animal models. It is clear that as the placenta is constantly growing and differentiating throughout gestation the timing of an insult may have profoundly different effects at different stages of gestation. Disturbance of the normal gestational-age specific developmental pathways of the placental vasculature or the trophoblast barrier by hormonal, nutritional, hypoxic or oxidative stress insults, change structure/function leading to programming. Investigation of the role of imprinted genes is illustrating the integrative nature of events at the fetal/maternal interface.

INTRAUTERINE GROWTH RESTRICTION AND SMALL FOR GESTATIONAL AGE, MAKING THE DIFFERENCE IS IMPORTANT

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Intrauterine growth restriction (IUGR) is the inability of the fetus to express its growth genetic potential. Clinically it is the estimation of birth weight under the 10^{th} percentile according to gestational age. The aetiology of IUGR has multiple factors, including genetics, infection, chronic maternal diseases and placental insufficiency, although some times it could be a constitutionally small but healthy newborn. The later is clinically interesting since being small for gestational age (SGA) could lead to preterm interruption of pregnancy of a healthy fetus thus making extremely important to distinguish which small fetuses are really unhealthy. We currently have the technology required to study the fetus from an hemodynamic point of view and the sequence of events of fetal compromise are known in details as well as the ability of the fetus to adapt to an adverse intrauterine milieu. This allows the election of the adequate moment for delivery as well as to distinguish between IUGR and SGA fetuses. With the help of Doppler ultrasound fetal and placental vascular beds can be studied in detail. The sequence of events related to fetal compromise begin with alterations in the umbilical arteries, followed with cerebral middle artery vasodilatation as a compensatory mechanism and finally a cardiac failure that can be measured as alterations of venous Doppler parameters. The advantage of this method is that it is not invasive; it goes a few steps ahead of the classical clinical observations such as the fetal biophysical profile and non-stress test, allowing a better control of fetal wellbeing and perinatal outcome. The normal Doppler evaluation in fetuses under percentile 10 with normal growth rates allows these pregnancies to reach term and diminishing preterm delivery based on medical indications that were previously considered necessary.

Symposium 9: Topics for teaching in physiology

Coordinator: B. Ramírez (Chile)

RESEARCH UNITS AS A MOTIVATING CONTRIBUTION TO PROBLEM-BASED LEARNING IN SECOND YEAR MEDICAL STUDENTS

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In order to develop inquiring attitudes in medical students, to stimulate them to find answers by themselves, to integrate knowledge of different science fields and, most of all, to offer possibilities of amazement along the learning process, research units (RU) were introduced into regular medical undergraduate curriculum as part of a student-centered teaching methodology. **Methods:** Since 1993, 200-240 2nd year medical students were annually distributed into 68-70 working groups performing RU in either basic science or in clinical areas. Along a year, the students actively participate in every phase of scientific work, including project redaction, experimental and/or clinical observations, result evaluation and publication by oral communication and a written report. A tutor, experienced in the corresponding field, advised each working group. Thematic areas were covered by specialized coordinators (n=16) and evaluators (n=32). **Results:** Questionnaires revealed year for year a high approval (98%) of RU among students as well as by teachers. For established groups, RU represented a valuable contribution in questioning current working lines, performing observations and redaction of publications. RU students could thereby be often integrated into active working groups, as well as into the author list of publications at international level. **Conclusions:** RU represents a refreshed innovation in teaching methodology, based in stimulation of natural curiosity and learning.

PHYSIOLOGY: CURRICULUM PLANNING AND DESIGN FOR ACTIVE LEARNING

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The main points discussed at the Curriculum Planning and Design track at the 2005 IUPS teaching-workshop in Pali Mountain, Ca, USA are presented here. Discussion was centered on five points: Learning objectives, how do we teach to get our learning objectives, Assessment, Physiology as an independent discipline and Strategies for changing a curriculum. Student engagement is mandatory to get an effective learning and must be attained in each teaching style (lecture, laboratory, tutorial, other). Despite specific objectives are related to the interests of the students that attend the course some general objectives are essential for any physiology curriculum. Assessment must be aligned with these objectives. Each objective, as well as the education process, must be evaluated. Evaluation tools should be part of the curriculum. Integrated curricula versus independent disciplines were only tangentially discussed. It was clear, however, that to achieve successfully an integrated curriculum a strong institutional leadership is required. Before starting a fundamental curriculum change the need for that change must be addressed and the possibilities to implant the new curriculum must be carefully evaluated considering several aspects as target population, administration support, facilities, teaching skills, consensus for the change (academics, students, authorities) and financial aspects. Changes in the current curriculum should be always taken in account if they increase student participation in the learning process (increase active learning or student engagement).

RESEARCH IN PHYSIOLOGY EDUCATION: OUR CLASSROOMS ARE OUR LABORATORIES

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Research on how our students learn physiology best is a form of scholarship, defined as the application of the intellect in an informed, disciplined, and creative manner. This kind of classroom research involves planning a curriculum and making educational innovations, which require us to be well-informed not only about the content of the subject but also about appropriate and effective teaching and learning strategies. Intellectual discipline is needed to ensure that objectives, methods, and assessment are aligned and that curricular innovations are rigorously evaluated. We have to be creative to address the needs of our diverse student body within the limitations of our resources. An innovative, effective, well-evaluated educational program is as much a product of scholarship as is the findings of bench research, and we have an equal responsibility to report both. We can advance physiology education by presenting our educational innovations to our colleagues at our own universities, at professional meetings, and by publishing papers. This gives opportunities for peer review and allows more than our own students to benefit from our scholarship. As we become more scholarly and sophisticated about curriculum development, we need to educate ourselves about appropriate venues for reporting these results. The American Physiology Society publishes a journal that publishes peer-reviewed articles specifically about physiology education: *Advances in Physiology Education.* It is available free online at http://advan.physiology.org, and statistics show that thousands of our colleagues read it every month.

DOCTOR'S PROFILE FOR THE XXI CENTURY

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In few more years, people will be able to send a small blood sample, without having to move from their own homes, and the DNA sequence will allow specialists to predict the risk of presenting pathologies or to install genetic therapy if necessary. The contributions from Genome project and the developments in the molecular biology field will change the diagnosis, some procedures and therapies, health care systems and the medical education itself. In addition to that, scientific, technologic, cultural and social changes are so fast and powerful that the current professional careers will shortly become obsolete and isolated competences will be certified in order to have a better response to an always-changing working environment. Therefore, if we want the new generation of professionals to be capable to perform their jobs under these new conditions, it is necessary to adequate the professional training and the professional profile in order to consider the multiple variables that will influence the medical practice. To fulfill these objectives it is necessary to implement a semi – flexible curriculum, based on the development of competences, with interchangeable modules and with more than one path towards new and multiple alternatives in formation. New learning methodologies, reduction and addition of curricular contents such as molecular biology, genetic clinic practices, economy applied to medical field, among others must be taken into account. Particularly, horizontal and vertical integration have to be included, considering the real protagonist and subject of the medical task: the patient; who, in fact, has been truly forgotten in medicine's practice during the XX century. All of this must be based on a solid and integral ethical formation which allows the treatment of the patient, including his/her relatives, under a respectful and compassionate environment considering sexual, cultural and religious diversity as well as the principles of confidentiality, informed consent and patient's rights.

Symposium 10: Immune factors and reproduction

Coordinator: S. Daher (Brazil)

CD4+CD25+FOXP3+ T REGULATORY CELLS MEDIATE TOLERANCE AT THE FETAL-MATERNAL INTERFACE IN A MURINE MODEL

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Mechanisms underlying immune tolerance during pregnancy are poorly understood. We recently reported diminished number and function of Treg in abortion-prone mice. $CD4^+CD25^+$ Treg cells from normal pregnant and non-pregnant CBA/J mice could inhibit both; proliferation and IFN-γ secretion of lymphocytes from abortion-prone mice *in vitro*, while *in vivo* prevention of fetal rejection could only be achieved after adoptive transfer of Treg cells from normal pregnant mice. These data suggest that pregnancy-induced Treg cells might play a vital role in maternal tolerance to the allogeneic fetus. It is known that vaccination of CBA/J females ($H2^k$) with splenocytes from BALB/c male ($H2^d$) before pairing with DBA/2J ($H2^d$) prevents abortion. We observed that this treatment expanded the peripheral and thymus Treg populations. Moreover, Treg isolated from the mice rescued from abortion were able to prevent abortion if freshly transferred into DBA/2J-mated CBA/J females. Our results strongly suggest that Treg act in an antigen-specific manner during pregnancy. Regarding the mechanisms by which Treg would act, our data suggest that the transfer of Treg does not diminish the decidual levels of Th1 cytokines. The transfer of Treg dramatically up-regulated the expression of LIF, TGF-β and HO-1 at the fetal-maternal interface. Our data suggest that Treg-treatment cannot prevent T cell infiltration or high Th1 levels but is able to create a privileged tolerant microenvironment at the fetal-maternal interface.

RELATIONSHIP BETWEEN IMMUNOGENETIC FACTORS AND PREGNANCY

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Different pattern of immunologic disturbances have been associated with obstetric intercurrences, such as recurrent spontaneous abortion (RSA), preterm labor (PT) and preeclampsia (PE). Within this scenario, natural killer (NK) cells and cytokines figures as representative elements. Increased number and/or NK cells activities have been associated with pregnancy pathologies. Despite a direct causal relationship between cytokine production and miscarriage has not been determined, differences in its profile have been observed in RSA patients. Looking for factors that might contribute to such alterations, we evaluated polymorphisms of genes coding for NK receptors and cytokines. High and low cytokine production phenotypes have been associated with different genotypes. However, several factors may affect cytokine secretion. Concerning RSA some associations have been detected, including results of meta-analysis studies. On the other hand, no significant association was found for PT. Evaluation of PE patients' suggests ethnic influence on immune response. Besides cytokine, hormones such as progesterone also influence pregnancy development. It prepares endometrium to implantation, induces the production of Th2 cytokines and interferes with the trophoblast apoptosis. In addition, progesterone affects the production of vascular endothelial growth factor (VEGF). Search for the relationship between pregnancy loss and polymorphisms of gene coding for progesterone receptor and for VEGF have been considered. Implantation, placentation and successful fetal development, depend on adequate secretion of all these factors; thus, the identification of genetic characteristics or previous detection of abnormal levels, may help to elaborate preventive measures and create a more effective therapeutic protocol.

STUDY OF MOLECULAR MECHANISMS INVOLVED IN THE ETIOLOGY OF EMBRYO-FETAL LOSSES ASSOCIATED TO IMMUNOLOGICAL AND HEMATOLOGICAL PROCESSES

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During last years, Immunology and Hematology has been approached to Reproductive Medicine in other to explain mechanisms implicated in endothelial injury causing abortion. Current research has been focused on inflammatory process leading to coagulation involved in causation of fetal death by ischemia. Cytokine provoking vascular damage rather than direct action on trophoblast may explain abortion of genetically normal embryos. We have been demonstrated that inoculation of rIL-6 or low-molecular weight heparin was able to diminish abortion rate in a murine model. In the present work, the hypothesis that antioxidant supplementation is able to counteract toxic effect of oxidative stress on pregnancy has been postulated. We studied the effect of Vitamin E oral supplementation of CBA/J x DBA/2 pregnant females on abortion rate (IR), TNF-α, IL-6, IL-10 placental levels, as well as placental NO production. **Methods:** Different doses of natural Vitamin E were administered orally on CBA/J x DBA/2 females on days 0.5 up to 12.5 of pregnancy. Feto-Placental units were scored at 14.5 days of pregnancy and IR was scored. Cytokine concentration was determined by ELISA. Placental NO levels were measured by colorimetric procedure described by Griess. **Results:** 0,5mg/g of Vitamin E decreases IR up to 93% ($p<0,0001$) whereas increases placental IL-6 levels up to 23.6pg/mL with regards to 10.9 pg/mL of non-treated animals ($p=0,0004$). No differences were found on IL-10, TNF-α or NO levels. **Conclusions:** Vitamin E is able not only to prevent fetal wastage but also to modulate anti-inflammatory IL-6 placental levels. This protective effect seems to be independent of the antioxidant function. Supported by B 094 UBA 2004-2007.

THE MULTI-TASKING OF COMPLEMENT REGULATORY PROTEINS ON SPERM?

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Membrane cofactor protein [MCP; CD46] is a compement regulatory protein (CRP) expressed widely on human cells but, by sperm, solely as an unusual molecular isoform on the inner acrosomal membrane [IAM] which becomes surface exposed only after the acrosome reaction [AR]. Spermatozoal CD55 and CD59 are considered to be predominantly localized on the plasma membrane, although recently we have demonstrated CD55 is also markedly expressed on the IAM. Antibodies against CD46, but not CD55 or CD59, inhibit binding and penetration of human sperm to zona-free eggs. Antibodies specific for the first short concensus repeat [SCR] domain are most effective. New World monkeys express a CD46 isoform lacking SCR1 on all cells except the IAM of spermatozoa, supporting a role for the SCR1 ectodomain in fertilization. Also, in rodents, CD46 is not a systemic complement regulator but instead is solely expressed on spermatozoal IAM and no other cells. PCR and immunocytochemistry have indicated that outbred wood mice fail to express spermatozoal CD46 and, like CD46 knockout mice, are fertile and undergo an accelerated spontaneous AR. Proposed complement-dependent and -independent roles for CD46 on AR-sperm include protection from local complement activation, involvement in sperm-egg binding/fusion, acrosome stabilization and opsonization of redundant sperm.

AUTOIMMUNE ASPECTS OF RECURRENT ABORTIONS

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Recurrent spontaneous abortion (RSA) refers to three or more consecutive spontaneous abortions before the 20th week of pregnancy. One of its etiological factors is antiphospholipid syndrome (APS), which was described as the occurrence of thromboses, recurrent fetal deaths and thrombocytopenia associated with antibodies against negatively charged phospholipids. APS diagnostic is made when at least one clinical and one laboratorial criterion are present. One clinical criterion is a history of at least 3 consecutive spontaneous abortions before the 10th week of pregnancy. Laboratory criteria include detection of anticardiolipin IgG and/or IgM- isotope antibodies on two or more occasions at least 6 weeks apart, and/or the presence of lupus anticoagulant detected by nonspecific coagulation tests. The interaction of antibodies with a β2-GP1 present in endothelial cells leads to the expression and release of different molecules, as well as the interference with the coagulation cascade resulting in vasoconstriction, interference in the C/S protein and thrombomodulin systems and finally interference in the action of annexin-V which would enhance platelet activation. Possible mechanisms that lead to pregnancy loss in the APS seem to be related to enhancement of thrombosis, decreased trophoblast secretion of hCG and inhibition of phosphatidylserine. This inhibition is caused by the interaction of anticardiolipin antibodies with the β2GP-1, either directly or mediated by molecules such as prothrombins and annexin-V. Best gestational results are obtained through previous administration of aspirin and heparin. Patients with thrombocytopenia should also be treated with corticosteroids, receive meticulous prenatal Fetal surveillance should begin around the 26th week and include serial Doppler ultrasound. A multidisciplinary team should care for these patients.

TRYPTOPHAN-DEPENDENT MACROPHAGE AND NK CELL ACTIVITY IN MURINE PREGNANCY

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Tryptophan metabolism has been involved in explaining maternofetal tolerance through induction of anergy of T lymphocytes but its role in the modulation of macrophages and uNK cells is not clear. **Methods:** We isolated and characterized murine decidual macrophages expressing the specific marker F4/80, non-specific esterase and phagocytic activity similar to macrophages derived from splenic monocytes. **Results:** IFN-γ was able to induce mRNA expression of indoleamine 2-3 dioxygenase (IDO) by decidual macrophages but these cells did not induce intracellular production of tryptophan-dependent IL-1α, TNF-α or IL-12. Additionally, a possible role of tryptophan on cytotoxic activity and cytokine production by uNK cell was studied. There was an increase in the cytotoxic activity of uNK cells using a tryptophan-free medium ($p<0.01$) but not with tryptophan-supplemented or 1-methyl-tryptophan-supplemented medium (1-MT, IDO specific inhibitor). The results with splenic NK cells were statistically non-different. Regarding intracellular cytokine production of IFN-γ, and TNF-α by NK cells we did not find any effect related to the presence or absence of tryptophan or 1-MT. **Conclusions:** The model of tryptophan depletion in T lymphocyte anergy may not apply to uNK cell function in explaining pregnancy tolerance. These paradoxical findings could be interpreted by postulating that tryptophan plays a completely different role in NK cells, not down regulating but instead increasing citotoxicity. This adds to the controversy of the real effect of tryptophan in pregnancy. Financial support: Colciencias and University of Antioquia.

Symposium 11: Primate implantation, human and baboons

Coordinator: P. Cameo (Argentina)

CELLULAR AND MOLECULAR ASPECTS OF HUMAN IMPLANTATION

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Extra-uterine pregnancies are not rare in humans whereas they are almost unknown in other mammals. Implantation in the human is thus unique. This uniqueness is characterised on the maternal side by a spontaneous and massive decidualisation of the endometrium and on the embryonic side by an almost unlimited invasive potential. Human embryos express an intrinsic invasive potential, which allows them to implant almost anywhere except in the endometrium because it protects itself from implantation. Human implantation is only possible during a limited period known as the implantation window. This particular receptivity seems to be the property of the endometrial epithelial lining since experimental removal of the uterine epithelium allows the blastocyst to "implant" completely. Several animal or in-vitro models have been developed to study this process. We use in-vitro cultured human cytotrophoblastic cells (CTB) obtained from first trimester legal abortions to mimic the trophectodermal cells of the blastocyst because under normoxic conditions, CTB are spontaneously invasive. Tumour invasion and CTB invasion share the same biochemical mediators: the matrix metalloproteinases (MMPs) and their inhibitors. MMPs are a family of enzymes capable of digesting the extra cellular matrices of the host tissues. That MMPs are causally related to trophoblast invasion in the endometrium is shown by the fact that tissue inhibitor of metalloproteinases inhibits cytotrophoblastic invasion in vitro. In contrast to tumour invasion of a host tissue, trophoblastic invasion during implantation and placentation is stringently controlled in both space and time. The factors responsible for these important regulatory processes are unknown but in-vitro studies point to autocrine (trophoblastic) and paracrine (endometrial) controls by cytokines and growth factors.

PRIMATE MODELS FOR HUMAN REPRODUCTION

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Given the close phylogenetic relationship with humans, the non-human primate has been extensively used as a model to study both the physiology of human systems and also the pathology of many human diseases. The focus of our laboratory is to understand the mechanisms by which the early embryo modulates the receptive endometrium to ensure a successful implantation and the mechanisms by which endometriosis affects fecundity. Understanding the maternal-fetal dialog in vivo requires the development of appropriate model systems since studies of this nature are ethically and morally not permissible in the human. By developing both a stimulated and early pregnant model system in the baboon we have been able to clearly elucidate the role of chorionic gonadotrophin (CG) in modulating uterine receptivity and preparing the uterus to respond to the appropriate blastocyst signals during trophoblast invasion. Our data have demonstrated that the initial transformation of endometrial stormal cells requires an intermediate change in its phenotype that is induced by CG. This change in phenotype is a requisite to enable the stromal cells to transform into decidual cells in response to embryonic cytokines such as IL-1β. These changes using the simulated pregnant baboon model are similar to the responses that are observed in the presence of an embryo. Thus, this model system enables us to delineate carefully the potential function of individual embryonic signals in modulating a receptive endometrium. By developing an induced baboon endometriosis model, we have also been able to demonstrate that the presence of ectopic lesions alters the uterine environment during the receptive window. These changes are sequential and correspond to the development of the disease in vivo. The workshop will focus on describing the value of primate models to ask relevant questions as they pertain to the reproductive process and discuss the implications of an aberrant uterine environment because of pathology such as endometriosis, which may affect the fertility potential of women with this disease.

Symposium 12: Emergency contraception

Coordinator: A. Manzur (Chile)

CLINICAL STUDIES ON THE MECHANISM OF ACTION OF LEVONORGESTREL USED FOR EMERGENCY CONTRACEPTION

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The absolute contraceptive efficacy of levonorgestrel (LNG) remains undetermined and current estimates range from 60 to 89%. These figures indicate its efficacy is well below that of pills for regular contraception, therefore any explanation given on how it prevents a woman from becoming pregnant, must also account for the non prevented pregnancies. **Methods:** Nine, original research papers focused on the mechanism of action of LNG in women published in 2001-2005 and ongoing research are reviewed. **Results:** Five provide evidence that LNG interferes with ovulation. Two found no alteration in the endometrium that would support the hypothesis that LNG prevents implantation, and one found glycodelin increases earlier when LNG is given before ovulation. Two studies report minimal if any direct effect of LNG upon human spermatozoa in vitro. One paper reported effects on the mid luteal phase endometrium in a single woman who took doses several fold higher than used for EC. Since the difference between a medicine and a poison resides only in the dose, the findings of this report are meaningless for the current regimen. Our endometrial gene expression profiling studies do not support LNG interferes with endometrial receptivity. In a study conducted in Australia a blood sample was taken prior to the ingestion of LNG for measuring serum LH, E2 and progesterone levels, which allowed establishing whether the pill was taken before or after ovulation in 99 subjects. Three pregnancies occurred, all in subjects who took the pill shortly after ovulation. **Conclusions:** The evidence indicates that LNG can interfere with the ovulatory process if taken on time and thus prevent fertilization. Otherwise, if taken after ovulation has been triggered they are ineffective, accounting for the estimated 11-40% failure to prevent expected pregnancies.

EMERGENCY CONTRACEPTION AND SEXUAL AND REPRODUCTIVE RIGHTS

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Sexual and reproductive rights (SRR) are an essential part of human rights, deriving from the right to life and health, freedom of conscience and religion, equity, education, be free of torture or mistreatment and access the benefits of scientific progress, among others. SRR include the right of individuals and couples to make free and informed decisions about their sexual and reproductive life. This includes choice of partners, having children or not, how many and when to have them. Thus, they could reach high standard of sexual and reproductive health, to be free of discrimination, coercion or violence in their sexual life, and to with equal opportunity, respect and to share responsibilities in sexual and reproductive life. Access to contraception, including emergency contraception (EC), is essential for implementing SSR. In developing countries, the estimated daily unsafe abortions are 55.000, which cause 200 deaths. The adolescent pregnancy rates are 5-10 times higher in developing than in developed countries. EC has an important role in this context since it is the only contraceptive that a woman can use to prevent an unwanted pregnancy after an unprotected sexual intercourse, either forced or voluntarily. Although EC products are registered in around 120 countries, access is restricted because of opposition of conservative sectors; lawsuits against EC products; need of medical prescription; high price of EC products; lack of EC in public services and conscience objection of providers. Improving access to contraception, including EC, will protect the right to life and health, the right of children to be born being wanted and the right of people to free decisions regarding their sexual and reproductive life.

ADVANTAGES AND DISADVANTAGES OF ANIMAL MODELS TO ASSESS THE MECHANISM OF ACTION OF LEVONORGESTREL ADMINISTERED AS EMERGENCY CONTRACEPTION

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Most advances in medicine have emerged from experimentation with animal models. Although extrapolation from one species to another is limited by known and unknown differences among them, mammals afford useful models to investigate fundamental physiologic processes. There are more similarities than disparities among them even when the position in the zoological scale may be distant. Our studies in *Cebus* monkeys and rats have clearly demonstrated that like in women, acute treatment with levonorgestrel (LNG) interferes with the ovulatory process. Development and implantation of the embryo are unaffected in these animal models, and extrapolation of this result to the human is supported by the fact that the three species use the same basic processes to reproduce and the same hormones regulate them. LNG is a progestin (progesterone analog) that acts through progesterone receptors (PR) and mimics progesterone actions. The fundamental action of progestins, which justifies its name, is related to the enhanced endometrial receptivity to embryo implantation; they relax the myometrium and maintain decidual integrity once implantation has taken place. This is a universal property of progestins among mammals, as it is the development of endometrial receptivity in response to PR agonists. Since everything is possible in biology there is no guarantee that the same results will be obtained if experiments are performed in women, but current knowledge makes highly unlikely that it will not occur.

REFLECTIONS ON EMERGNCY CONTRACEPTION

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Reflection is developed en relation to five approaches: (1) That the discussion of aspects related to sexuality is beneficial for the society. (2) I start from the premise that person's life begins at the moment of fertilization and from there on, is subject to all human rights and care. (3) The Catholic Church considers as anthropologically allowed, the use of techniques that restrain the ovulation or prevent the fertilization in case of violation. (4) Doubts of the people who believe that in relation to peri ovulatory period exist post conceptive mechanisms, will conclude as soon as the investigation progress takes place, consequently to support that the pre-conceptive machanism is the action to be taken. (5) Aware call on the concept that the greatest use of emergency contraception does not come from victims of abuse.

Symposium 13: Epithelia functional heterogeneity

Coordinator: L. Sobrevia (Chile)

TRANSPORT PROCESSES IN THE PLACENTA: LESSONS FOR EPITHELIAL PHYSIOLOGY

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The placental syncytiotrophoblast performs two major epithelial functions: it forms a barrier between the maternal and fetal circulation and at the same time permits transport of solutes into or out of the fetus. As in other epithelia, such vectorial transport requires a cellular polarity with regard to the presence and /or activity of transport systems in the membranes facing the opposite poles of the cell. This asymmetry has been revealed in recent years in the syncytiotrophoblast by studies on isolated membrane vesicles and immunohistochemistry. The transcellular transport occurs against limited gradients or sometimes downhill, a situation rarely found in other epithelia. In addition, endocytosis and transcytosis seems to play a significant role in nutrient transfer to the fetus, a fact often disputed in other epithelia. The syncytiotrophoblast is also undergoing a continuous rearrangement of plasma membrane components-thereby adding another example for the dynamics of epithelial cell polarity. These dynamics involve intracellular trafficking of transporters combined with a regulation of the transport activity as demonstrated recently in a variety of other epithelial cells. Thus, similarities and differences exist between the syncytiotrophoblast and other epithelial cells that optimize the cell for its unique functional requirements.

ROLE OF NUCLEOSIDE TRANSPORTERS IN CELL SIGNALLING

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Uptake of nucleosides via CNT (Concentrative Nucleoside Transporter) and ENT (Equilibrative Nucleoside Transporter) transporters has been traditionally understood as a basic mechanism to support nucleoside salvage processes, particularly in most cell types lacking endogenous purine and pyrimidine nucleotide biosynthesis. Nevertheless, since some of these transporter proteins are efficient adenosine carriers, a putative role in cell signalling has also been postulated. Extensive work on the ENT contribution to modulate extracellular adenosine levels in endothelia comes from Sobrevía and colleagues. At our laboratory we have focused on the CNT protein family and, particularly, on CNT2, the concentrative transporter that shows the highest affinity for adenosine. We have been able to demonstrate that CNT2 expression in selected epithelia (i.e. along the nephron) does not particularly correlate with an absorptive role exclusively, but instead can be associated with renal purinergic modulation. Indeed, CNT2-related transport activity is under purinergic control in hepatocytes, via A1 type receptors, by a mechanism that is mediated by Katp channels and, accordingly, sensitive to the energetic status of the cell. Adenosine taken up by CNT2 is indeed implicated in a rapid activation of the AMPK signalling pathway, thus establishing an additional link between CNT2 function and energy control. Taken together, these data suggest that selected nucleoside transporters might play other physiological roles beyond substrate salvage. Supported by Ministerio de Sanidad y Consumo, Ministerio de Educación y Ciencia, Generalitat de Catalunya, Universitat de Barcelona, Fundación Ramón Areces, Fundación "la Caixa" y FIPSE.

TRANSCRIPTIONAL REPRESSION OF THE EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 IN HUMAN UMBILICAL VEIN ENDOTHELIUM FROM GESTATIONAL DIABETES

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Human umbilical vein endothelium (HUVEC) from gestational diabetes (GD) show increases nitric oxide (NO) synthesis by an adenosine receptor A_{2a} dependent mechanism. A requisite for A_{2a} signaling in GD is an increase in extracellular levels of adenosine by down regulation of the expression and activity of the equilibrative nucleoside transporter 1 (hENT1). We studied the transcriptional activity of the *hENT1* promoter in HUVEC from GD. **Methods:** Primary culture of HUVEC from normal and GD pregnancies were maintain up to passage 2 in medium 199 (3.2 mM L-glutamine, Ethic committee approval and informed patient consent obtained). *hENT1* gene promoter activity was measured in HUVEC exposed to adenosine (10 μM, 4 h) following transfection by electroporation with pGL3 (luciferase reporter gene) plasmids carrying -3100, -2056 and -1016 bp of the promoter sequence (320 Volt, 30 ms, 8-10% efficiency). hENT1 protein content in HUVEC was determined by Western blot. **Results:** Transcriptional activity of *hENT1* promoter sequence from -2050 up to -3100 bp is decreased in HUVEC from GD compared to normal cells. This effect was also observed in normal cells exposed to adenosine. hENT1 protein content is reduced by ~50% in HUVEC exposed to adenosine. **Conclusions:** Down regulation of hENT1 in GD is mediated by a decreased transcriptional activity in the *hENT1* promoter. In addition, hENT1 expression is repressed at transcriptional and post-translational level by adenosine in HUVEC. Supported by FONDECYT 1030781/1030607/7050030 (Chile) and Fundación Andes (C-14060/50).

DIFFERENTIAL INSULIN SIGNALLING IN EPITHELIA AND ENDOTHELIA OF THE HUMAN PLACENTA

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Both surfaces of the human placenta are exposed to different metabolic and endocrine environments i.e., the syncytiotrophoblast (epithelium) to the maternal and the endothelium to the fetal circulation, respectively. Maternal diabetes mellitus is a condition associated with changes in both circulations. Maternal hyperinsulinemia is a feature of insulin-treated women. Hyperinsulinemia in the fetal circulation results from the stimulation of the fetal pancreas by diabetes-associated increase in levels of insulin secretagogues. Therefore, diabetes in pregnancy represents a condition in which insulin will act *in vivo* on the two different surfaces of the placenta. Maternal and fetal insulin may compete in their effects and even discordantly alter placental function if the responses are not controlled and coordinated. Recent studies demonstrated that one mechanism of control involves the separation of effects in time, i.e., gestational age, and space, i.e., the epithelial and endothelial surface. Insulin effects that were differentially regulated in placental epithelia and endothelia were related to gene expression as well as to glycogen synthesis suggesting a general difference in insulin receptors or downstream signalling mechanisms or both. Trophoblasts and placental endothelial cells express different insulin receptor isoforms resulting in the activation of different insulin signalling pathways. The MAP-kinase pathway is preferentially activated in trophoblasts that express the long insulin receptor isoform. As a downstream effect, insulin stimulates an increase in mRNA levels of some early response genes (*fos, jun*). The expression of distinct insulin receptor isoforms on the two surfaces of the placenta i.e., epithelial trophoblast vs. endothelium, therefore, represents a mechanism to provide diverse regulatory functions of maternal and fetal insulin in its surface target cells. Supported by Jubilee Fund 10053 and 10896 (Vienna, Austria).

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

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Up-regulation of pro-inflammatory cytokines, cyclooxygenase (COX-2) and prostaglandins is a critical factor driving human term labour and inflammation-associated preterm labour. Nuclear factor kappa B (NF-κB) is activated in response to a number of inflammatory mediators, including cytokines and lipopolysaccharide (LPS). The aims of this study were: (i) to investigate if TNF-α and LPS activate the NF-kB pathway; and (ii) to use short interfering RNA (siRNA) targeted against human inhibitor κB kinase (IKK)-β to indisputably confirm a functional role of the NF-κB pathway in the regulation of pro-inflammatory mediators in human placental JEG-3 cells. **Methods:** JEG-3 cells (n=3) were (i) incubated in the presence or absence of 10 μg/ml LPS or 20 ng/ml TNF-α, or (ii) transfected with 100 nM IKK-β siRNA. After 48 h incubation, cells were collected and cytoplasmic and nuclear protein extracted, and incubation media collected for analysis of IL-6 and $PGF_{2\alpha}$ release by ELISA. Western blot analysis was used to determine the expression of COX-2, IKK-β, IκB-α and phosphorylated IκB-α in cytoplasmic extracts, and NF-κB subunits in nuclear extracts. **Results:** LPS and TNF-α increased the expression of cytoplasmic IKK-β and phosphorylated IκB-α, and the nuclear expression of the NF-κB proteins p50 and p65, an effect associated with an increase in COX-2 protein, and release of IL-6 and $PGF_{2\alpha}$ ($p<0.05$). Treatment of cells with BAY 11-7082 (50 μM) significantly inhibited both basal, LPS and TNF-α induced NF-κB and COX-2 protein expression, and IL-6 and $PGF_{2\alpha}$ release ($p<0.05$). Transfection of JEG-3 cells with siRNA duplexes targeted against human IKK-β transcripts knocked down levels of the endogenous IKK-β protein in human placental JEG-3 cells, which was associated with a 40% and 48% reduction in IL-6 and $PGF_{2\alpha}$ release ($p<0.05$). **Conclusions:** Pro-inflammatory mediators regulate the NF-κB transcription pathway in human JEG-3 cells, and the IKK-β/NF-κB pathway is a regulator of inflammatory mediators in human placental JEG-3 cells. The control of NF-κB activation may therefore provide an alternative therapeutic strategy for reducing the release of pro-inflammatory mediators in infection associated preterm labour.

Symposium 14: Fetal hypoxia: a model of multisystemic response

Coordinator: H. Muñoz (Chile)

FETAL HYPOMETABOLISM: A MECHANISM TO WITHSTAND HYPOXIA

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The fetal llama has walked for millions of years by the thin oxygen trail of the Andean *altiplano*. We hypothesize that a pool of genes has been selected in the llama *(Lama glama)* that express very efficient mechanisms to withstand hypoxia. In the brain, there is no increase in cerebral blood flow during acute hypoxemia in the llama fetus, decreasing brain oxygen delivery *pari passu* with the decrease in carotid artery O_2 content. In spite of this lack of increase in brain blood flow, there is no increase in O_2 extraction across the brain. Thus, a decrease in cerebral oxygen consumption occurs during hypoxemia in the llama fetus. The fetal electrocorticogram (ECoG) mirrors this substantial reduction in cerebral oxygen consumption, since it remains flat during part of the hypoxemic insult, returning to normal in the post-hypoxemic period. With a more prolonged hypoxemia (24h), there is a decrease in the fetal brain temperature with a reduction in activity of the Na^+-K^+-ATPase in the cerebral cortex, accompanied by a lesser expression of voltage-gated Na^+ channels. No signs of brain cell apoptosis were observed. Furthermore, the fetal llama responds to hypoxemia with intense peripheral vasoconstriction. All these responses could be the expression of a marked hypometabolism, both in the brain as well as in the whole body. How this hypometabolism is produced and how the cells are preserved during this condition remains to be elucidated. FONDECYT 1010636, 1050479, The Wellcome Trust, CRIG 072256.

Symposium 15: Physiological effects of prenatal exposure to environmental polutans

Coordinator: A.M. Ronco (Chile)

EFFECT OF ORGANOPHOSPHATE PESTICIDES THAT CROSS THE PLACENTA ON BIOMARKERS: *IN VITRO AND IN VIVO* STUDY

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The placental transfer of pesticides has been established by the presence of metabolites of these compounds in the meconium of the newborn. Our objective was to evaluate, by *in vitro* and *in vivo* studies, the effects on biomarkers of the post-placental passage of organophosphate pesticides, their action upon acetylcholinesterase (AChE) activity, and their genotoxicity. **Methods:** The patients that we studied resided in the agricultural zone of Chihuahua, Mexico. To compare the AChE sensitivity *in vitro* of women and newborns to ethyl paraoxon [EP], we determined the inhibitor concentration yielding a 50% enzymatic activity (IC_{50}). In order to determine the genotoxic effect of the EP on lymphocytes we used the micronuclei (MN) technique and comet assay. **Results:** The enzymatic activity of the AChE produced during the time period free of the application of pesticides was 5.78±1.17 U/mL, whereas that tested during intense pesticide application, was 5.15±1.16 U/mL ($p < 0.01$). We identified no difference between the maternal IC_{50} and that of the newborns, but our test results did indicate an increase in deoxyribonucleic acid migration with a dose effect response ($p < 0.01$). Only the MN frequency showed an increase with high doses ($p = 0.02$). **Conclusions:** Our results suggest that, in pregnant women who reside in agricultural zones, AChE activity falls during times of pesticide application. The sensitivity of immature organisms exposed to EP is apparently not related to the intrinsic properties of the AChE enzyme. The test data collected suggest an EP-generated, dose-responsive genotoxic effect.

MOLECULAR EFFECTS OF DICHLORODIPHENYLTRICHLOROETHANE IN A HUMAN CHORIOCARCINOMA CELL LINE

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Progesterone (P4) is known to be essential for the establishment and maintenance of pregnancy including the prevention of labor. Any substance that can decrease P4 production during pregnancy may induce changes that can lead to preterm labor. Recently, two different groups proposed that dichlorodiphenyltrichloroethane (DDT), blocks P4 production by a granulosa cell line. Additionally, high serum levels of DDT and dichlorodiphenyldichloroethylene (DDE) have been associated with an increased rate of preterm labor. Thus, the aim of this study was to determine the ability of DDT or it metabolites to inhibit P4 production by a human choriocarcinoma cell line (BeWo). **Methods:** BeWo cells were cultured in the presence of different concentrations of DDT, DDE or DDD alone or in combination with cholera toxin, a protein kinase A activator that stimulates progesterone synthesis. P4 was determined by ELISA and viability of the BeWo cells during incubation were assessed by measuring lactate dehydrogenase (LDH) leakage into the medium. **Results:** Exposure of BeWo cells to 10, 100 and 1000 nM DDT, DDE or DDD for 24 hours resulted in no loss of cell viability. DDT but not DDE or DDD inhibited BeWo cell progesterone production in a concentration-dependent manner. In addition, DDT blocks the stimulatory effect of cholera toxin, after 24 hours incubation. **Conclusions:** The results of the present study indicate that DDT but not DDE or DDD inhibits BeWo cell steroidogenesis. Supported by IMSS/FOFOI FP-2004-003.

CYTOGENETIC DAMAGE IN FEMALE CHILEAN AGRICULTURAL WORKERS EXPOSED TO MIXTURES OF PESTICIDES

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The VIII Region of Bio-Bio is a major fruit-growing area of Chile that makes intensive use of agricultural pesticides. The cytogenetic damage associated with exposure to mixtures of pesticides was evaluated by comparing peripheral blood lymphocyte micronucleus (MN) frequencies in a group of 64 female agricultural workers and 30 female controls. The exposed subjects worked during the spring and summer in thinning and pruning fruit trees and in harvesting and packing different fruits, such as raspberries, grapes, apples, and kiwis. They did not use any protective measures during their work activities. A significant increase in the frequency of binucleated cells with micronuclei (BNMN) was found in the exposed women as compared with the controls (36.94 ± 14.47 vs. 9.93 ± 6.17 BNMN/1000 BN cells, $P<0.001$). The frequency of BNMN varied as a function of age in both the exposed and control groups, but no correlation was found between BNMN frequency and the duration of exposure. Also, smoking and other habits had no effect on MN frequency. Our study confirms that occupational exposure to pesticide mixtures results in cytogenetic damage. Supported by Dirección de Investigación Universidad de Concepcion 201.031.089-1.

INCREASED LEVELS OF METALLOTHIONEIN-2 ISOFORM IN PLACENTA OF SMOKERS. ASSOCIATION WITH FETAL GROWTH AND PLACENTAL TRACE ELEMENT LEVELS

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Cigarette consumption during pregnancy produces deleterious effects in both, mother and fetus through still unknown mechanisms. The aim of this research was to determine placental concentrations of zinc, cadmium, total and specific metallothionein (MT) isoforms MT-1 and MT-2 in smokers and nonsmokers to correlate these parameters with neonate´s birth weight and height. **Methods:** Placental cadmium and zinc of both groups were analyzed by atomic absorption spectrometry (AAS) and neutron activation analysis respectively; total MT was determined by western blot; MT-1 and MT-2 in cadmium saturated samples were separated by high performance liquid chromatography (HPLC) and their respective concentrations calculated by cadmium measurement by AAS - inductively-coupled-plasma (AAS-ICP). **Results:** Smokers delivered neonates with low birth weight (LBW), a parameter significantly correlated with placental cadmium concentrations. Zinc, cadmium and total MT were higher in smoker's placentas. MT-2 concentrations increased by seven fold in smokers' placentas (1.16 vs. 0.15 nmol/g wet tissue), being MT-1 not significantly modified. **Conclusions:** MT-2 is the main cadmium-induced isoform found in placenta of smokers suggesting a role in retaining cadmium inside the placenta, and thus protecting fetus of this toxic element. MT-2 may also be able to retain zinc reducing the transference of this essential element to the fetus, which in turn may affect fetal growth and development leading to LBW. Supported by International Atomic Energy Agency (IAEA, grant 11527/RO/RBF).

DELAYED EFFECTS OF PRENATAL EXPOSURE TO VARIOUS CHEMICAL COMPOUNDS: A POSSIBLE CAUSE OF ADULTHOOD PATHOLOGIES

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The development of cervicovaginal adenocarcinoma in young women whose mothers were treated with diethylstilbestrol during pregnancy allowed G. Csaba to develop the hypothesis of hormonal imprinting, explaining irreversible alterations induced by perinatal exposure to abnormal doses of various hormones. We reported that prenatal exposure of rats to diethylstilbestrol changed responses to estrogen at older age. In further studies we reported that prenatal exposure to non-hormonal compounds, such as lead and other pollutants, pharmaceuticals, substances of abuse, food additives and natural food components, may also induce the imprinting mechanism. Evidence for these mechanisms is presented. It can be concluded that perinatal exposure to several agents causes irreversible changes in cell programming, which determine health conditions during adulthood. Diseases developed during adulthood were probably determined during early stages of life by maternal exposure to substances or the effect of maternal diet during pregnancy. Among them, cancer, immune depression, autoimmune diseases, infertility, alteration in the action of hormones, changes in personality such as aggressive behavior, tendency to drug addiction, delinquent behavior, and other neurobehavioral alterations. Regulations to avoid these early exposures may contribute to an important improvement of health conditions of humankind.

Symposium 16: Thrombophilia

Coordinator: R. Barini (Brazil)

INTRAVENOUS HUMAN IMMUNOGLOBULIN INDICATIONS IN PREGNANCY LOSS

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Recurrent abortions and *in vitro* Fertilization (IVF) failures are interest issue in human reproduction. In these patients, several factors must be evaluated: genetics, infection, hormonal, anatomic and immunologic. During implantation, the embryo becomes intimately connected with the maternal endometrium or decidua. *T-Helper* 2 immune response is observed in a normal interaction between trophoblast cells and the maternal immune system. Autoimmune factors (anti-thyroid antibodies, anti-nuclear antibodies, anti-DNA antibodies, anti-phospholipids antibodies), alloimmune factors (HLA compatibility), cellular factors (decrease of suppressor cells, increase activity of NK cells), *T-Helper* 1 immune response are observed in couples with bad gestational results. The immunotherapy, active (lymphocytes immunization) and passive (Intravenous Human Immunoglobulin – IVIg), is an indication for those couples. Use of Intravenous Human Immunoglobulin for patients with a history of reproductive failure has gained popularity over the past decade. Nowadays, IVIg is indicated in cases of fetal alloimmune thrombocytopenia, idiopathic and immunological recurrent miscarriage and for patients with a history of IVF failures. There are different protocols for IVIg therapy in literature. Researches must be done to identify the best IVIg protocol (patient selection, time to start, composition, dosage, duration).

THROMBOPHILIA AND INFERTILITY

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Thrombophilia has been exhaustively studied in severe clinical conditions, such as venous and arterial thrombosis, obstetric complications as recurrent abortion and infertility. Patients with in vitro fertilization implantation failure may have thrombotic disorders. Special treatment for these conditions may increase implantation rates for this selected group. A prevalence study of the antiphospholipid syndrome (anticardiopilin antibody and/or lupus anticoagulant) and inherited thrombophilia (protein C and S deficiency, anti thrombin III, Factor V Leiden gene mutation, Factor II G20210A prothrombin gene mutation, C677T metilenotetrahidrofolate reductase gene mutation) was performed. We analyzed also the prevalence of these conditions in a group of a normal woman with at least one normal healthy baby and no abortion. A comparative analysis was performed between the two groups, which showed a higher frequency of C677T MTHFR gene mutation and protein S deficiency in the infertile group. This may indicate that anticoagulant prophylaxis for these patients may benefit implantation rates in IVF cycles.

THROMBOPHILIA AND RECURRENT ABORTION

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The presence of acquired and inherited thrombophilic factors has been associated with recurrent spontaneous abortion (RSA). Several studies were realized to evaluate this association. The most important factors associated with pregnancy complications as pré-eclâmpsia, intrauterine growth restriction, fetal death, infertility and recurrent abortion are the anticardiolipin antibody (ACA), lupus anticoagulant (LA), protein C, S and antithrombin III (ATIII) deficiencies, Leiden factor V, mutation G20210A in prothrombin gene and mutation C677T in methylene tetrahydrofolate reductase (MTHFR) gene. Several results of these factors evaluation in pregnancy complications, mainly in recurrent abortions, will be described.

Symposium 17: Transport in placenta

Coordinator: R. Marín (Venezuela)

SYNTHESIS, METABOLISM AND REGULATION OF VITAMIN D IN HUMAN PLACENTA

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Human placenta synthesizes and metabolizes 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] through the expression of 25-hydroxyvitamin D_3-1α-hydroxylase and $1,25(OH)_2D_3$ 24-hydroxylase, the two key enzymes for vitamin D metabolism. Our laboratory has investigated the presence of 25-hydroxyvitamin D_3 1α-hydroxylase (1α-(OH)ase) gene expression products in cultured human syncytiotrophoblasts. Primary cultures exhibited 1α-(OH)ase activity and a transcript for this gene could be demonstrated in these cells. Northern blot analysis revealed the presence of a 2.5 kb product, similar in size to that previously reported in kidney and RT-PCR analysis demonstrated the presence of a single transcript with nucleotide sequence identical to that previously reported for human 1α-(OH)ase cDNA clones. The control of $1,25(OH)_2D_3$ production represents a critical step in the regulation of calcium metabolism and may also be important for the growth and differentiation of placental cells. We investigated the effects of cAMP and $1,25(OH)_2D_3$ on the activity and expression of 1α- and 24-hydroxylases in normal human syncytiotrophoblast cells in culture, including their enzymatic activities in an effort to understand the regulation and biological implications of vitamin D metabolism in placenta. Calcitriol and cAMP supress 1α-hydroxylase expression, whereas both of them upregulate 24-hydroxylase mRNA. The mechanistic differences in gene transcription regulation by a cAMP-dependent pathway, of these two key enzymes involved in the metabolism of vitamin D in placenta and kidney, respectively are probably mediated by cAMP via CREs and/or via additional tissue specific transcription factors at the level of individual promoters.

ROLE OF AQUAPORIN-9 IN HUMAN PLACENTA

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The expression and localization of aquaglyceroporins (AQP3, AQP7, AQP9) in the syncytiotrophoblast of human placenta were previously reported by us. In particular, the increase of AQP9 expression levels in preeclamptic placentas were not followed by water and mannitol transport. It is known that progesterone (P_4) and the hypoxia inducible factor-1 (HIF-1) are augmented in preeclampsia. Therefore in the present study we studied the expression of AQP9 in explants from normal placentas treated for 24 h with 300ng/mL P_4 or either 250μM $CoCl_2$, an inducer of HIF-1. Explant viability was controlled by β-hCG level in the extracellular medium. AQP9 mRNA expression was measured by semi-quantitative RT-PCR. Unidirectional fluxes of water, mannitol and urea were measured by radiolabeled techniques. AQP9 expression was higher in both, $CoCl_2$ and P_4 treatments than controls. Water and mannitol uptake decreased in explants treated with $CoCl_2$ (82% and 51%) whereas they were increased in explants treated with P_4 (40% and 63%). In addition, the uptakes in both conditions were significantly inhibited by 0.3mM $HgCl_2$ *($P<0.05$).* In conclusion the increase in AQP9 expression was not always coincident with its function, suggesting that $CoCl_2$ and P_4 triggered different mechanisms for AQP9 regulation. In the case of hypoxia there is no correlation between AQP9 expression and water permeability as we previously observed in preeclampsia. These data suggest that HIF-I may dysregulate AQP9 in pregnancy disorders associated to hypoxia like preeclampsia. ANPCYT PICT 9508/02.

ARE THE SERUM LIPID PEROXIDES OF THE PREECLAMPTIC WOMEN ABLE TO CROSS THE SYNCYTIOTROPHOBLAST?

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Several investigators have previously showed increased products of lipid peroxidation in the serum of both preeclamptic mothers and their neonates. Oxidative stress of the syncytiotrophoblast of preeclamptic mothers could be responsible for the increase in the level of lipid peroxidation of both mothers and their neonates. **Methods:** Venous blood was obtained by venipuncture of 12 nulliparous normotensive and 12 preeclamptic pregnant women. After cesarean delivery, blood samples were obtained from the umbilical cord of the same patients. Red blood cell ghosts were prepared and assayed for TBARS and conjugated dienes. Placentas from normotensive women were obtained and tissue fragments were exposed to hypoxia. The TBARS in the incubation medium were determined. Red cells from normotensive women were preincubated with the medium of the hypoxic placentas and the level of lipid peroxidation of the ghosts was measured. **Results:** The serum TBARS of preeclamptic pregnant women are higher than those of normotensive pregnant women. Similar results were obtained for the serum of their neonates. The level of TBARS and conjugated dienes of red cell ghosts are significantly increased with preeclampsia in mothers and their neonates ($p<0.001$ and $p<0.005$ respectively). The tissue fragments of placentas from normotensive women, incubated under hypoxia, generated enough lipid peroxidation products to increase the level of lipid peroxidation of the red cell membranes. **Conclusions:** Preeclamptic mothers and their neonates show an increased level of lipid peroxidation of their red blood cell ghosts. Lipid peroxidation by-products liberated by the syncytiotrophoblast of the preeclamptic women would contribute to the oxidative stress of the neonates. Supported in part by grant No. H9/181/R427, Project 96350, from the World Health Organization.

LIPID PEROXIDATION AND Na-K-ATPase ACTIVITY IN PLASMA MEMBRANES OF HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

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Lipid peroxidation and its by-products can severely damage the plasma membrane of different cells, affecting in this way the activity of membrane enzymes such as the Na-K-ATPase (NKA) and the plasma membrane Ca-ATPase (PMCA). The syncytiotrophoblast is one of the few epithelia without a polarity in the distribution of the NKA, showing the NKA activity in both the basal plasma membranes (fetal side) and the microvillous plasma membranes (maternal side). In the present work we evaluated the sensitivity to lipid peroxidation of the NKA activity of membranes from syncytiotrophoblasts. **Methods:** Plasma membranes lipid peroxidation was induced by UV irradiation (254 nm) at 4°C. TBARS and NKA activity were measured after UV irradiation of the membranes. Fragments of the central cotyledon of placentas from normotensive women were exposed to hypoxia at 37°C and TBARS and NKA activity were measured in plasma membranes from the syncytiotrophoblast. **Results:** The NKA activity of basal plasma membranes (fetal side) is highly susceptible to UV irradiation when compared to that of microvillous membranes. Similar results were obtained for tissue fragments incubated at 37° under hypoxia. **Conclusions:** Microvillous plasma membranes from syncytiotrophoblasts are more resistant to lipid peroxidation than the basal plasma membranes (fetal side), maintaining higher levels of their NKA activity. Supported in part by grant No. H9/181/R427, Project 96350, from the World Health Organization.

Symposium 18: Physiology of transition metals

Coordinator: M.T. Núñez (Chile)

ANTIOXIDANT VERSUS PRO-OXIDANT EFFECTS OF THIO- AMINO ACIDS IN THEIR INTERACTION WITH COPPER IONS: IN VITRO STUDIES

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Interactions of thiol compounds with copper ions induce both pro- and antioxidant responses. In fact, in promoting Cu^{2+} reduction, thiols give rise to the highly oxidant Cu^{1+} that generates the radicals $O_2^{\cdot-}$ and $OH^{\cdot}$. For the contrary, by trapping and neutralizing free radicals, thiols work as effective anti-oxidants. In this work we postulate that the thio-amino acids homocysteine (Hcys) and cysteine (Cys), besides their capacity of neutralizing free radicals, have the property of binding copper ions thus forming a redox-inactive complex. Thus, these thiol-Cu complexes would have a double antioxidant function. To test this hypothesis, we 1) investigated the capacity of Hcys and Cys to react with the free radical $ABTS^{\cdot+}$, 2) characterized the interaction of Cu^{2+} ions with Hcys and Cys in terms of the redox state of Cu, 3) quantified the capacity of Hcys and Cys to protect SH-titrable groups against oxidation by Cu^{2+}, 4) characterized the capacity of Hcys and Cys to diminish Cu^{2+} mediated production of free radicals ($O_2^{\cdot-}$ and $HO^{\cdot}$), 5) studied the capacity of Cu^{2+} to induce ascorbic acid oxidation in the presence or absence of Hcys and Cys. We conclude that the interaction of Cu^{2+} with Hcys and Cys generates complexes that present a clear capacity to trap free radicals of the $ABTS^{\cdot+}$ type while at the same time differ in their reactivity towards oxygen, hydrogen peroxide, and ascorbic acid. Supported by FONDECYT 1040736 (Chile).

CELLULAR METABOLISM OF COPPER IN PRO- AND EUKARYOTES: COMPARATIVE ANALYSIS OF THEIR TRANSCRIPTIONAL REGULATION MECHANISMS

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Copper is an essential micronutrient required for cell function but in excess is toxic. Both prokaryotes and eukaryotes have developed common strategies to maintain optimal copper levels. The fact that the proteins involved in copper homeostasis are highly similar in phylogenetically distant organisms has allowed for a wide variety of biological models in the study of copper homeostasis. Our laboratory has studied the expression of genes involved in copper metabolism in five biological models (bacteria: *Enterococcus hirae* and *Enterococcus faecalis;* yeast: *Saccharomyces cerevisiae;* plant: *Arabidopsis thaliana*; rodent: *Mus musculus* and human: *Homo sapiens*) using different experimental approaches (micro- and macro-arrays; qRT-PCR), when exposed to different copper concentrations. A comparative analysis of the result will be presented in this Symposium, together with a general model for the homeostatic regulation of cell copper.

HEREDITARY HEMOCHROMATOSIS: A DISEASE OF IRON AND COPPER METABOLISM

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Hereditary Hemochromatosis (HH), caused by point-mutations in the protein HFE, is characterized by increased intestinal iron absorption, which leads to progressive iron overload. Based on the observation that ectopic expression of HFE strongly inhibits apical iron uptake, a negative regulation of HFE on the apical membrane transporter DMT1 was proposed as a mechanism by which HFE regulates iron absorption. **Methods:** To advance in this hypothesis, we investigated: i) the effect of HFE antisense oligonucleotides on apical iron uptake by polarized Caco-2 cells, ii) the apical/basolateral membrane distribution of HFE, β-2-microglobulin (β2m) and DMT1 and, iii) the putative molecular association between HFE and DMT1. **Results:** We found that HFE antisense treatment reduced HFE expression and increased apical iron and copper uptake, whereas transfection with wild-type HFE inhibited iron and copper uptake. Selective apical or basolateral biotinylation indicated preferential localization of DMT1 to the apical membrane and of HFE and β2m to the basolateral membrane. Ectopic expression of HFE resulted in increased distribution of HFE-β2m to the apical membrane. The amount of HFE-β2m in the apical membrane inversely correlated with apical iron uptake rates. Immunoprecipitations of HFE or β2m with specific antibodies resulted in the co-precipitation of DMT1. **Conclusions:** These results sustain a model by which direct interaction between DMT1 and HFE-β2m in the apical membrane of Caco-2 cells results in down-regulation of apical iron uptake activity. Financed by Fondo Nacional de Ciencia y Tecnología grants 1051006 and 1040448.

COPPER AND ZINC AS MODULATORS OF NEURONAL EXCITABILITY

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Copper and zinc are released during synaptic events in some regions of the central nervous system, particularly in glutamatergic neurons. It has been proposed that these metal ions could play a role as normal modulators of neuronal excitability. Our aim was to gather information to help testing this hypothesis. **Methods:** We characterized the effects induced by a wide concentration range of these divalent metal ions on the action potential firing rate of *in situ* olfactory epithelium neurons and on the inward sodium currents in isolated olfactory neurons. **Results:** At low concentrations values (1-50 nM for Cu^{2+} and 1-50 μM for Zn^{2+}), these divalent cations induced a concentration-dependent increase in the firing rate, speeded-up the activation and inactivation kinetics of the inward sodium current and increased its amplitude. At higher concentrations (0.1-5 μM for Cu^{2+} and 100-500 μM for Zn^{2+}) however, these metal ions decreased the firing rate and inhibited the inward sodium currents. The biphasic effect of copper and zinc on the neuronal excitability may be explained by the interaction of these metal ions with high and low affinity sites in sodium voltage gated channels. **Conclusions:** Our results suggest that copper and zinc may regulate excitability by modifying the neuronal firing rate. Cells exposed to low concentrations of these cations could become more excitable and those exposed to high concentrations would become less excitable. Funded by Fondecyt grant 1040681.

PHYSIOLOGY AND PATHOLOGY OF IRON IN NEURONAL FUNCTION

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Iron is necessary for the development of cognitive functions. Iron-deficient children had significantly lower values in comprehension tests and in full scale IQ compared to controls. In contrast, iron accumulation is involved in the generation of oxidative damage in several neurodegenerative diseases such as Parkinson's and Alzheimer's disease. In this study we examined the relationships between iron homeostasis and neuronal function utilizing SHSY-5Y neuroblastoma cells and hippocampal neurons. We found that both iron excess and iron deficit produced marked changes in electrical function. Beneficial effects could be traced to iron stimulation of the ERK 1/2 pathway by a mechanism involving reactive oxygen species and stimulation of calcium channels. We studied evoked field response to paired-pulse stimulation in hippocampal slices. We found that iron depletion induced a lasting depression of the CA1 area responses to CA3 area stimulation. By contrast, iron induced a lasting potentiation. We also characterized neuronal function as a function of cell iron load. We found that iron accumulation killed a large proportion of cells, but a sub-population became resistant to iron accumulation developing an adaptative mechanism intended to decrease intracellular iron content and to increase GSH content. The decrease in iron content was traced to the regulation of import and export iron transporters while the increase in GSH content was traced to increased expression of the catalytic and regulatory sub-unit of glutamate cysteine ligase. Financed by ICM project P99-031 and by grant 1040448 from Fondo Nacional de Ciencia y Tecnología, Chile.

Oral Communications (Incorporations SCHCF)

REMODELING OF THE EXTRACELLULAR MATRIX DURING DECIDUALIZATION

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In rodents, several molecular and cellular modifications occur in the uterus during pregnancy, probably to provide an adequate microenvironment for embryo implantation and development. The expression of some components of the extracellular matrix and disappearance of others during this period are probably associated with decidual transformation. Previous studies showed extensive modifications of collagen fibrils during decidualization in mice. In these species, an increase of the collagen fibril diameter occurs exclusively in the decidualized areas that surround the implantation sites of the uterus. We observed that in mice pregnant endometrium the proteoglycan decorin is associated with the thin collagen fibrils whereas biglycan is associated with thick collagen fibrils, suggesting that biglycan is involved in the fibrillogenesis of collagen. Curiously, decidualization in rats does not promote thickness of collagen fibrils. In rats the distribution of decorin was quite similar to that observed for mice. In this specie, however, no expression of biglycan was detected in the decidua. These results show that the expression of biglycan in the decidua is species-specific, and biglycan is probably related with the collagen fibrillogenesis in uteri of mice. Considering the present results, we conclude that molecular changes observed during the embryo implantation period, together with the redifferentiation of endometrial fibroblasts into decidual cells, comprise a sequential and coordinate remodelation of proteoglycans and collagen in the endometrial stroma during the embryo implantation. Fellow from FAPESP, Brazil.

oxLDL EFFECTS IN L-ARGININE/NITRIC OXIDE PATHWAY IN T-LYMPHOCYTE AND HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

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Endothelial dysfunction is associated with pathological vascular conditions including high plasma level of low density lipoprotein (LDL) or high D-glucose in atherosclerosis. The oxidatively modified form of low-density lipoprotein (oxLDL) is recognized as a major cause of endothelial dysfunction and it have been associated to an impaired activity of L-arginine transport systems (high and low affinity) and nitric oxide (NO) synthesis. The aim of this research was to determine the effects of oxLDL and D-glucose in the L-arginine/NO pathway in human endothelial cells and T-lymphocyte. **Methods:** Human T-lymphocytes were obtained from peripherical mononuclear cells and endothelial cells from human umbilical vein (HUVEC). The cells were cultured in absence or presence of oxLDL and D-glucose. Kinetic parameters of L-arginine transport and eNOS activity were measured. ROS and SOD activity were determined as oxidative status. **Results:** oxLDL decreased the V_{max} of L-arginine transport, but did not alter K_m apparent. D-glucose (25 mM) increased the maximal transport capacity (V_{max}/K_m) in Lymphocyte and HUVEC. This effect was affected in presence of oxLDL only in the high affinity system for L-arginine transport in Lymphocytes. In parallel, we demonstrated an increase in ROS and reduced eNOS and SOD activity. **Conclusions:** Our results suggest that oxLDL and high D-glucose impaired the L-arginine/NO pathway. These effects could be associated to oxidative stress signals mediated by oxLDL. Supported by DIUC 204074037-1 and FONDECYT 1030781 (Chile).

RECOMBINANT PROTEIN bFGF INDUCES EARLIER EXPRESSION OF DEVELOPMENTAL MORPHOGENES IN RAT ACUTE RENAL FAILURE

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Recovery for acute renal failure requires the replacement of injured cells with new cells to restore tubule epithelial integrity. The morphology of the process have been described, but not the cellular events and its regulation. The expression pattern of nephrogenic proteins such as Vimentin, Ncam, bFGF, Pax-2, BMP-7, Noggin, Lim-2, BMP-7, Engrailed, Smad, p-Smad, HIF-1α, VEGF and Tie-2, have been previously reported after renal damage, in a similar way as observed in normal kidney development. This suggests that renal cells can differentiate in a cellular type similar to metanephric mesenchymal and express morphogenes before differentiating into epithelia. With the hypothesis that renal regeneration is accelerated by bFGF, we studied the expression pattern of these morphogenes in rats with acute renal failure treated with recombinant bFGF. **Methods:** Male SD rats were submitted to 30 min. renal ischemia and injected with bFGF (30 µg/Kg) followed by reperfusion. The rats were sacrificed at 24, 48, 72 and 96 h. after ischemia-reperfusion. The expression of nephrogenic proteins was studied by immunohistochemistry, Western blotting and RT-PCR. **Results:** After ischemia-reperfusion, rats treated with bFGF showed an earlier (12-24 h) local expression of morphogenes than not treated rats. Although the expression of morphogenes was earlier, a similar pattern of distribution of morphogenes was observed in kidneys from treated and non-treated rats. **Conclusions:** These results indicate that the treatment with bFGF can accelerate the regeneration process after acute renal failure. Supported by Fondecyt 3050075 and 1050977.

KINETIC STUDIES AND SITE-DIRECTED MUTAGENESIS OF HUMAN ARGINASE II

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The arginase catalyzes the hydrolysis of L-arginine to L-ornithine. In mammalian tissues contain two distinct isoenzymic forms: arginase I, which is highly expressed in the liver and it has been traditionally associated with ureagenesis, and the extra-hepatica arginase II, which is thought to provide a supply of L-ornithine for proline and polyamine biosynthesis. Particularly interesting has been a possible role of arginase II in regulating the availability of L-arginine for nitric oxide synthesis. The aim of this research was to contribute to a better understanding of arginase II. **Methods:** The arginase II cDNAs were directionally cloned into the pBluescript II K (+) vector and the enzymes were expressed in *E. coli* strain .The mutant forms of human arginase II were obtained, using the QuickChange site-directed mutagenesis kit. The enzyme activities were determined by measuring of urea. **Results:** 53% and 95% of wild-type arginase activity were expressed by fully manganese-activated species of the His120Asn and His145Asn variants, respectively. The K_m for arginine was not altered and the wild-type and mutant enzymes. In contrast, the Asn149Asp mutant expressed almost undetectable activity on arginine, but significant activity on agmatine. After dialysis against EDTA, the His120Asn variant was totally inactive and contained <0.1 Mn^{2+}subunit^{-1}, whereas wild-type and His145Asn enzymes were half active and contained 1.1 ± 0.1 Mn^{2+}/subunit^{-1} and 1.3 ± 0.1 Mn^{2+}/subunit^{-1}, respectively. **Conclusions:** In addition to substantiate the participation of His120 and His145 as ligands for the manganese ions , our results have provided additional evidence for the differences between the active sites of this enzyme and arginase.

PROGRAMMING OF FEMORAL ARTERY ENDOTHELIAL DYSFUNCTION IN ADULT RATS EXPOSED TO CHRONIC INTRAUTERINE HYPOXIA

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Foetal origin hypothesis proposes that stress events that occur during foetal life, such as hypoxia, induce permanent modifications that can be observed later in adulthood and could be involved in chronic diseases such as hypertension. Hypertension is caused, in part, by endothelial dysfunction measured as a low level or absence of nitric oxide (NO) after stimulation with acetylcholine (Ach). The aim of this study was to determine the function of system Ach-NO-Relaxation, as a marker of endothelial dysfunction, in adult rats exposed to hypoxia during the last week of foetal life. Methods: Pregnant rats were exposed to hypoxic condition (Fraction inspired of O_2 = 0.1) for the last week of pregnancy (n=6), and a control group to normoxia (n=6). After delivery all animals were maintained in normoxia until the third month of life, when they where sacrificed and femoral artery resistance (3th branch) was isolated and mounted on small vessel myographs (Multi-Myograph DMT). Concentration response curves (CRC) were performed for KCl, Ach and sodium nitroprusside (SNP). Maximal response (Rmax) were expressed as percentage of KCl and sensitivity (PD_2), values were analysed. Results: Mayor Rmax was observed in hypoxic group in comparison with the normoxic group. In precontracted arteries (60 mM KCl) Ach induced contraction starting at 10^{-10} M. Furthermore, SNP induced contraction since from 10^{-10} M, and Rmax observed was minor than normoxic group. Conclusions: Vasoconstriction induced by Ach and SNP at low concentration (nM) was observed in adult rats whose mothers were exposed to severe hypoxia during the last week of pregnancy. These results suggest that foetal hypoxia induces endothelial dysfunction in adult life. Partially supported by VRA-UDP 10636.

EDUCATIVE INNOVATION IN MEDICAL PHYSIOLOGY

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The School of Medicine at Universidad Catolica del Norte (UCN) adopted progressively from its beginning (2003) the didactic methodology known as mixed Problem-Based Learning (mixed-PBL) in order to stimulate active and participative learning in small groups work (7-12 students). In the mixed-PBL methodology, "pure" PBL is reinforced with traditional lectures and practical experiences. The student-centered mixed-PBL dynamic generates a positive learning climate where students develop more self assurance, confidence and life-long learning by deeply engaging in their studies and keeping their interest high. The highly motivated students develop critical skills to search new valid information, to learn to learn and to teach each other the new information. The students also develop social skills, tolerance, capability to work in group, critical thinking, communicational abilities and in general, attitudes and values, things that could hardly be developed in the traditional (lecture-based) method. The physiology course organized with the mixed-PBL methodology is an example of curricular innovation where the activities are designed to motivate and facilitate in the students several ways of learning (student-centred) and also to ensure the proper traditional teacher lecture-review of the key themes. The physiology course evaluation included: 1) self-evaluation of individual performance in small group work; 2) cross evaluation between classmates of the same group; 3) tutor evaluation of the student work in the small group; 4) weekly individual sumative test; 5) group evaluation during a debate at the end of the course; 6) evaluation of physiology conceptual map; 7) individual final exam. The student evaluation of the physiology mixed-PBL course was positive; they enjoy learning in this way. Our institutional evaluation of this didactic method is also positive. Clearly, engaged students will perform better.

SUB-ACUTE EXPOSURE TO DICHLORVOS FAVORS LONG-TERM POTENTIATION TRHOUGH A MECHANISM THAT INVOLVES □$_7$ NICOTINIC RECEPTORS AND ACYLEPTIDE HYDROLASE INHIBITION

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Dichlorvos (DDVP) is the active molecule of the pro-drug metrifonate used to revert the cognitive deficits associated with Alzheimer disease. A few years ago it has been reported that DDVP inhibits the enzyme acylpeptide hydrolase (ACPH) at lower doses than those necessary for inhibit acetylcholinesterase (AChE) to the same extent. So, the aim of our investigation was to test out the hypothesis that DDVP is involved in the enhancement of synaptic plasticity processes through a mechanism that involves ACPH. **Methods:** We used long-term potentiation (LTP) in rat hippocampal slices as a model of synaptic plasticity. DDVP was applied by injection to the perfusion fluid during 20 min (10 min before and after the stimulation protocol for elicit LTP), yielding a final concentration of 50 □M in the recording chamber. **Results:** DDVP enhances LTP in approximately 100%. This effect was reverted by the □$_7$ nicotinic receptor antagonist methyllicanonitine (MLA). Surprisingly, the enzymatic activity of AChE from slices exposed to DDVP was not affected. Instead, ACPH activity was 50% inhibited by DDVP. **Conclusions:** These results support the notion that ACPH could be a target of relevancy related to synaptic plasticity processes. Supported partially by Fondecyt 1030220.

CLONING AND STUDIES OF THE SEASONAL EXPRESSION OF CARP NUCLEOLIN GENE

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Seasonal acclimatization in the carp (*Cyprinus carpio*) induces a segregation of the nucleolar components, which morphologically reflects a temporary inactivation of ribosomal gene expression in winter-adapted fish. Nucleolin has been proposed as a factor imply in the regulation of ribosomal gene transcription. We hypothesise that nucleolin may be one of the key regulatory protein implicated in the modulation of rRNA synthesis during the acclimatization process in carp. **Methods:** Carp nucleolin gene and cDNA clones were isolated by screening of genomic and liver cDNA libraries from carp. The liver mRNA content was seasonally quantificated by real time PCR. Nucleolin protein levels were detected by Western blot and immunohistochemistry. The interaction of nucleolin with nuclear factors was carried out by co-immunoprecipitation. **Results:** We cloned three cDNA, which encoded nucleolin isoforms and represent the expression of different genes in the carp. The organization of one of these nucleolin genes show that the higher number of acidic regions in the carp proteins results of additional exons. The nucleolin gene encodes the snoRNAs U20, U23 and U82 in its introns. The mRNA content was differentially affected by seasonal acclimatization, where two transcript isoforms were higher in winter-adapted carp. Total protein expression and content of phosphorylated nucleolin were higher in winter. Also a differential interaction with nuclear factors was seasonally observed. **Conclusions:** A higher content and regulated activity by phosphorylation and interaction with nuclear factors involve to nucleolin in the temporary inactivation of ribosomal synthesis in winter-adapted carp. Support: FONDECYT 1970633, MIFAB (Chile).

STIMULATION OF Na^+/H^+ EXCHANGER ACTIVITY IN A COLON CELL LINE BY ARSENIC TRIOXIDE

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The Na^+/H^+ exchanger (NHE) plays an important role in promoting cell proliferation in addition to its function mediating intracellular (pH and salt) homeostasis. Since arsenic trioxide has also been involved in cell proliferation, the aim of this research was to determine whether the effect of arsenic trioxide (AsIII) could be mediated by altering the activity of NHE in the T84 colon derived cell line. **Methods:** Confluent T84 cells cultures on glass slides were used for pH determinations by ratiometric fluorescence microscopy, using BCECF as a probe. Phosphorylated p^{44}/p^{42} MAP kinase and p^{44}/p^{42} MAP kinase were determined by Western blot analysis. **Results:** The basal pH in control cells no exposed to AsIII was 7.19±0.02 (n=9), and the rate of pH recovery after an acid NH_4Cl pulse was 0.13±0.01 (n=9) pH units/min. Cells pretreated with 0.5 mM AsIII for 48 h showed a basal pH of 7.82±0.06 (n=9) and the rate of pH recovery was 0.27±0.02 (n=9) pH units/min. When the cells were incubated in AsIII (0.5 mM) plus genistein (30 µM) the basal pH was 7.27±0.05 (n=6), and the rate of pH recovery was 0.16±0.01 (n=6) pH units/min. The exposure of cells to 0.5 mM As(III) for 48 h resulted in a large increase in phosphorylated p^{42}/p^{44} MAPK. **Conclusions:** Our results suggest that As(III) stimulates the activity of NHE and therefore increase the intracellular pH. Protein tyrosine kinase and ERK pathways could mediate these effects. Supported by PEI-1308-04 University of Antofagasta.

CLONING AND SEASONAL EXPRESSION OF SHORT PROLACTIN RECEPTOR ISOFORMS IN A TELEOST FISH

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Eurythermal fish have evolved compensatory responses to cyclical seasonal changes of the environment. This adaptation requires a neuroendocrine cascade that translates environmental signals to conform the systemic compensation of the organism. In carp (*Cyprinus carpio*) seasonal acclimatization requires signaling by PRL, whose expression is significantly induced in pituitary gland of summer acclimatized fish. We studies if PRL signaling requires one or more forms of its receptor (PRLr) whose expression will respond to summer-winter-summer cycles. **Methods**. Total RNA was isolated from gills of winter-acclimatized carp. Transcript isoforms of the PRLr were cloned by 3'RACE-PCR using gene specific primers derived from carp receptor cDNA. The PRLr contents in membrane protein extracts from kidney, gills and intestine of summer- and winter-acclimatized carp were detected by western blot. **Results**. We cloned two new PRLr transcript isoforms from carp. These transcripts encodes for shortest receptor proteins, cPRLra S_1 and cPRLra S_2, containing intracellular domain extension of 71 and 118 residues, compared to 350 aa into the long form of the receptor in the fish. The newly cloned mRNAs arise from alternative splicing in the carp *PRLRA* gene transcripts. We detected that short forms of the receptor were significantly expressed in osmoregulatory tissues compared to the long forms. Seasonally, the PRLr proteins content was higher in winter-acclimatized carp tissues. **Conclusions.** The seasonal acclimatization requires differential expression of PRL and PRLr isoforms in an ectoterm organism. Supported by DI-UNAB 22-03 and MIFAB (Chile).

REGIONAL CHANGES OF NaKATPase ACTIVITY IN THE BRAIN OF THE HYPOXEMIC LLAMA FETUS

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We have reported evidence of brain hypometabolism as a mechanism to prevent hypoxia–induced neuron damage in the fetal llama (FLL)(*Lama glama*), a species adapted to live in chronic hypoxia. The aim of this study was to compare the ability of different regions of the FLL brain to undergo hypometabolism when exposed to hypoxia. **Methods:** A fetal femoral catheter and a carotid flowmeter were used to measure blood gases and carotid blood flow (CBF), respectively. Four animals were submitted to 24 h of hypoxia to reach hypoxemia (PO_2~12 Torr) while the control group was normoxemic (n=4). CBF (as brain perfusion index) was continuously recorded. NaKATPase activity from brain cortex, mesencephalon and brain stem (as index of metabolic state) was measured at the end of the experiments. **Results:** A consistent increase of CBF from 7 h to 24 h of hypoxemia was detected. We found a decrease of 48% and 39% of NaKATPase activity in the brain cortex and mesencephalon respectively, whilst in the brain stem the enzyme activity remained without significant changes in the hypoxemic FLL. **Conclusions:** These results suggest that brain cortex and mesencephalon from FLL are protected from hypoxemia by hypometabolism, whilst the brain stem seems to preserve its metabolism, probably by a selective increase of its blood flow. FONDECYT 1020599, The Wellcome Trust 072256.

PIASγ REPRESSES NURR1-DEPENDENT TRANSCIPTIONAL ACTIVITY

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Nurr1, a transcription factor belonging to the nuclear receptor superfamily, is essential for the generation of midbrain dopaminergic neurons during embryonic development. Mechanism regulating Nurr1 transcriptional activity is unknown. The aim of this study was to search for regulatory factors of Nurr1 function. **Methods:** yeast-two-hybrid screening of an embryonic mouse library, co-immunoprecipitation and reporter assays in transient cell transfection studies were used to search and validate regulatory cofactors of Nurr1. **Results:** we identified the SUMO-E3 ligase PIASγ, as a Nurr1 interacting partner. Nurr1 has two consensus SUMO domains; replacing lysine 91 by arginine in one of the SUMO domains significantly enhanced Nurr1 transcriptional activity, while replacing lysine 577 by arginine in the second SUMO domain significantly decreased Nurr1 transcriptional activity. Double mutant behaved as the second-SUMO mutant, suggesting a dominant role for this domain in the regulation of Nurr1-dependent transactivation. Co-expression of PIASγ with Nurr1 or the Nurr1-SUMO-mutants resulted in potent repression of transcription. This effect was reverted over-expressing a peptide corresponding to the PIASγ interacting domain of Nurr1. Because PIASγ interacts constitutively with histone deacetylase 1 (HDAC) we tested increasing concentrations of the HDAC inhibitor sodium butyrate. The results showed that the inhibitory effect of PIASγ on Nurr1-dependent transcription is independent of HDAC. **Conclusions:** Our studies indicate that PIASγ repressed the transcriptional activity of Nurr1 independently of the presence of SUMO domains in Nurr1 and of the hystone deacetylases activity. Support by: FONDECYT # 1030496.

ISOLATION OF MICROVASCULAR ENDOTHELIUM FROM HUMAN PLACENTA

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Endothelial cells are morphologically and functionally heterogeneous, with differences between macro- and micro-vessels. The aim of this study was to isolate and culture human placenta microvascular endothelial cells (hPMEC). **Methods:** Human placentas were collected from normal pregnancies (Ethics committee approval was obtained). Placental tissue was minced in Hank´s balanced salt solution containing 100 µg/ml of gentamicin. Samples were digested with 0.25% trypsin (10 min, 37°C) and 0.2 mg/ml collagenase (2 h, 37°C). Digested tissue was resuspended in medium 199 (M199) with 40% sera and filtered (nylon mesh, 55 µm pore size). Cell suspension was cultured (1% gelatin, 37°C, 95% air/5% CO_2) and positive immuno-selection was performed using Dynabeads©CD31 (DYNAL, Norway). hPMEC phenotype was characterized by staining for von Willebrand Factor (vWF; 1:400) and α-smooth muscle actin (1:500), using peroxidase-coupled biotin-straptavidin with di-aminobencidin as substrate. L-Arginine transport (0-1000 µM, 37°C, 1 min) and L-[^{3}H]citrulline formation from L-[^{3}H]arginine (2 µCi/ml, 30 min, 37°C) were measured. **Results:** hPMEC phenotype in culture resembles thin cords. Dynabeads©CD31 treatment was associated with 4-fold enrichment for vWF and 75 ± 8% decrease for α-smooth muscle actin. L-Arginine transport showed an apparent K_m ~200 µM, and L-[^{3}H]citrulline formation was inhibited by N^G-nitro-L-arginine methyl ester. **Conclusions:** We have isolated, purified and cultured hPMEC. These cells take up L-arginine and synthesise nitric oxide. Support: FONDECYT 1030781/1030607/7050030 (Chile). C. Escudero holds a MECESUP-PhD fellowship.

PROINFLAMMATORY CYTOKINES AND CARDIOVASCULAR DAMAGE IN CHRONIC HEMODIALYSIS PATIENTS

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Cardiovascular disease is a major cause of mortality in hemodialysis (HD) patients. Recently, proinflammatory mechanisms mediated by aldosterone have been implicated in the development of cardiovascular damage. We evaluated the relation of the immunological status of HD patients with their cardiovascular function and structure. **Methods:** We investigated 8 chronic HD patients and 8 healthy controls matched for age and sex. The patients were anuric for at least six months, non diabetic. Lymphocyte subpopulations as well as in-vitro proliferation and activation in whole blood were determined by flow cytometry. Expression of IL-1α, IL-6, TNF-α and COX-2 mRNA levels in peripheral blood mononuclear cells (PBMC) was quantitated by real-time RT-PCR. Clinical laboratory tests and cardiac and carotideal echographic measurements were performed in all HD patients. **Results:** We found no significant differences for absolute numbers or percentages in CD3, CD4, CD8, CD19, CD56 lymphocyte subpopulations among HD patients and controls. Similarly, lymphocyte activation and proliferative responses were not altered in HD patients. We detected no significant differences in COX-2 expression or pro-inflammatory cytokines, including IL-1α, IL-6 and TNFα. HD patients presented increased left ventricular mass index and arterial damage, but no correlations were found when comparing immunological variables with serum aldosterone or echocardiographical indexes of left ventricle function, ventricular mass, carotideal wall thickness or plaques. **Conclusions:** In this preliminary study, the cardiovascular damage of HD patients was not correlated with serum aldosterone or changes in the immune function. Supported by FONDECYT 1050265 (Chile).

GM-CSF PARTICIPATION IN MALE GERM CELL SURVIVAL

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Spermatogenesis is a complex process that involves cell proliferation, genomic recombination and differentiation, which implies a drastic reorganization of cell structures to complete the formation of a highly specialized and mobile cell as spermatozoa. Several growth factors and cytokines have been described to be involved in this process, promoting proliferation, differentiation and survival, but their mechanisms have been poorly understood. **Methods:** In this study, the expression of both subunits of the GM-CSF receptor and its ligand was demonstrated by Western blot analysis, inmunocytochemistry and RT-PCR in rat seminiferous epithelium. **Results:** GM-CSFr α partially colocalizes with GLUT3 and caveolin-1 in detergent-resistant membrane domains (DRMDs) isolated from male germ cells. The action of GM-CSF promotes a fast increase of 2-deoxiglucose uptake in these cells. Cholesterol depletion with methyl-beta-cyclodextrin blocks the glucose uptake stimulated by GM-CSF. **Conclusions:** Our results suggest that localization of GM-CSFr in lipid rafts/caveolae and association with caveolin-1 enables this receptor subunit to have a close contact with GLUT3, suggesting an important role of caveolae in glucose uptake signalled by GM-CSF. GM-CSFr forms a complex with downstream signalling molecules like PI-3K/Akt, which are only dissociated upon GM-CSF stimulation. A role for the PI-3K/Akt pathway in GM-CSF-stimulated delay of germ cells apoptosis is suggested by the ability of PI-3K inhibitors to attenuate this process. (Grants: FONDECYT 199-0994, DID-UACh 2000-01)

Contributed Oral Communications

HUMAN ERYTHROCYTE GLYCERALDEHYDE-3P-DEHYDROGENASE MEMBRANE BINDING AND ACTIVITY IN HYPOBARIC HYPOXIA

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Previous studies have shown that erythrocytes under oxidative stress release membranebound glyceraldehyde-3P-dehydrogenase (GPDH) to the cytosol. This has suggested that membrane binding of GPDH modulates NAD+/NADH balance for metHb reduction and to keep glutathion in a reduced state. We investigated the effect of hypobaric hypoxia (HH), known to produce a mild oxidative stress in erythrocytes, in relation to GPDH activity and its membrane binding. **Methods:** Erythrocytes were obtained from blood of subjects Ander HH during 20 min. at an equivalent altitude of 4500m and after the reoxygenation period. *In vitro* hypoxia was reached by submitting erythrocytes to a 11% O2 atmosphere. GPDH membrane binding was assessed by immunoblotting. GPDH activity and carbonyl groups were spectrophotometrically determined. **Results:** HH produced an increase in carbonyl groups of membrane proteins with no decrease of membrane-bound GPDH. Reoxygenation produced a larger increase in carbonyl groups and a decrease of GPDH activity associated to the membrane. It is proposed that this is due to its inactivation by direct oxidation since no release of GPDH to the cytosol was observed. The same results are observed in *in vitro*produced hypoxia. However in the presence of azide, a catalase inhibitor, *in vitro* hypoxia produced a decrease in membrane-bound GPDH. **Conclusions:** We suggest that erythrocytes under HH show a decrease in membrane-associated GPDH activity by a direct oxidative effect rather than by a release of GPDH to the cytosol. Supported by FONDECYT 1030033, DIPUV 10-2003, DIPUCV 125794.

CHANGES IN PLASMATIC PGI_2/TXA_2 RATIO IN MENOPAUSAL WOMEN: RELATIONSHIP TO BLOOD PRESSURE

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In menopausal women (MW) hypostrogenemia has been associated to endothelial dysfunction and arterial hypertension. Since estrogens affect the vascular synthesis of prostacyclin (PGI_2) and thromboxane-A_2 (TXA_2), we investigated whether changes in the plasmatic levels of these prostanoids of antagonist hemodynamic effects, alter PGI_2/TXA_2 ratio in MW compared with normotensive fertile age women (NW). **Methods:** The study was carried out in 10 control women (NW), with normal arterial blood pressure (BP, 109/70 ± 2 mmHg) during the menstrual cycle, and in 20 MW. MW was divided into two groups: 10 normotensive menopausal women (N-MW, BP 124/80 ± 3 mmHg), and 10 MW who developed systemic arterial hypertension (H-MW BP, 160/104 ± 4 mmHg). Blood pressure was measured according to international standard methods. Plasmatic concentration of 6-ketoPGF$_{1\alpha}$ and TXB_2 (stable hydrolysis metabolites of PGI_2 and TXA_2, respectively), were measured by radioimmunoassay (RIA) after extraction of the acidic lipids with cyclohexane/ethylacetate (1/1 v/v). **Results:** a marked increase of plasmatic levels of 6-ketoPGF$_{1\alpha}$ and TXB_2 was observed in MW ($p<0.001$). Moreover, in H-MW, TXB_2 levels were higher than 6-ketoPGF$_{1\alpha}$ and the 6-ketoPGF$_{1\alpha}$/TXB_2 ratio decreased by 56% and 53% compared to NW and N-MW respectively. **Conclusions:** These results support the concept that, the imbalance between the vasodilator-PGI_2 and the vasoconstrictor-TXA_2 in favor to TXA_2, may explain hypertension observed in menopausal women and could be used as a predictive factor for this pathology. Supported by FONDECYT 1277-91 (Chile).

PARAVENTRICULAR-COERULEAR INTERACTIONS: ROLE IN HYPERTENSION INDUCED BY PRENATAL MALNUTRITION IN THE RAT

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Cells of the paraventricular nucleus (PVN) excite locus coeruleus (LC) neurons via corticotrophin releasing hormone (CRH) while LC projections activate PVN neurons via α_1 adrenoceptors, suggesting the existence of reciprocal excitatory PVN-LC connections. Prenatally malnourished rats develop hypertension when adults, which can be associated with increased hypothalamic CRH expression. **Methods:** At day 40 of age, in normal and prenatally malnourished rats, we studied the effect of CRH intra-LC (0.5 µg/0.5 µl) and/or the anti-hypertensive drug prazosin intra-PVN (1.2 µg/0.5 µl) on multiunit activity recorded simultaneously in PVN and LC, as well as on arterial pressure and heart rate. **Results:** (i) Prenatal malnutrition increases neuronal activity, systolic pressure and heart rate, (ii) CRH stimulated LC and PVN neurons and increased systolic pressure and heart rate only in normal rats, (iii) Prazosin decreased LC and PVN neuronal activity, systolic pressure and heart rate only in malnourished rats, (iv) In normal rats, prazosin prevents the stimulatory effect of CRH only in PVN activity; in malnourished rats, prazosin allowed CRH regain its stimulatory effect. **Conclusions:** Results point to the existence of an excitatory PVN-LC closed loop, which is hyperactive in prenatally malnourished rats; this loop could be responsible, at least in part, of hypertension and tachycardia developed by these animals. Supported by FONDECYT 1030626 (Chile).

AN ESTRADIOL METABOLITE ACCELERATES OVUM TRANSPORT IN CYCLIC RATS THROUGH NON–GENOMIC PATHWAYS

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Estradiol accelerates ovum transport (OT) through non-genomic pathways in cyclic rats and through genomic pathways in pregnant rats. Mating decreases mRNA level of Catechol-O-Methyltransferase in the oviduct, an enzyme that generates 2-methoxyestradiol (ME) a metabolite that produces nongenomic actions in other cell systems. We postulate that ME activates non-genomic pathways in cyclic rats and that mating silences these pathways. The objective of this study was to determine 1) The effect of 2-Methoxyestradiol (2ME) on OT 2) the requirement of protein synthesis and estrogen receptor (ER) for 2ME-induced OT acceleration and 3) The effect of ethynylestradiol (EE) administrated via intraoviductal (io) or systemic (sc) injection on OT. **Methods:** 1) Rats on day 1 of cycle (C1) or pregnancy (P1) were injected intrabursally (ib) with 2ME o vehicle. 2) Rats on C1 were injected with 2ME sc ±Actinomycin D or ±ICI 182780 (ER antagonist) or vehicle ib. 3) Rats on C1 or P1 were injected sc or io with vehicle or EE. In all groups, the number of oviductal eggs was recorded 24 hours later. **Results:** 2ME accelerated OT in C1, but not in P1. This effect was dependent of ER, but did not require protein synthesis. EE accelerated OT in C1 and P1, but io was more effective than sc administration only in P1. **Conclusions:** These results suggest local metabolism of estrogens in the oviduct is necessary to accelerate OT through non-genomic pathways in C1. Supported by FONDECYT 1030315, 1040804 (Chile).

REGULATION OF SINGLE RYR CHANNELS FROM BRAIN BY ENDOGENOUS MODULATORS

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Ryanodine receptor (RyR) channels can participate in the amplification of calcium signals, as they are activated by μM increases in cytoplasmic free [Ca^{2+}]. The aim of this research was to determine the calcium dependence of brain RyR-channels at near physiological [ATP] and/or [Mg^{2+}] and after exposure to NO donors. **Methods:** Endoplasmic reticulum vesicles from rat brain cortex were fused with planar bilayers and channel activity was recorded at various cytoplasmic [Ca^{2+}]. **Results:** In the presence of 3 mM [ATP], RyR-channels were activated at lower [Ca^{2+}], reaching a higher plateau of maximal activity and were inhibited at higher [Ca^{2+}]. The change in Ca^{2+} dependence induced by ATP can be interpreted as a decrease in $K_{0.5}$ for calcium activation and an increase in $K_{0.5}$ for calcium inhibition. Addition of 0.5 mM free [ATP] and 0.8 mM free [Mg^{2+}] to the cytoplasmic compartment shifted the Ca^{2+} response curve to higher [Ca^{2+}]. This combined effect of Mg^{2+} and ATP can be explained by an increase in both $K_{0.5}$ for activation and $K_{0.5}$ for inhibition by Ca^{2+}. Addition of the NO donor SNAP increased channel activity primarily decreasing $K_{0.5}$ for activation by Ca^{2+}, both at PO_2 of 10 or 150 mm Hg. **Conclusions:** Our results suggest that RyR-channels can amplify calcium signals at physiological [ATP] and [Mg^{2+}], especially when critical SH residues are modified. Supported by FONDECYT 1040717 & FONDAP 15010006 (Chile).

ANTIIFLAMMATORY (TGF BETA-1/IL-10) AND PROINFLAMMATORY (TNF/ IL-1BETA) CYTOKINES IN MATERNAL COLOSTRUM AND SERUM FROM MOTHERS OF TERM AND PRETERM NEWBORNS

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Breast milk provides the newborn the essential nutrients and gives protection against multiple infectious and non-infectious diseases. Cytokines, small polypeptides secreted by leukocytes and other cells, favor the growth of newborn's organs and systems, including the immune system. In this study we compared the concentrations of anti-inflammatory and proinflammatory cytokines (TGF-β1/ IL-10 and TNF-α / IL-1) in colostrum and serum from mothers of term and pre-term newborn. **Methods:** Concentrations of TGF-β1, IL-1 β, TNF-α and IL-10 in serum and colostrum from 49 mothers (25 preterm and 24 term) were determined by ELISA and Flow Citometry techniques. **Results:** TGF-β1 was significantly higher ($P<0.05$) in colostrum and serum in term than in pre-term mothers. On the other hand, IL-10 and TNF-α were significantly higher ($P<0,001$) in colostrum of preterm than in term newborns, while, they presented similar sera concentrations. Concentrations of IL-1 β in both groups of mothers were similar in serum and colostrums. **Conclusions:** Our results suggest a significant local production of TGF- β1, IL-10, TNF α and IL-1 β, and that cytokine concentrations vary in serum and/or colostrum according to weeks of gestation (preterm and term labour). Elevated concentrations of anti-inflammatory and proinflammatory cytokines are present in colostrum. The differences in their concentrations in serum and colostrum suggest that they are supplied to the newborn through breast milk favoring the idea of biological adaptation of breast milk to the newborn's needs. Supported by CDCH-UC 984-2004.

PLACENTATION IN THE SPINY MOUSE AND THE EFFECT OF DEXAMETHASONE ON PLACENTAL DEVELOPMENT

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Exposure of the pregnant mother to synthetic glucocorticoids alters placental gene expression and leads to hypertension in the adult offspring. The Spiny mouse has a 40 days gestation period. We describe its placenta ontogeny and examine the effects of maternal dexamethasone treatment on placental development. **Methods:** Fetuses and placentas were weighed from days 14-39 of gestation. Each placenta was bisected, half was frozen and half was fixed and processed to paraffin. Mid-sagittal sections were stained and the proportions of labyrinth (exchange) and junctional (germinative) zone quantified. Dexamethasone (D) (150μg/kg for 60hrs, days 20-23 of gestation via mini osmotic pump) and saline (S) treated females (n=5) were killed at days 23 and 37 and placentas processed. **Results:** Placental growth is linear from at least day 14 and occurs predominantly in the junctional zone until day 18 when the labyrinth differentiates and expands until it occupies ~55% of placental volume. Fetal growth increases rapidly after differentiation of the labyrinth. Fetal (day 23 - S, 0.63g±0.04 n=10; D, 0.54g±0.04 n=13, day 37 - S, 5.04g±0.26 n=7; D, 4.88g±0.12 n=10) and placental (day 23 - S, 0.21g±0.01 n=7; D, 0.21g±0.01 n=10, day 37 - S, 0.46g±0.03 n=7; D, 0.50g±0.01 n=10) weights at day 23 or 37 were not significantly different following D treatment, but the fetal to placental weight ratio (S, 2.56±0.16; D, 2.95±0.18; P=0.03) was increased by day 37. **Conclusions:** Our data hint that dexamethasone may alter placental structure and function, affecting the growth and development of the fetus, resulting in permanent physiological changes.

ENDOCRINE CHANGES IN THE HYPOPHYSIS-GONAD AXIS INDUCED BY HYPOBARIC HYPOXIA IN MALE RATS

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The mechanisms that underlie changes in testicular function under hypobaric hypoxia (HH) are not known, and could involve the hypophysis-testicle endocrine status. We have tested the hypothesis that in rats subjected to HH, endocrine (FSH, LH, testosterone) changes related to testicular physiology take place. **Methods:** Male Wistar rats (247 ± 16g) were maintained in normobaric (Nx) or hypobaric conditions (428 torr, 4,600m). At days 0, 5, 15 and 30 days post-exposure, 12 rats were anesthetized with ketamine, and blood was collected in a heparinized syringe to obtain blood plasma. These rats were subsequently sacrificed and the testicles were fixed in 4% formaldehyde and processed for routine histological analysis. **Results:** Our results show that the height of the seminiferous epithelium decreased significantly at day 5 post-hypoxia and thereafter, indicating a decreased spermatogenesis. Consistent with these data, FSH levels rose by day 5 post-exposure. In subsequent days, FSH levels decreased in HH but had a tendency to remain higher than the Nx group. LH plasma levels underwent a decrease in rats exposed to HH. Consistent with the decrease in LH levels, plasma testosterone showed a tendency to decrease in HH rats. **Conclusions:** The integrated analysis of hormonal changes in rats subjected to HH allows us to conclude that LH decreased in HH, while FSH transiently rose under this condition. Although no conclusive changes of plasma testosterone levels can be derived from our data, the effects of HH on spermatogenesis can be in part derived from changes in the hypophysis-gonad hormonal axis.

EXPRESSION PATTERN OF MT1 AND MT2 MELATONIN RECEPTOR ISOFORMS IN RAT ADRENAL

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Binding of the pineal hormone melatonin to its cognate MT1 receptor inhibits ACTH-stimulated cortisol production in the primate adrenal cortex. In the rat adrenal, there is conflicting evidence on the presence of melatonin binding sites as well as on a regulatory role of melatonin on adrenal corticosterone production. This prompted us to investigate the expression pattern of both MT1 and MT2 melatonin receptor isoforms (MT1r and MT2r) in the rat adrenal. **Methods:** Ex-vivo rat adrenals were processed to obtain total RNA samples, membrane protein extracts and histological sections. These samples were subjected to RT-PCR and cDNA sequencing using PCR primers for MT1r and MT2r, and to Western blot and immunocytochemistry using antibodies against human MT1r and MT2r, respectively. **Results:** Expression of the mRNAs encoding for MT1r and MT2r in the rat adrenal was demonstrated by amplification of the predicted PCR fragments, which identity was established by cDNA sequencing. The results of the Western blot analysis indicated the presence of the expected 40/60 kDa MT1r and MT2r polypeptides. Immunocytochemistry revealed that MT1r was expressed in cortical cells from the fasciculata layer and in a small population of cells from the medulla, whereas the MT2r was expressed exclusively in the medulla. **Conclusions:** These findings are the first evidence indicating MT1r and MT2r expression in the rat adrenal, suggesting a direct role of melatonin on adrenal physiology, particularly on corticosterone production. Supported by FONDECYT 1050389 & DID-UACh S-200433 (Chile).

ALTERED LIPID LEVELS IN THE FETO-PLACENTAL UNIT FROM DIABETIC RATS: MODULATORY EFFECTS OF PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR A (PPARA) AGONISTS

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Diabetes induces alterations in maternal lipid metabolism and affects feto-placental development. PPARα is a nuclear receptor involved in lipid homeostasis in different tissues. We aimed to evaluate the levels of triglycerides (TG), cholesterol (CHOL), cholesteryl esthers (ECHOL) and phospholipids (PL) and to determine whether PPARα agonists regulate lipid metabolism in the feto-placental unit from control (C) and neonatal-streptozotocin-induced diabetic rats (D). **Methods:** Fetuses and placenta (day 13.5 of gestation) were incubated for 3 h either with or without LTB_4 (an endogenous PPARα agonist, 0.1 μM) or clofibrate (a pharmacological PPARα agonist, 20 μM). Lipids were evaluated by TLC and densitometry. LTB_4 was measured by EIA. **Results:** In D placenta we found increased TG (50%, $p<0.05$) and ECHOL (93%, $p<0.01$) levels when compared to C. In C placenta, no effects of PPARα agonists on lipid levels were found. In D placenta, LTB_4 reduced CHOL (21%, $p<0.05$) and PL (47%, $p<0.02$) levels and clofibrate reduced levels of all lipids evaluated ($p<0.05$). In D fetuses, there were increased PL levels (138%, $p<0.01$) when compared to C, while CHOL, ECHOL and TG levels remained unaltered. PPARα agonists reduced ($p<0.05$) CHOL, ECHOL and TG levels and did not modify PL levels in C and D fetuses. LTB_4 levels were reduced in D fetuses (58%, $p<0.05$) and placenta (82%, $p<0.01$) when compared to C. **Conclusions:** Our results provide evidence of a novel role of PPARα in the regulation of lipid levels in the feto-placental unit. LTB_4 is reduced in D fetuses and placenta and is likely to be an important regulator of lipid levels in these tissues.

IMBALANCE OF TOXIC AND ESSENTIAL METAL ELEMENTS IN PLACENTAS OF WOMEN DELIVERING LOW BIRTH WEIGHT NEONATES IS NOT RELATED TO OXIDATIVE STRESS

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Balance of nutrition-pollution factors is involved in placental function. The aim of this study was to evaluate essential (Fe, Cu, Zn and Se) and toxic (As, Pb, Cd and Hg) elements in placentas from mothers delivering normal (NBW) and low birth weight neonates (LBW) and relate these concentrations with placental oxidative stress. **Methods:** the study utilized placentas from mothers delivering LBW (1,000-2,500 g) and NBW (> 3,000 g) neonates. Elements were determined by Atomic Absorption Spectrometry (Cu, Fe, Pb and Cd) and Instrumental Neutron Activation Analysis (Se, Zn, As and Hg). Total antioxidant activity (TAS), thiobarbituric acid reactive species (TBARS; a marker of lipoperoxidation), glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were evaluated by spectrophotometric assays. **Results:** concentrations of toxic elements Cd and Pb were significantly higher in placentas associated to LBW neonates. Placental Cd was directly correlated with LBW (r -0.695). Among essential elements, Fe was clearly lower in placentas associated to LBW neonates. Oxidative stress parameters GSH, TBARS, TAS, GPx and SOD were not different in both groups of placentas; only GSH and SOD had a tendency to be elevated in placentas from mothers delivering LBW and NBW respectively. **Conclusions:** Abnormal balance of placental toxic/essential metal elements may be associated to placental dysfunction leading to LBW neonates. However, a correlation between this condition and a deleterious placental redox environment is not clear. Supported by International Atomic Energy Agency (Grant 11960/RO/RBF)

MEDIATORS OF TH1/TH2-TYPE IMMUNE RESPONSE MODULATES MMP-9 PRODUCTION IN HUMAN CYTOTROPHOBLAST CELLS

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Preeclampsia (PE) is the major cause of maternal mortality worldwide and their etiology is not known. PE is characterized by defective trophoblast invasion and incomplete spiral artery remodeling. The immunological origin of such implantation defects characterized by abnormal balance of Th1/Th2 mediators has been suggested as a central mechanism. We investigate the effect of Th1-cytokines (IL-2 e IFN-γ) and Th2 cytokines (IL-4 and IL-10) on the secretion of matrix metalloproteinase-9 (MMP-9) and chorionic gonadotrophin (hCG) by human cytotrophoblast (CTB). **Methods:** CTB cells were isolated from term pregnancy placentas by enzymatic digestion. CTB were cultured 4 days in presence of the cytokines. Amount of MMP-9 and hCG secreted during CTB culture was measured by specific ELISA. **Results:** CTB cells spontaneously differentiated to syncytiotrophoblast after four culture days. Stimulation with IL-2 and IFN-γ inhibited the production and activation of MMP-9, while IL-10 e IL-4 did it only at the final stage of culture. IL-10 diminished the MMP-9 activation but not synthesis. **Conclusions:** The cytokine effect observed on MMP-9 may have a correlation with the event of trophoblast invasion in PE. An immune response mediated by Th-2 mediators influences the production and activation of an invasion marker, which points to a direct effect of this immune arm on trophoblast invasion. A combination of low levels of IL-2 and high levels of IFN-γ, resulted in the maximum inhibition of MMP-9 trophoblasts production. Supported by CONACYT-21117.

ELECTRICAL SIGNALING IN PANCREATIC β-CELLS FROM *PSAMMOMYS OBESUS*, AN ANIMAL MODEL OF TYPE 2 DIABETES: IMPLICATIONS FOR GLUCOSE HOMEOSTASIS

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Glucose-induced insulin secretion from pancreatic islet β-cells depends on membrane depolarization and consequent Ca^{2+} influx. This process has been studied extensively in mouse β-cells, but data from models of diabetes are lacking. The desert gerbil *Psammomys obesus* (fat sand rat) rapidly develops obesity, insulin resistance and type 2 diabetes when fed a high-energy diet. The objective of this work was to determine if sand rat β-cells manifest defects in electrical signaling that could contribute to dietary-induced diabetes. **Methods:** Membrane potential (Vm) was recorded from islet cells of non-diabetic sand rats and mice with microelectrodes, and cytosolic Ca^{2+} ($[Ca^{2+}]c$) was measured by ratiometric imaging. **Results:** We observed three distinct patterns of electrical activity in sand rat islets. Most cells (68%) displayed glucose-dependent Vm oscillations ('bursts'), similar to mouse β-cells but with longer period ($p<0.01$) and lower glucose threshold ($p<0.02$). The second pattern was glucose dependent but non-oscillatory. Third, some cells exhibited a spiking electrical activity that persisted in the absence of glucose. All three patterns were generated by cells expressing ATP-sensitive K^+ channels, as evidenced by sensitivity to diazoxide. In accord with bursting being the predominant electrical pattern, 8 of 8 sand rat islets displayed glucose-dependent slow $[Ca^{2+}]c$ oscillations. **Conclusions:** Electrical responsiveness to glucose is heterogeneous in sand rat islet cells. The relative hypersensitivity to glucose, including glucose-independent activity in some cells, could contribute to dietary-induced hyperinsulinemia in *P. obesus*. Supported by FONDECYT 1050571 (Chile).

MODULATING EFFECT OF CHLORIDE EFFLUX ON hCG-INDUCED PROGESTERONE RELEASE IN PRIMARY CULTURES OF HUMAN GRANULOSA CELLS

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Human chorionic gonadotrophin (hCG) activates steroidogenesis in human luteinized granulosas cells (lGC). In several steroidogenic cellular models the gonadotrophins activate a chloride conductance. Our aim was to determine the effect of extracellular chloride removal and chloride channel inhibitors on progesterone (P_4) release in primary culture of human lGC. **Methods:** Primary cultures of human lGC were incubated for 2 hours with hCG (10 UI/ml) or 25OH-cholesterol (10 μM) in the presence or absence of 17β-estradiol-BSA (5 μM), tamoxifen (10 μM) or DIDS (500 μM). hCG stimulation was also performed in the absence of external chloride by equimolar glutamate replacement for chloride, in the presence or absence of DIDS. The P_4 accumulated in the culture medium was determined by RIA. **Results:** hCG or 25OH-cholesterol increased 2.5-fold P_4 release (basal: 118.6 ± 41.5 ng/ml). Extracellular chloride substitution increased hCG-induced P_4 release, effect that was inhibited by DIDS. hCG-stimulated, but not the 25OH-cholesterol-induced P_4 release, was inhibited by 17β-estradiol-BSA, tamoxifen and DIDS. **Conclusions:** Our results suggest that chloride efflux is necessary for hCG-induced steroidogenesis in primary cultures of human lGC. The absence of effect of Cl^- channel inhibitors on the response to 25OH-cholesterol suggests that these drugs may act upstream of the cholesterol translocation in the mitochondria, the rate limiting step of steroid biosynthesis, controlled by the steroidogenic acute regulatory protein (StAR). Supported by FONDAP 15010006 and MECESUP UVA106.

HYPOXIA INDUCES CALCIUM-DEPENDENT ERKs ACTIVATION IN SKELETAL MUSCLE CELLS

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Skeletal muscle responds to exercise, electrical stimuli, or to hypoxia, with changes in gene expression including structural proteins and energetic metabolism enzymes. Early mechanisms involved in changes on hypoxia-induced gene expression in skeletal muscle remain to be clarified. The response to hypoxia in many cell systems is a complex event involving regulation of multiple signaling pathways and coordinated expression of perhaps hundreds of genes. The aim of this work was to study the possible role of calcium in the early events leading to changes in muscle cells gene expression. **Methods:** Primary cultures of rat skeletal muscle cells were exposed to hypoxic conditions (1-2%O_2) for 10 to 60 min and ERK 1/2 phosphorylation was determined by Western blot analysis. The calcium role was assessed by exposing cells to hypoxia in the absence or presence of external calcium (3mM), or in cells incubated with 10 μM nifedipine, an L-type calcium channel inhibitor, or with 50 μM ryanodine to block intracellular calcium release. **Results:** ERK 1/2 phosphorylation increased threefold after 10-30 min under hypoxic conditions. External calcium absence and ryanodine or nifedipine, partially reduced this activation. Meanwhile, external calcium absence and ryanodine abolished the ERK 1/2 phosphorylation increase. **Conclusions:** Our results suggest that ERK 1/2 phosphorylation induced by hypoxia is totally calcium-dependent. Supported by FONDAP 15010006 and FONDECYT 1030988 (Chile).

EFFECT OF ANTIOXIDANTS IN OVINE PLACENTA AND NEWBORN WEIGHT IN PREGNANCIES UNDER NATURAL HYPOXIA: COMPARISON BETWEEN LONG AND SHORT-TIME RESIDENCES AT HIGH ALTITUDE

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Pregnancy at high altitude (HA) results in intrauterine growth retardation, associated with changes in placental morphology. Adverse effects of HA might be associated with oxidative stress. Our aim was to compare the preventive action of antioxidants on newborn body weight and placental traits between pregnant ewes with long- and short-time residence at HA. **Methods:** The study was performed at INCAS (3580 m.a.s.l.). Twenty pregnant singleton ewes were natives to HA (group HH) and twenty to low altitude (LA; 500 m.a.s.l.), but moved to HA for mating and gestation (group LH). Ten ewes of each group were daily treated with vitamins C (500 mg) and E (350 IU) during mating and pregnancy (group +Vit). After parturition, newborns and placentas were weighed. Cotyledons count, cotyledons diameter, and estimation of the surface area of cotyledon-caruncule contact were obtained. Cotyledons were processed for conventional histology to measure the cotyledon area occupied by the vasculature. **Results:** Newborn body weights in the LH group were lower than those observed in vitamin-treated animals which were the highest. HH ewes showed less cotyledons number, greatest surface of cotyledon-caruncle contact, and a trend to greater placental weight and cotyledon area occupied by vasculature. **Conclusions:** Antioxidants administration in pregnant ewes diminished the effect of HA on newborn body weight and placental morphological traits. Despite, HH ewes maintained best pregnancy outcomes due to adaptation to HA. Supported by FONDECYT 1020706 (Chile).

A CRITICAL ANALYSIS OF THE VASCULAR RELATIONSHIP BETWEEN THE HUMAN EMBRYO AND ITS YOLK SAC

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Early embryo survival depends of nutrients that are transported from the yolk sac (YS) through the vitelline stalk. The mechanism for this transport has not been clearly defined in human. There are no clear data about the human YS vascular organization and its interrelationship with the embryo vascular system. The presence of an arterial and venous circulation in the human YS wall before week 7 of embryonic development is generally accepted. However, at present there is no confident experimental evidence to sustain such assumption. Hence, the main objective of this work was to investigate the structure of human vitelline stalk through the embryonic period. **Methods:** Five normal YS from embryos (4th - 8th weeks) collected from salpingectomies performed due to tubal ectopic pregnancies were analyzed. The YS and its vitelline stalk were removed, fixed in glutaraldehyde and treated according to conventional procedure for light and scanning electron microscopy. Serial epon semi thin section were also prepared and stained with toluidine blue. **Results:** The vitelline stalk in 4 week embryos has a vitelline duct that communicates the YS cavity with the intestinal cavity. This communication was no more present in vitelline stalk after the end of 5th week. Two vitelline vessels were observed in the vitelline stalk closer to the YS, but in that portion close to the embryo body only a single blood vessel was observed. These structures showed a very dynamic pattern through these development weeks. **Conclusion:** We conclude that only a single blood vessel moves through the human yolk stalk after the end of week 5 of development. This finding is in contradiction with what has been supported since many decades. Supported by DICYT (Chile).

EFFECTS OF CHOLINERGIC AGENTS ON CAROTID CHEMOREFLEXES

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We studied the effects of nicotinic agonists (ACh, nicotine, epibatidine) on carotid body (CB) chemosensory discharges and chemoreflexes in control conditions and after cholinergic block by mecamylamine (Mec), dihydro-β-erithroidine (DH□E) and methyllycaconitine (MLA). **Methods.** In pentobarbitone-anesthetized adult cats, the frequency of chemosensory discharges (f_χ) was recorded from one carotid nerve; mean arterial pressure (Pā), from a cannulated femoral artery; heart frequency (f_H), from an ECG; tidal volume (V_T) and respiratory frequency (f_R), from pneumotachography. Intravenous (iv) and intracarotid (ic) injections were given via catheters in saphenous vein and thyroid artery, respectively. **Results:** ACh, nicotine and epibatidine given ic produced fast and transient increases in f_χ (ED_{50}'s: epibatidine << ACh ~ nicotine) or reflex increases in V_T, f_R, Pā and f_H if the ipsilateral carotid nerve was intact. Mecamylamine strongly and extendedly blocked nicotinic effects, while MLA and DH□E partially and reversibly blocked such effects; all three initially increased basal f_χ. No effect was observed on chemosensory excitation or ventilatory chemoreflexes elicited by hypoxic hypoxia or iv injections of cyanide, but cardiovascular chemoreflexes were suppressed by efferent ganglionic block. **Conclusions:** Epibatidine >> nicotine ~ ACh excite chemosensory activity; mecamylamine >> MLA > DH□E block nicotinic excitation of CB, more effectively than in autonomic ganglia. None of these antagonists blocks hypoxia-induced chemoexcitation or ventilatory chemoreflexes, while cardiovascular reflexes are blocked at ganglionic level. Work supported by FONDECYT 1010951.

INCREASED IGF-II EXPRESSION AND MMP-2 ACTIVITY IN HYDATIDIFORM MOLE IN COMPARISON WITH FIRST TRIMESTER HUMAN PLACENTA

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Optimal placental growth and function are determined, in part, by the extent to which extravillous trophoblast (EVT) invades the uterus and remodels its vasculature to establish an adequate exchange of key molecules between the maternal and fetal circulations. EVT cell migration is stimulated by insulin-like growth factor-II (IGF-II) although the involvement of IGF-II receptors in that effect has not been elucidated. Hydatidiform mole (HM) is a form of gestational trophoblast disease characterized by an abnormal proliferation and invasiveness of trophoblast cells. The aim of this study was to understand the involvement of IGF-II and the metalloproteinase-2 (MMP-2) in the pathogenesis of HM**. Methods:** Ten patients with HM (gestational weeks 8 through 20) with clinical diagnosis and high serum gonadotropin (hCG) levels and five patients with spontaneous abortion (SA) (weeks 8 through 12) were analyzed. Tissue mRNA levels of the different genes were analyzed by RT-PCR and expression was normalized to GAPDH as housekeeping gene. MMP-2 activity was measured by zymography in gelatin-containing SDS-PAGE. **Results:** IGF-II mRNA expression was higher in HM in comparison with SA, whereas IGF-II receptor levels were reduced in HM. MMP-2 transcription levels showed no difference between mole and abortion but at the protein level the enzyme activity was higher in HM. **Conclusions:** These results suggest that the elevated IGF-II expression in hydatidiform mole might be associated with increased generation of mature forms of MMP-2, an effect that may not involve IGF-II receptors. This study was supported by Colciencias (Colombia).

PLATELET-DERIVED GROWTH FACTOR (PDGF) INCREASES INTRACELLULAR ROS BY NAD(P)H OXIDASE ACTIVATION IN ENDOTHELIAL CELLS

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Intracellular production of reactive oxygen species (ROS) in cells stimulated with growth factors has been widely demonstrated. However, the mechanism(s) by which PDGF increases intracellular ROS in endothelial cells remains poorly understood. The aim of this study was to investigate the mechanism(s) of PDGF-induced intracellular ROS production. **Methods:** Cells from the HUVEC-derived cell line EA hy926 were grown at 37ºC in DMEM supplemented with 10% FBS, 2 mM L-glutamine, 50 U/ml penicillin-streptomycin, and 2.5 µg/ml amphotericin medium. Detection of ROS was measured by DCF fluorescence. Cells were incubated with 10 µM DCFH-DA for 30 min at room temperature and then exposed to 1-30 ng/ml of PDGF or buffer alone for 20 min. **Results: E**ndothelial cells exposed to PDGF increased intracellular DCF fluorescence (1.5 to 1.9-fold increase). This effect was completely inhibited by the PLC inhibitor U73122 (5 µM), PI3-K inhibitor Ly 204002 (10 µM) and the generic PKC inhibitors BIM-I (0.5 µM) and chelerythrine (1 µM). Furthermore, selective inhibitors of conventional and novel PKCs (Gö 9676, 10 µM and rottlerin 10 µM, respectively) produced the same inhibition as the one observed with the generic PKC inhibitors. On the other hand, cells pre-treated with the Akt specific inhibitor NL-71 (50 µM) or the inactive PLC inhibitor U73323 (5 µM) did not increase DCF fluorescence. Also, the NAD(P)H oxidase inhibitors DPI (5 µM) and apocynin (100µM) inhibited DCF fluorescence. **Conclusions:** Our results suggest that PDGF-induced ROS production is mediated by NAD(P)H oxidase activation, by a mechanism dependent on PLC, PI3-K and PKC (conventional and novel) and Akt independent. Supported by FONDAP 15010006.

THE CAPUCHIN MONKEY ADRENAL HAS AN INTRINSIC CAPACITY TO OSCILLATE IN VIVO

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In primates, plasma cortisol shows a circadian rhythm characterized by higher concentrations during early morning. A component of this rhythm is the circadian rhythm of plasma ACTH. A second component may be an ACTH independent intrinsic oscillatory capacity of the adrenal, suggested by the circadian expression of clock genes in this gland (peripheral oscillator). We investigated the presence of circadian rhythms of plasma cortisol in the absence of ACTH and in response to exogenous ACTH. **Methods:** Five animals (2.2 ± 0.2 years) were treated with dexamethasone (Dex 5 mg, i.m.) to inhibit the endogenous ACTH. Blood samples were drawn at -15, 0, 30, 60 and 120 min post ACTH 1-24 (cortrosyn, 125 µg/kg weight, i.v.) at 08, 14, 20 and 02 h. Cortisol was measured by RIA. **Results:** Dex lowered plasma cortisol to 0.2% of normal values, but values at 08 were higher than at the other hours. The response to ACTH was higher at 08 than at 14 h. (1.8 ± 0.3 vs 1.3 ± 0.3 µg/ml x 120 min; area under the curve, $P<0.05$ ANOVA). **Conclusions:** The maintenance of a circadian rhythm of plasma cortisol in absence of ACTH plus the observation of a circadian response to exogenous ACTH support the hypothesis that the primate adrenal gland is a peripheral oscillator. FONDECYT 1050833, MECESUP PUC-0211.

EVALUATION OF HISTOPATHOLOGY CRITERIA, DNA POLIMORPHISM AND GENE EXPRESSION FOR THE DIAGNOSIS OF GESTATIONAL TROPHOBLASTIC DISEASE

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Complete and partial hydatidiform moles are two types of abnormal pregnancies originated in a failure of the fertilization process. Complete mole has a diploid set of chromosomes, both of paternal origin and with a high progression toward persistent illness or malignancy. Partial mole has a triploid set of chromosomes with two of them of paternal origin and shows less frequency of persistence or malignancy. Not always is possible to find the typical morphological characteristics classically described, that allow the distinction between the two types of mole as well as the non molar hydropic abortion. The present work was performed to establish the accuracy of genetic polymorphisms and gene expression, beyond clinical and histological methods, in the diagnosis of mole. **Methods:** Genetic polymorphisms test were carried out in abortion samples of 100 women as well as in blood samples of the parents with the purpose of establishing the parental origin in each case. Pathologic analysis and expression of the genes Igf1, and igf2, was measured too. Statistical concordance among pathologists, among geneticists and between both groups of observers was determined. **Results:** The concordance between pathologists for diagnosis of mole was 56%, and between geneticists was 86%. **Conclusions** We suggest that the clinical diagnosis of mole begin to be confirmed using a strategy that combines the pathologic studies, STR polymorphisms and expression of the gene igf-2, in a sequential way according to the resolution level in each individual situation. Sponsored by COLCIENCIAS (Colombia).

LEPTIN MODULATES LIPID METABOLISM IN HUMAN TERM PLACENTA

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Leptin has significant effects on appetite, energy expenditure, lipid mobilization and reproduction. Leptin and its receptor are expressed in human placenta suggesting autocrine/paracrine functions of this hormone. The aim of the present study was to evaluate the role of leptin in lipid metabolism in human placenta. **Methods:** Human placental explants were obtained after delivery and incubated for 3 h either with or without leptin additions (1-30 nM). Lipid levels were assessed by thin layer chromatography and revealed with iodine. Lipid synthesis was evaluated by ^{14}C-acetate incorporation on the distinct lipid species. Lipid catabolism was analyzed by quantification of glycerol release. **Results:** Leptin diminished triglycerides (51%, $p<0.01$) and cholesterol placental levels (26%, $p<0.001$), but did not modify the cholesteryl esters and phospholipids levels. Leptin additions did not modify incorporation of ^{14}C-acetate to any of the lipids evaluated. When we tested leptin effects on placental lipid catabolism we found that leptin increased glycerol release (93%, $p<0.01$). **Conclusions:** In human placenta, leptin decreases in lipid mass may be due to a stimulatory effect on lipid catabolism but not to modulation of the *de novo* lipid synthesis. These findings provide evidence of a role of leptin in lipid metabolism, a placental function that may condition the transfer of nutrients to the developing fetus.

ERYTHROCYTES BAND 3 ANION TRANSPORT IS INHIBITED BY PEROXYNITRITE

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It has been reported that erythrocytes exposure to peroxynitrite anion ($ONOO^-$) results in a nitrated, crosslinked and phosphorylated anion exchanger band 3 protein. However, it has not been evaluated how these modifications affect its anion transport capacity. Due to the importance of erythrocyte anion transport in O_2 delivery to tissues we have characterized its alteration when cells are exposed to $^{\cdot}ONOO$. **Methods:** Erythrocytes (Hto: 1%) were exposed to $ONOO^-$ at 25ºC for 3 min. Sulphate influx kinetics was evaluated by measuring intracellular sulphate at different times up to 2h at 25ºC. Tyr-nitration, Tyr-phosphorylation and crosslinking of band 3 protein were evaluated by immunoblotting. **Results:** Sulphate influx rate constant was decreased by 14%, 61% and 74% in erythrocytes exposed to 100µM, 200µM and 300µM $ONOO^-$ respectively (n=3). No sulphate influx was observed in the presence of DIDS (n=6). $ONOO^-$ decomposed products account for less than 10% of anion transport inhibition. Tyr-nitration, Tyr-phosphorylation and crosslinking of band 3 protein were concentration dependent (n=3) and its importance is discussed. **Conclusions:** $ONOO^-$ inhibits anion transport, an effect that is probably taking place at physiological conditions. Inhibition is produced by $ONOO^-$ and not by its decomposition products. Anion transport inhibition is located at band 3 protein. Supported by FONDECYT 1030033, DIPUV 10-2003, DIPUCV 125.794 (Chile).

Poster Communications (Incorporations SCHCF)

THE COP9 SIGNALOSOME OF *TRYPANOSOMA CRUZI*

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T.cruzi is the causative agent of Chagas´disease, a major public health problem in many countries of South America. A crucial step after invasion is the cell differentiation from trypomastigotes to amastigote. We have previously reported that cell differentiation is proteasome and phosphatase 2A dependent. In eukaryotic cell, COP9 signalosome (CSN) is known as interphasic component between proteolysis and cell signaling, but its presence and function are not known in parasites. The aim of this research was identify the presence and biological role of CSN in *T.cruzi*. **Methods:** Firstly we purify CSN by FPLC chromatography. 20S Proteasome was immunoprecipited using rabbit polyclonal antibody. The immunoprecipitate was electrophoresed and transferred to PVDF. Immunoblot was development using polyclonal antibodies raised against CSN2 and CSN3 subunits. In addition CSN5 was cloned by PCR using degenerate primers. We also studied the effect of curcumin (CSN inhibitor) on cell proliferation and differentiation. **Results:** CSN displayed a protein pattern that resembles the observed in complex isolated from other eukaryotic cell. In addition, proteasome immunoprecipitated were recognized by anti-CSN antibodies suggesting that CSN coprecipited with proteasome. Cell proliferation was strongly inhibited by curcumin. On the other hand, transformation from trypomastigote to amastigote was blocked by curcumin at concentration of 5 µM. Finally, CSN5 was cloned and the predicted protein displayed a 42% of identity with human, mouse, frog and rat CSN5. **Conclusions:** COP9 signalosome is present in *T. cruzi* and appears to be involved in cell proliferation and cell differentiation. Supported by FUNDACIÓN ANDES C-13955/17 & FONDECYT 1051045.

CARDIOVASCULAR RESPONSES TO ACUTE HYPOXEMIA IN LOW ALTITUDE (LA) AND HIGH ALTITUDE (HA) FETAL SHEEP (FS)

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Chronic hypoxia during pregnancy is one of the major causes of fetal and neonatal mortality and morbidity. We hypothesized that due to changes in the vascular reactivity produced by chronic hypoxia, basal (B) and acute hypoxemic (H) blood flows are different between high altitude (HA) fetal sheep (FS) and low altitude (LA) FS. **Methods:** Under general anesthesia, 6 LAFS (580 m) and 5 HAFS (3,580 m) were chronically instrumented with vascular catheters and transonic flowprobes. Four days after surgery, the fetuses were submitted to 3 h protocol: 1 h B, 1 h of H (PaO_2 12 ± 1 mmHg) and 1 h of recovery (R). We measured heart rate (HR), systemic arterial pressure, carotid (CBF), femoral blood flow and organs blood flow with fluorescent microspheres. **Results:** HAFS showed higher HR in B and H when compared to LAFS, which could be produced by enhanced beta-adrenergic receptor density in the heart. Furthermore, CBF increased during B and R in HAFS differing from LAFS. In addition, HAFS did not reduce kidneys blood flow as LAFS did during H, suggesting a blunted vasoconstrictor activity in this vascular bed. **Conclusions:** These data shows a distinct cardiovascular response to hypoxia in the late gestation fetal sheep, which have undergone gestation at high altitude. Whether these changes are adaptative or a sign of malfunction remain to be elucidated. The Wellcome Trust CRIG 072256 (UK).

HIGH D-GLUCOSE REDUCES PROMOTER ACTIVITY OF HUMAN EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Reduction of adenosine uptake by human equilibrative membrane transporters 1 (hENT1) in human umbilical vein endothelial cells (HUVEC) from gestational diabetes, or in HUVEC from normal pregnancies exposed to high extracellular D-glucose, is associated with reduced hENT1 mRNA expression. We studied the effect of high D-glucose on the transcriptional activity of the promoter region of *SLC29A1* gene (for hENT1) in HUVEC. **Methods:** Cells were isolated and cultured in medium 199 (Ethics committee approval and informed patient consent were obtained). Fragments of *SLC29A1* promoter (-3100, -2056, -1016 and -697 bp from ATG) were subcloned in pGL3 vector, upstream *firefly* luciferase reporter gene. Cells were co-transfected with hENT1-promoter constructs and pRL-TK vector by electroporation (320 V, 20 ms) and exposed to 5 or 25 mM D-glucose (24 hrs). **Results:** *firefly/renilla* luciferase activity was similar in all constructs transfected in 5 mM D-glucose. However, 25 mM D-glucose was associated with reduced transcriptional activity of sequences -697 to -1016 bp and -2056 to -3100 bp. **Conclusions:** These results suggest that the reduced hENT1 mRNA level detected in HUVEC exposed to high D-glucose could result from altered transcriptional activity of *SLC29A1* promoter, most likely related to activation of repressor sequences of this gene. Supported by FONDECYT 1030781/1030607/7050030. M Farías holds Faculty of Medicine- and CONICYT-PhD fellowships.

INSULIN INHIBITS THE STIMULATORY EFFECT OF D-GLUCOSE ON L-ARGININE TRANSPORT IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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L-Arginine transport is mainly mediated by y^+/hCAT-1 membrane transporters in Human Umbilical Vein Endothelial Cells (HUVEC). Incubation of HUVEC with D-glucose (25 mM, 24 h) or insulin (0.1 nM, 8 h) increases L-arginine transport and nitric oxide (NO) synthesis. We determined whether insulin alters high D-glucose effect on L-arginine transport in HUVEC. **Methods:** Cells were isolated (0.2 mg/ml collagenase)(Ethics Committee approval and informed patient consent were obtained) and cultured in medium 199 with 20% sera. L-[^{3}H]Arginine transport (0-1000 µM, 2 µCi/ml, 37°C, 1 min) was measured in presence or absence of D-glucose (25 mM, 24 h) and/or insulin (0.001-10 nM, 8 h). **Results:** D-Glucose increases the maximal velocity (V_{max}) of L-arginine transport from 3.9 ± 0.9 to 10.1 ± 4.3 pmol/µg protein/min. This effect of D-glucose was blocked by insulin with an EC_{50} = 0.15 ± 0.04 nM, reaching a maximal inhibition at 1 nM insulin (V_{max} = 3.1 ± 1.8 pmol/µg protein/min). Apparent K_m was not significantly altered by insulin, and absolute values were in the range of L-arginine transport mediated by system y^+/hCAT-1. **Conclusions:** We propose that insulin is a physiological modulator of the effect of high D-glucose on L-arginine transport in HUVEC. We also suggest that insulin effect is mainly, if not completely, mediated by activation of insulin receptors (activated by <1 nM insulin) rather than insulin-like growth factor receptors (activated by >1 nM insulin) as described in other endothelia. Supported by FONDECYT 1030781/1030607/7050030 (Chile). M González holds a CONICYT PhD-fellowship.

MAXI CHLORIDE CHANNEL IS A CANDIDATE FOR APICAL ATP RELEASE IN HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

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Recently it has been demonstrated that extracellular ATP plays a fundamental role as a paracrine or autocrine messenger in epithelial cells. In trophoblast cells ATP stimulates a Ca^{2+}-activated K^+ efflux suggesting a role in cell volume regulation. Furthermore, syncytiotrophoblast microvillous membrane (MVM) responds to a hyposmotic stimulus by activating both K^+ and DIDS-sensitive anion conductances. The electrogenic translocation of ATP via Maxi Chloride Channel has been suggested as one possible mechanism of its release during regulation of volume decrease in mammary and macula densa cells. We have reported the properties of a DIDS-sensitive Maxi Cl^- Channel of syncytiotrophoblast. The purpose of this work was to demonstrate that this channel could be an ATP conductive pathway in syncytiotrophoblast. **Methods:** MVM was purified and reconstituted in giant liposomes suitable for patch clamp technique. Single channels were recorded from high resistance seals of excised patches. **Results:** Typical biophysical properties from Maxi Cl^- Channel were detected in symmetrical Cl^- solutions. Replacement of Cl^- by 100 mM ATP in the bath solution shifted reversal potential from 0 mV to ~ -17 mV. The relative permeability ratio for ATP over Cl^- was 0.05 ± 0.006 (mean ± S.E.M.; n=4). Lower ATP concentrations blocked chloride currents with a Kd of 11.4 ± 1.8 (n=3). **Conclusions:** We demonstrate that the apical membrane Maxi Cl^- Channel is permeable to ATP. These results suggest that this channel is a candidate for ATP release involved in volume regulation of human placental syncytiotrophoblast. Supported by FONDECYT 1040546, Beca AT-403031 CONICYT and Beca PG/48/2004 U. de Chile. (Chile).

CARBON MONOXIDE (CO): A KEY VASODILATOR IN THE PULMONARY CIRCULATION IN THE LLAMA NEONATE

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Hemoxygenase 1 (HO-1) and 2 (HO-2) produces CO in the arteries, a vasodilator operating through the activation of sGC and potassium channels. The newborn llama (NBLL), a highland species does not develop pulmonary hypertension (PHT) in the *altiplano*, while newborn sheep (NBSH) does. We hypothesize that CO plays a key role in avoiding PHT, therefore we study the CO production and HOs protein expression by the lung in NBLL and NBSH at high (HA) and low altitude (LA). **Methods:** Under ketamine anesthesia (15 mg/kg i.m.) 10 LA NBSH (580m), 6 HA NBSH (3600 m), 7 LA NBLL (580 m) and 6 HA NBLL (3600m), between 8-12 days old, were catheterized in femoral artery and pulmonary artery (PA) (Swan Ganz). We measured PA pressure (PAP), CO content in PA and aorta, cardiac output and calculated the CO production by the lung. We determined lung HO-1 and HO-2 expression by Western blot. **Results:** The NBLL showed a higher lung CO production than NBSH. Accordingly, HO-1 and HO-2 expression in the HA NBLL was increased, whilst PAP was the same than LA NBLL. In contrast, HA NBSH presented a higher PAP than the rest of the groups, with a lower HO-1 expression than LA NBSH. **Conclusions:** The NBLL has a highly developed HO-CO system as a key mechanism to compensate pulmonary hypoxic vasoconstriction. FONDECYT 1050479(Chile); The Wellcome Trust CRIG 072256(UK).

POSSIBLE ROLE OF CATIONIC CONDUCTANCES IN *TRYPANOSOMA CRUZI* APOPTOTIC PROCESS

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Ionic conductances have a significant role in apoptosis induction. Changes in K^+ homeostasis facilitate protease and nuclease activation. *Trypanosoma cruzi*, the agent of Chagas' disease, is a protozoan with a digenetic life cycle that develops in two hosts; hematophagous insects and mammals. The aim of this work was describe apoptosis in *T. cruzi* and establish if cationic conductance activation is a crucial event in this process. **Methods:** We have evaluated morphological and biochemical changes in epimastigotes, induced by natural and synthetic drugs. Caspases activation was detected by western blot and specific substrate cleavage. TUNEL and agarose gels were used to detect DNA fragmentation. Also, we have isolated plasma membranes of *T. cruzi* epimastigotes. The purified membranes were reconstituted in giant liposomes and registered by patch-clamp. **Results:** Death process present typical features of apoptosis, like cystein-protease activation, PS exposure and DNA fragmentation. We have identified two cationic conductances, the more frequent channel showed a non-linear current-potential relationship. Unitary conductance was 65±4 pS (n=12) and 45±1.9 pS (n=12) for positive and negative potentials respectively. The channel was slightly more permeable to potassium than sodium and the permeability sequence was $NH_4^+ \cong Cs^+ > K^+ > Na^+ > Li^+$. **Conclusions:** We demonstrated that *T. cruzi* share evidences of apoptotic death. On the other hand, this is the first electrophysiological evidence of single channels activity in this parasite. Cationic channel described could correspond to efflux pathways responsible for ionic unbalance in apoptosis induction, suggested in other trypanosomatids. Support: Anillo ACT 29, FONDECYT 1040546 (Chile), SIDA/SAREC Network.

MELATONIN BLOCKS NORADRENALINE-INDUCED VASOCONSTRICTION IN MIDDLE CEREBRAL ARTERY IN LOWLAND FETAL SHEEP

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In adults, melatonin (Mel) is considered to be a modulator of the vascular reactivity, acting either as vasoconstrictor or vasodilator. Maternal Mel crosses the placenta, but its effects on fetal vascular reactivity are not known. We hypothesized that Mel reduces the noradrenaline-induced vasoconstriction in middle cerebral artery (MCA) from sheep fetuses. **Methods:** Four lowland (580 m) sheep fetuses at 0.85 gestation, were delivered by cesarean section, anesthetized with sodium thiopentone and euthanized with saturated KCl. Two mm of MCA were dissected and mounted in a myography bath. Cumulative dose-responses curve were obtained using: (1) K^+ from 6 to 125 mM; (2) noradrenaline (NA) from 10^{-10} to 10^{-3} M; (3) 10 pM Mel 30 min before NA as in 2; and (4) 1 µM luzindol (luz)(antagonist of Mel receptor MT1 and MT2) with 10 pM Mel before NA. **Results:** NA induced a vasoconstriction of 113% respect maximum K^+-induced response (K^+_{max}), but this response decreased to 7.62 % of K^+_{max} after 10 pM Mel preincubation. However Mel itself did not change K^+ response. Furthermore, Mel effect was not reverted by preincubation with luz. **Conclusions:** Mel blocks the adrenergic vascular response in MCA and this response is not mediated by the known Mel receptors. We speculate that melatonin could play a key role in blood flow redistribution during fetal stress. Wellcome Trust CRIG 072256, UK.

CHRONIC HYPOXIA INDUCES CHANGES IN NITRIC OXIDE SYNTHASE PROTEINS LEVELS IN CAROTID BODY CULTURED CELLS

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Chronic hypoxia enlarges the carotid body (CB) and increases its chemosensory response to hypoxia. Considering the time required by hypoxia to produce morphological and functional modifications in the CB, changes in the pattern of gene expression are to be expected. Our previous results showed that chronic hypoxia modifies the expression of nitric oxide synthase (NOS) genes in CB cells. However, less is known about the effect of chronic hypoxia on the protein levels of NOS isoforms in CB cells. Therefore, we studied the protein levels of the endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) NOS isoforms in cultured CBs maintained in hypoxic conditions. **Methods:** CB cells obtained from anesthetized male rats were cultured in normoxic (21% O_2) or hypoxic (O_2 < 2%) conditions for one week. Proteins were extracted from normoxic and hypoxic cultured CB cells and their levels were measured using SDS-PAGE Western Blot method. **Results:** Exposure to chronic hypoxia increases eNOS and iNOS protein levels, whereas the level of nNOS decreased. **Conclusions:** Present results indicate that NOS isoforms changes theirs protein level to adjust to the lower and prolonged oxygen deficit in cultured CB cells. Our results agree and extended our previous findings showing an increase in the eNOS and iNOS gene expression in the whole rat CB, as well in cultured cells. Supported by FONDECYT 1030330 and DIPUC.

SHORT-, BUT NOT LONG-TERM STIMULATION OF L-ARGININE TRANSPORT BY D-GLUCOSE INVOLVES TGF-β1 IN HUMAN FOETAL ENDOTHELIUM

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Human umbilical vein endothelial cells (HUVEC) exposed to high D-glucose show increased L-arginine transport. High D-glucose is also associated with increased release of Transforming Growth Factor β1 (TGF-β1). We here studied the role of TGF-β1 on D-glucose stimulated L-arginine transport. **Methods:** HUVEC from normal pregnancies were exposed to 5 or 25 mM D-glucose in absence or presence of TGF-β1 (0.01-10 ng/ml, 0-24 h). L-[^{3}H]Arginine transport (15-1000 μM) was measured and human Cationic Amino acid Transporter 1 (hCAT-1) mRNA was quantified by real time PCR. TGF-β1 extracellular level was measured by ELISA. Phosphorylated and total p42/44^{mapk} protein level was determined by Western blot. **Results:** L-Arginine transport was increased by TGF-β1 [half-maximal effect = 0.28 ng/ml] between 1-6 h, and by 25 mM D-glucose, or 25 mM D-glucose + TGF-β1 between 1-24 h. D-Glucose and TGF-β1 effect on L-arginine transport was associated with higher maximal velocity (1.5 to 2.9-fold), without significant changes in the apparent K_m. hCAT-1 mRNA number of copies was also increased (~8-fold) by D-Glucose and TGF-β1. Active TGF-β1 level was increased at 6 h, but was decreased at 24 h by 25 mM D-glucose. Phosphorylation of p42/44^{mapk} was increased by 25 mM D-glucose (6 and 24 h), and TGF-β1 (6 h). **Conclusions:** High D-glucose stimulation of L-arginine transport at 6 h could result from a mechanism involving TGF-β1 and p42/44^{mapk}. However, long-term effect of D-glucose (24 h) seems to be mediated by a TGF-β1 independent mechanism. Supported by FONDECYT 1030781/1030607/7050030 (Chile). R. Vásquez holds a DIPUC-PhD fellowship.

ENDOTHELIN-1 CONTRIBUTES TO THE ENHANCED CAROTID BODY CHEMOSENSORY RESPONSES INDUCED BY CHRONIC INTERMITTENT HYPOXIA

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Chronic intermittent hypoxia (CIH) enhances reflex cardiorespiratory responses to acute hypoxia, which are initiated in the carotid body (CB). Endothelin-1 (ET-1), a potent vasoactive excitatory modulator of CB chemoreception is upregulated in sustained hypoxia. Thus, we studied the possible contribution of ET-1 to the enhancement of CB chemosensory responses in cats exposed to CIH. **Methods:** We studied ET-1 plasma levels and CB immunoreactivity (ET-ir) in cats exposed to CIH for 4 days. We assessed the effects of ET-1 and bosentan, a non-selective ET_A and ET_B receptor antagonist ($ET_{A/B}$-R) on CB chemosensory discharges (f_x) induced by acute hypoxia in CBs perfused *in vitro*. **Results:** CIH increased local ET-ir in the CB leaving ET-1 plasma levels unchanged. Exogenous ET-1 produced a long-lasting dose-dependent f_x increase. The dose-response ET-1 curve was rightward-shifted in CIH-treated CBs. In addition, CIH increased basal f_x and enhanced chemosensory response to hypoxia. These effects are reduced by $ET_{A/B}$-R blockade with 50 μM bosentan. **Conclusions:** ET-1 is locally upregulated in the CB of CIH-treated cats, and exerts a tonic excitatory effect in CB basal and hypoxia-evoked chemosensory discharges. The chemosensory potentiation is reduced in CIH-treated CBs by $ET_{A/B}$-R blockade. Thus, ET-1 may contribute to an enhanced CB-mediated cardiorespiratory responsiveness in our CIH model. Supported by FONDECYT 1030330.

PARADOXICAL EFFECT OF L-NAME ON CAT CAROTID BODY EXPOSED TO CHRONIC INTERMITTENT HYPOXIA

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Chronic intermittent hypoxia (CIH) increases carotid body (CB) chemosensory discharges and responses to acute hypoxia, effects that may be attributed to a reduced vascular inhibitory tone in the CB. Nitric oxide (NO) produced within the CB is an inhibitory modulator of hypoxic chemoreception. Accordingly, pharmacological inhibition of nitric oxide synthases (NOS) increases CB basal discharges and responses to acute hypoxia. Thus, we studied the effect of the blockade of the tone exerts by NO on CB chemosensory response to acute hypoxia in cats exposed to CIH. **Methods:** We assessed the effect of the non-selective NOS inhibitor N-ω-nitro-L-arginine methyl ester (L-NAME) on carotid chemoreception in perfused *in vitro* CBs from cats exposed to 2-min hypoxic cycles (PO_2 ~75 Torr) repeated 10 times/hour during 8 hours for 4 days. **Results:** Perfusion with 0.5–1.0 μM L-NAME in control CBs increased both basal discharges and chemosensory responses to acute hypoxia (PO_2 ~25 Torr). However, in the CIH-treated CBs, L-NAME failed to increase both basal and hypoxic-evoked discharges. Moreover, in 3 out of 6 CBs, L-NAME reduced basal chemosensory discharges. **Conclusions:** Results showed that contrarily to what was expected, blockade of NOS did not increase basal and hypoxic-evoked discharges, suggesting that NO may exert an excitatory tone in CIH-treated CBs. Supported by FONDECYT 1030330.

Contributed Poster Communications

TROPHOBLAST EXPRESSION OF KALLIKREIN, BRADYKININ B2 RECEPTOR, AND ENDOTHELIAL NITRIC OXIDE SYNTHASE IN NORMAL GESTATION, PREECLAMPSIA AND PLACENTA ACCRETA

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Hypothesizing that paracrine vasodilator factors contribute to placentation and to enhance placental blood flow, we studied the expression of tissue kallikrein, the bradykinin B2 receptor (B2R) and endothelial nitric oxide synthase (eNOS) in syncytio (STB) and extravillous trophoblasts (EVT) in normal pregnancy (N, n=11), preeclampsia (PE, n=15), and placenta accreta (PA, n=6). **Methods:** Placentas were immunostained with antibodies to kallikrein, B2R and eNOS using biotin-streptavidin/DAB. The intensity of the signal was graded by a blinded observer from 0=absence to 3=intense. **Results:** The STB expressed kallikrein in N, PE and PA, being the immunostaining higher in PA [2.0 (2.0-2.2 interquartile range) vs. 1.0 (1.0-1.5) for N and PE; $p < 0.05$], while B2R and eNOS were similarly expressed in N, PE and PA. The EVT expressed kallikrein in PA [0.9 (0.5-1.3)] while no signal was detected in N and PE ($p < 0.001$), while the B2R signal was enhanced in PE [2.5 (2.0-2.7) vs. 1.0 (0.7-2.2) and 2.0 (2.0-2.2) in N and PE respectively, $p < 0.5$]. Endothelial NOS was higher in the EVT in PA [2.0 (1.9-2.0) vs. 0.5 (0.5-1.0) in N and PE, $p < 0.001$]. **Conclusions:** The upregulation of kallikrein in STB, and of kallikrein and eNOS in EVT in PA, a condition of exaggerated trophoblast invasion, suggests the participation of both vasodilator factors in trophoblast invasion and in placental perfusion. The absence of this response in PE supports the view that a defective trophoblast function contributes to altered placentation. Fondecyt 1020705 &1050707 (Chile).

FUNCTIONAL AND STRUCTURAL CARDIAC RESPONSES TO SPIRONOLACTONE IN UREMIC RATS

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Recent evidence implicates aldosterone as an important pathogenic factor in progressive cardiovascular disease observed in uremic patients. The present study was designed to investigate aldosterone receptor-dependent cardiac changes in a model of uremia. **Methods:** Sprague Dawley rats were rendered uremic by 5/6 nephrectomy (NPX) or sham operated (SHAM). Half of uremic rats received spironolactone treatment (Spi). After six weeks, before the animals were euthanized, arterial blood pressure and eco-cardiographic indexes were measured,. 11□HSD2 activity was measured by the rate of conversion of corticosterone to 11-dehydrocorticosterone. Total RNA, extracted from left ventricle, was quantified by real time RT-PCR for 11□HSD2 and mineralocorticoid receptor (MR) expression. **Results:** All uremic rats developed significant proteinuria and high plasma creatinine. Plasma aldosterone and corticosterone were not different between the three groups. Nephrectomy induced significant hypertension at 5 weeks (systolic blood pressure: 150±6 mmHg vs 108±3 mmHg control; $P<0.05$). Spi did not normalize blood pressure (133±7mmHg). Transthoracic echocardiography demonstrated increased left ventricle posterior wall thickness in the NPX group (2.00±0.08, $P<0.001$) that was prevented by Spi ($P<0.05$ NPX *vs.* NPXspi). Spi significantly decreased aortic diameter in NPX rats (SHAM=3.69±0.07; NPX=4.04±0.10; NPXspi=3.73±0.06; $P<0.05$ NPX *vs.* NPXspi). Left ventricular heart weight/body weight ratio was higher in NPX than SHAM rats. Spi significantly reduced ventricular hypertrophy in the NPXspi rats ($P<0.01$ *vs* NPX alone). 11□HSD2 activity and mRNA expression were elevated in NPXspi group. MR mRNA expression was not significantly different between the three groups. **Conclusions:** These observations indicate a vascular and heart protective effect of spironolactone in rats with renal failure. Supported by FONDECYT 1040338 (Chile).

ADRENOCORTICAL RESPONSE TO A SUPERIMPOSED EPISODE OF ACUTE HYPOXIA IN HIGH ALTITUDE FETAL LAMBS

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Acute hypoxia increases cortisol plasma concentration in fetal lambs. Nevertheless, chronic hypoxia of pregnancy at high altitude (HA) blunts fetal cortisol response to an ACTH challenge compared to lowland fetal lambs (LAFL). We hypothesized that chronic hypoxia blunts cortisol response to acute hypoxia in fetal lambs which have undergone gestation at HA. **Methods:** Under general anesthesia 7 HA (3,580 m) and 3 LA (580m) fetal lambs were chronically catheterized at 134±1 days GA. Four days after surgery, the lambs were submitted to experiments based on a 3h protocol: 1h basal (B), 1h of acute hypoxia (H; PaO_2 12±1 mmHg) and 1h of recovery (R). Cortisol plasma concentrations were measured by RIA. **Results:** HAFL have a marked decrease in plasma cortisol (ng ml^{-1}) basally and during acute hypoxia (B 22.9±2.9[a]; H 31.2±5.2[a]; R 22.8±4.2[a]) compared to LA (B 40.8±10.1; H 81.6±12.4*; R 62.4±5.3*) (*$P<0.05$ vs B and [a]$P<0.05$ vs LA). **Conclusions:** These data show lower basal cortisol plasma concentration that goes together with a blunted adrenal response to acute hypoxia in chronically hypoxic fetal lambs gestated in the high altitude of the Andean altiplano. We speculate that this blunted adrenal function in chronic hypoxic fetal lambs may help to avoid premature organ maturation and preterm delivery. The Wellcome Trust CRIG 072256, FONDECYT 1050479 (Chile).

ROLES OF NITRIC OXIDE AND PROSTAGLANDINS (PG) IN LIPOPOLYSACCHARIDE-INDUCED EMBRYONIC RESORPTION (ER) MODEL IN MICE

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Nitric oxide (NO) at high concentrations, as those observed in sepsis, has toxic effects on embryos either itself as a free radical or producing peroxinitrite, a potent oxidant. PG are abortive too since they stimulate uterine contractility. In our murine early embryonic resorption model, lipopolysaccharide (LPS) increased NO and PG synthesis at implantation sites. This low dose of LPS does not endanger the survival of the mother and produces complete embryonic resorption (100%) at 24 h. The aim of this study was to determine whether changes in both mediators are associated with ER prevention. **Methods:** Balb/c females were injected with LPS, COX and NOS inhibitors at day 7 of pregnancy. Six hours later (when NO production is maxim) uterine and decidual tissues were removed to determine PG level (RIA) and NOS activity (Bredt & Snyder). Five days post administration of endotoxin the percentage of ER reversion was determined. **Results:** Indomethacine (a non-selective COX inhibitor) reduced ($p<0.001$) basal and stimulated prostanoid levels *in vivo* and Aminoguanidine (an iNOS inhibitor) increased them in decidua but not in uterus. We found that the same inhibitors were capable of reducing ER from 100% to 48% (iNOS inhibitor, $p<0.001$) and 10% (COX inhibitors, $p<0.001$), being 10% the spontaneous ER rate in our laboratory. **Conclusions:** These results suggest that (:) NO and PG play a major role in LPS-induced ER in mice. Supported by PICT 05-10901 (Argentina).

LUMINESCENCE ASSOCIATED TO THE INTERACTION OF TRYPTOPHAN RESIDUES WITH SINGLET OXYGEN

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Biological oxidations lead to the emission of visible chemiluminescence (CL). This emission has been employed to monitor, in real time, oxidation processes in organelles, tissues, and whole animals. However, the origin of this CL has not been established. We show in the present work that singlet oxygen mediated oxidation of tryptophan residues produces peroxide like compounds that emit an intense visible CL. **Methods:** Peptides and proteins bearing Tryptophan residues were exposed to singlet oxygen generated in the photolysis of Rose Bengal. Irradiations were performed at low (4 ºC) in order to minimize the decomposition of the CL intermediates. After irradiation, the samples were introduced in a liquid scintillation counter operating in the out-of-coincidence mode in vials maintained near 0 ºC. **Results:** Intense CL, that lasted by several minutes, was observed when the Rose Bengal was irradiated in the presence Ala-Trp dipeptide, Ala-Trp-Ala tripeptide, and BSA. Negligible CL was observed for free Trp or Trp-Ala dipeptide. The CL was totally suppressed by ebselen addition, but was not affected by Trolox addition. The reported results show that the reaction of singlet oxygen with Trp groups that do not bear a free carboxylic group produces peroxy-like intermediates that generates an intense CL. **Conclusion:** The sensitivity of the methodology allows for the evaluation of small degrees of oxidation of Trp groups in complex structures and raises the interesting possibility that, at least part of the CL associated to biological oxidations is due to this type of process. This work has been financed by FONDECYT (Project 1030033)

PLACENTAL PATHOLOGY IN LUPUS PATIENTS

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To examine whether there are characteristic histological features in placentas from pregnancies of patients with systemic lupus erithematosus (SLE), associated with other collagen diseases or antiphospholipid antibody syndrome (SAF). **Methods:** Thirty–five placentas were retrieved with a diagnosis of SLE from the Pathology Service of Hospital Universitario Pedro Ernesto (State University of Rio de Janeiro) between 2002 to 2004. Twenty samples had only SLE , and 6 complicated with arterial hypertension, 2 associated with hypothyroidism, 1 with Raynaud, 1 with hyperthyroidism,and 1 systemic sclerosis (CLE, n=11) and 4 with SAF one with Sjögren (SLS, n=4). Placentas were from 2nd and 3rd trimesters, and were grouped by weight. Acute atherosis (AA), fibrinoid necrosis (FN), vascular thrombosis (VT), obliterative arteriopathy (AO), chorioangiosis (CA), dismaturity (DM) and the number of vasculo-syncytial membranes (VS) were evaluated semi-quantitatively. **Results:** Only 2 cases had AA, 4 had FN and those were related to placentas with less than 300g, 3 had VT, all cases exhibited AO and no differences were found among SLE, CLE and SLS or among weight groups. CA was observed in 31samples and the intensity was more important in large placentas ($p = 0.0285$). All samples from CLE and SLS had DM with was more intense in SLS ($p = 0.0493$). Thirty cases exhibited low number of VS that were found in placentas with low weight. **Conclusion:** There are no specific histopathologic placental abnormalities related to the SLE alone or complicated. Decidual vasculopathy was prominent in SLE with SAF and resulted in premature delivery. (Supported by FIOCRUZ, CNPq and FAPERJ)

PLACENTAL PATHOLOGY IN LUPUS AND SAF PATIENTS

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Pregnancy in women with auto-immune diseases is frequently associated with placental insufficiency, fetal death, pre-eclampsia, premature delivery or thrombosis. The aim of this study was to compare the histopathological features of placentas from patients with systemic lupus erithematosus (SLE), associated with other collagen diseases or antiphospholipid antibody syndrome (SAF). **Methods:** Sixty–three placentas were retrieved with a diagnosis of auto-immune diseases from the Pathology Service of UERJ between 2002 to 2004. Thirty-one samples had SLE alone (n=20) and 11 were complicated or associated with other auto-immune diseases. Fourteen placentas were from patients with SAF alone or with pre-eclampsia (n=3). Five patients had cutaneous lupus (CAL), 9 presented other auto immune diseases. Four had SLE with SAF. Placentas were grouped by weight. Fibrinoid necrosis (FN), vascular thrombosis (VT), obliterative arteriopathy (AO), chorioangiosis (CA) and the number of vasculo-syncytial membranes (VS) were evaluated semi-quantitatively. **Results:** Eleven cases had FN and those were related to placentas with less than 300g ($p=0.049$). Twelve had VT and the cases were from SLE+SAF ($p=0.050$) or with SAF ($p=0.002$). Only 3 cases did not exhibited AO, intensity of the lesion was associated with SLE ($p=0.036$). CA was mild in 39 cases and intense in 15 was more important in large placentas ($p=0.005$) and in 100% of CAL. Fifty cases had decreased VS presented in low weight placentas ($p=0.028$). **Conclusions:** Placentas from autoimmune diseases have many vascular abnormalities, in this series only VT was positively correlated to SAF. (Supported by FIOCRUZ, CNPq and FAPERJ)

MULTIPARITY INCREASES VEGF IN MATERNAL-FETAL INTERFACE. SUBNORMAL BEHAVIOUR OF THE CBA/J X DBA/2 CROSSBREEDING

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We previously reported that placentae from multiparous females showed an increased invasive trophoblast tissue. The aim of this work was to study the influence of multiparity on the presence of VEGF, VEGF-R1 and sVEGF-R1 in placenta and serum. **Methods:** *CBA/J x CBA/J, CBA/J x BALB/c* and the abortion-prone *CBA/J x DBA/2* mouse combinations were divided into three groups: Primiparous Young (PY): 3.0 ± 0.5 months old, n =10; Primiparous Old (PO): 8.5 ± 0.5 months old, n =10 and Multiparous Old (MO): 8.5 ± 0.5 months old with 4 pregnancies, n =10. Sera and placentae were obtained at term pregnancy. Placental VEGF expression was investigated by IHC. VEGF-R1 levels in PS and sera were measured by ELISA. **Results:** All groups showed similar quantity of VEGF+ cells in the whole trophoblast. Among the PY groups, *CBA/J x DBA/2* presented low VEGF+ cells in the area next to decidua ($p<0.01$). In this zone, all MO combinations showed higher expression of VEGF ($p< 0.001$). In non- pregnant CBA/J mouse sera VEGF-R1 level was 559 ± 172 pg/ml but at term of the first pregnancy, the *CBA/J x Balb/c* group showed a high increase (PY: 5900 ± 532 / PO: 8062 ± 1221) respect to *CBA/J x DBA/2* sera (PY: 3003 ± 553 / PO: 3134 ± 238). Sera from MO mice showed lower levels (*CBA/J x Balb/c*: 2212 ± 360 and *CBA/J x DBA/2*: 1015 ± 230). PS did not show significant differences. **Conclusions:** Multiparity status induces an increase of VEGF expression by invasive trophoblast. The *CBA/J x DBA/2* combination showed a lower effect. Our data suggest that VEGF could be involved in the invasion process. The diminished serum VEGF-R1 level found in MO females would also increase VEGF concentration. The biological significance of this systemic effect has to be further investigated. Supported by UBA (B097).

PARADOXICAL EFFECT OF ETHANOL ON Ca^{2+} CURRENTS VERSUS INTRACELLULAR Ca^{2+} IN ENDOTHELIAL CELLS OF BRAIN CAPILLARIES

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It has been well established that a link between chronic alcohol intake and predisposition to stroke exists. This is due to vasospasm and ischemia provoked by alcohol on the cerebral blood flow. Since the mechanism by which the alcohol affects blood flow is not well understood, **Methods:** we investigated the ethanol (EtOH) effect on low voltage-activated Ca^{2+} channels and intracellular Ca^{2+} in endothelial cells of rat brain capillaries by using the patch-clamp and the Fluo-3 fluorescence technique. **Results:** EtOH at concentrations between 10 to 100 mM depressed reversibly Ca^{2+} currents with maximal inhibition of about 80% at 100 mM. Although this inhibition was reversible at all concentrations we found, that over 40 mM the patch became worse and leaky. The EtOH inhibition was modulated by external Mg^{2+} ions. Reducing Mg^{2+} from 1.2 to 0.3 mM, enhanced the EtOH inhibition. When monitoring intracellular Ca^{2+}, paradoxically EtOH provoked a Ca^{2+} increase in dependence of high extracellular Ca^{2+} concentration (10 mM) but not when external Ca^{2+} was 0 or 2.5 mM. **Conclusions:** Since chronic alcohol intake is associated with vasospasm and ischemia in the brain blood vessels, we hypothesize that EtOH should exert a more complex cellular mechanism on cerebral endothelial cells rather than only inhibition of T-type Ca^{2+} channels as much as it induced also intracellular Ca^{2+} increase by an unknown mechanism. The enhanced inhibition produced by low Mg^{2+} is related to a negative membrane screening that alters the kinetic of the Ca^{2+} channel and makes it more sensitive to EtOH.

REVISING PSEUDOPREGNANCY AS *IN VIVO* EXPERIMENTAL MODEL OF PREGNANCY

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Pseudopregnancy (PP) has longer been used to study uterine environment without the embryo influences. In the present work it was revised the protocol and evaluated the best condition to simulate the normal pregnancy (P) with special attention on uterine NK (uNK) cells. **Methods:** Plant (Arachis) or mineral (paraffin) oils were inoculated into the uterine lumen of female mice on 4th PP day (PPd) after mating with vasectomized males. Groups of animals from each treatment were ovarectomized and supplied with exogenous ovarian hormones or chorionic gonadothropin hormone (CGH). Uterine samples were collected from 9th to 12th PPd and processed for histological and ultrastructural evaluations and compared with normal pregnancy. **Results:** All uterine samples collected on 9th PPd showed decidual reaction, being those induced by plant oil containing phytosterols stronger than mineral oil or normal P. Ultrastructure of decidual cells were similar and the uNK cells were found in all samples, but the fully differentiated granule reach uNK cells were found only in the mineral oil induced, progesterone or CGH supplied animals. The CGH treatment, rather than conventional treatment by supporting the ovarian corpus luten mimic the normal pregnancy-like uterine environment both for the decidua and uNK cells since they survive longer than 12th PPd, but degenerate after 14th PPd. **Conclusions:** Only mineral oil injection and up-regulation of ovary by CGH during the first half period of PP closely mimic the uterine environment of normal P. After 12th PPd additional factors from developing fetus should be the conditioned factor necessary to use the PP as *in vivo* experimental model. Supported by CAPES.

ALPHA-2 MACROGLOBULIN EXPRESSION IN THE MOUSE UTERUS DURING EARLY PREGNANCY

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Alpha-2 Macroglobulin (A2M) is a tetrameric glycoprotein involved in many organic processes such as protease inhibition, growing factor and cytokine binding, tissue remodeling, and the control of trophoblast invasion. **Methods:** The aim of this study was to investigate through immunohistochemistry the expression of human A2M by the pregnant endometrium between days 5.5 and 7.5 of pregnancy. **Results:** Our results showed that the mesometrial and anti-mesometrial decidual cells, uterine natural killer cells, and the myometrium were positive for A2M in all analyzed periods. However, the A2M staining observed on the mesometrial decidual cells was slightly higher than that from antimesometrial decidual cells. From day 6,5 of pregnancy, endothelial cells of the mesometrial sinusoids were strongly positive for A2M. In the interimplantation sites, the expression of A2M were observed in the uterine and glandular epithelium, and the muscular layer of the vessels, but were negative in the stromal cells. In the Liver the staining for A2M was weak and homogeneous in the hepatocytes. In the ovary, the corpus luteum, granulosa, cumulus cells, smooth muscle cells of the vessels, and fatty cells also showed positivity for A2M. **Conclusions:** These findings suggest that the decidual cells, uNK cells, and the myometrium may be the likely A2M sources in the pregnant uterus, and may participate in the rapid and intense tissue remodeling that occurs in the endometrium during early pregnancy. Financial support from FAPESP 01/09019-9.

EFFECT OF TRAINING BREATHING 100% OXYGEN IN HYPERBARIC AMBIENT ON PHYSICAL PERFORMANCE

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Skeletal muscle activity for a long period, delaying muscular fatigue, depends mainly on mitochondrial ATP from oxidative phosphorylation. Oxygen (O_2) utilization by muscle mitochondria depends on alveolar ventilation, oxygen alveolus-capillary diffusion, lung and muscle blood flow, O_2 diffusion from blood to muscle fibers, and partial pressures gradients between alveoli, blood and muscle fibers. **Methods:** We studied the effect of physical training in cycloergometer, breathing 100% O_2 at 2 atm.. Twelve male soccer players, ~18 years old, were subjected, to a daily 30 min cycloergometer (70 rpm, 75% of V'O_2max intensity). Control group (n=6) developed this exercise in Normoxic Normobaric (NN) conditions and the experimental group (n=6) in Hyperoxic Hyperbaric (HH) conditions (breathing 100% oxygen at 2 ATA). Each individual was evaluated before and after a three-week training period, performing a standardized exercise in cycloergometer, at NN conditions, until reaching V'O_2max. Respiratory, blood and oxidative stress metabolic parameters were measured on the evaluations. **Results:** In HH conditions an increase in V'O_2max and time required to reach it, was observed. No changes were observed in acid-base equilibrium parameters or in the oxidative stress indicators. **Conclusions:** Thus, improvement in physical performance as a consequence of training in HH conditions is observed, likely due to optimization of mitochondrial metabolic processes responsible for generating energy.

MORPHOMETRIC STUDY OF PLACENTAL VILLOUS AND VESSELS IN MATERNAL HYPERGLYCEMIA, GESTATIONAL AND OVERT DIABETIC PREGNANCIES

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Morphometric specific changes in diabetic placenta are contradictory and not always related to glycemic levels and diagnostic methods. This study compared morphometric results of terminal villi and villous vessels in placenta of pregnant women with varied intensity hyperglycemia, diagnosed by glucose tolerance test (GTT) or glycemic profile (GP). **Methods:** 207 pregnant women with minimum gestational age of 34 weeks, were classified in control group (n=56), hyperglycemia group (n=51), gestational diabetes group (n=59) and overt diabetes group (n=41). Placental samples were randomized for morphometric study in image analyser. Villous areas and numbers as well as their respective vessels were assessed. Statistical analysis was done through Qui-square and Fisher tests, variance analysis and stepwise, with $p \leq 0.05$. **Results:** Glycemic levels were lower in the control group (86.2mg/dL), followed by the hyperglycemia group (98.9mg/dL), the gestational diabetes group (114.1mg/dL) and the overt diabetes group (122.1mg/dL). In the hyperglycemia group, the placentas had higher villous area, same vascular area, higher number of vessels, and capillarization index similar to the control group. In the gestational diabetes and overt diabetes groups, the villous characteristics and number of vessels were equivalent to the control group, with smaller vascular area. Capillarization index was lower in the overt diabetes group. **Conclusions:** Maternal hyperglycemia with varied origin (GTT or GP) and intensity determines placental morphometric changes, related with benefit or damage of maternal-fetal exchanges.

DOPPLER VELOCIMETRIC STUDY OF UMBILICAL ARTERY AND MORPHOMETRIC CHANGES OF PLACENTAL VILLI AND VESSELS IN PREGNANT WOMEN WITH HYPERGLYCEMIA

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Diagnostic accuracy of Doppler velocimetry of umbilical arteries is not defined in pregnancies complicated by diabetes. Controversies are also common when the relation between Doppler velocimetry studies of umbilical artery and maternal metabolic control are evaluated. This study compared pulsatility index (PI) from umbilical arterial Doppler velocimetry with morphometric characteristics of terminal villi and villous vessels in placenta of pregnant women with hyperglycemia. **Methods:** One hundred and forty-three pregnant women with 34 weeks minimum gestation were submitted to Doppler velocimetry within 10 days of delivery; they were classified into groups: G1 normal glycemia and PI (n = 26), G2 hyperglycemia with normal PI (n = 102), and G3 hyperglycemia with altered PI (n = 15). Placental samples underwent randomized morphometric study using an image analyzer. Villous areas, number and their respective vessels were assessed. Statistical analysis was by Qui-square and Fisher tests, analysis of variance, and stepwise, with $p \leq 0.05$. **Results:** Average maternal glycemia levels were different, 85.8, 103.0, and 116.8mg/dL for G1, G2, and G3 respectively. G2 placentas had a higher number of smaller terminal villi than G1 but villous area was similar. The high number of small villous vessels defined a smaller vascular area and lower capillarization index in this group. Terminal villi and their respective vessel characteristics in G3 were similar to G1. **Conclusions:** Results showed that maternal hyperglycemia with varied origin and intensity determines placental morphometric changes, and is responsible for normal or altered umbilical artery PI values and for resistance in uteroplacental flow.

DISTRIBUTION OF ACTIVIN A IN THE MOUSE ENDOMETRIUM DURING THE OF PREGNANCY

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The embryo implantation in rodents induces decidualization of the endometrial stroma forming a new structure in the uterus, denominated the decidua. Besides other functions the decidua may act as a provisional endocrine gland controlling maternal and fetal adaptations required for the establishment and maintenance of pregnancy. However, as soon as the definitive placenta starts to be formed, the antimesometrial decidua degenerates probably by the action of the glycoprotein Activin A. There are biochemical evidences from cultures of mice decidual cell, correlating the expression of the activin A and caspases with the surge of decidual cells apoptosis. In this study we followed the expression of Activin A in mice uterine tissues from day 4 to 14 of pregnancy. **Methods:** Fragments of implantation and interimplantation sites were collected from pregnant uteri of Swiss mice. The samples were fixated in methacarn, embedded in paraplast and cut into 5□m transverse serial sections. Samples were immunocytochemically stained for detection of Activin A and the reaction was developed with 3,3`- diaminobenzidine. **Results:** On day 9 of pregnancy many degenerating decidual cells were observed in the antimesometrial (AM) decidua. On the 13th day the antimesometrial decidua was completely involuted. Accordingly, immunoreaction for Activin A was present in the cytoplasm of AM decidual cells from day 9 to day 12 of pregnancy. **Conclusion:** Activin A was not expressed in decidual healthy cells. However, Activin A starts to be expressed by dying cells of the AM decidua indicating a role of this molecule in the degenerative process of the AM in mice. LC was Fellowship from FAPESP. Grant from FAPESP.

15-DEOXY $\Delta^{12,14}$PROSTAGLANDIN J_2 MODULATES THE LIPID SYNTHESIS IN TERM PLACENTAS FROM HEALTHY AND GESTATIONAL DIABETIC PATIENTS

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Diabetes mellitus affects placental lipid metabolism. 15-deoxy $\Delta^{12,14}$prostaglandinJ_2 (15dPGJ$_2$) is a high affinity endogenous ligand for the peroxisome proliferator-activated receptor γ (PPARγ), which regulates the transcription of genes involved in lipid and glucose homeostasis. The aim of this study was to determine whether 15dPGJ$_2$ is able to regulate lipid metabolism in term placentas from healthy patients (CP) and gestational diabetic patients (GDP). **Methods:** Placental tissues from CP and GDP were obtained at term. Villous samples were taken and incubated for 3 h either with or without 15dPGJ$_2$ ($2x10^{-6}$ mol/l) for further evaluation of both lipid mass (by TLC and image analysis) and the *de novo* lipid synthesis (by determining the incorporation of ^{14}C-acetate to lipids). **Results:** The synthesis of cholesteryl ester (CE) ($p<0.05$), triglycerides (TG) ($p<0.01$), free fatty acids (FFA) ($p<0.05$) and phospholipids (PL) ($p<0.05$) was decreased in placentas from GDP compared with CP. No differences were found in lipid mass from both groups. The addition of 15dPGJ$_2$ decreased the synthesis of TG ($p<0.05$) and cholesterol (CHO) ($p<0.05$) in placentas from CP and GDP. In GDP the synthesis of CE ($p<0.01$), and FFA ($p<0.001$) was also reduced. 15dPGJ$_2$ did not modify the levels of the lipids evaluated in both groups. **Conclusions:** Gestational diabetes reduces the ability of placental *de novo* lipid synthesis, a pathway that serves to the accretion of placental lipids from carbon moieties, and that is negatively regulated in both CP and GDP by the PPARγ agonist 15dPGJ$_2$.

EXPERIMENTAL EXPOSURE TO LEAD IN BALB/c MICE: EFFECTS ON PLACENTAL MORPHOLOGY

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Several studies have shown that high doses of lead (recognized as a toxic substance), could induce abortion. In addition, lead exposure in uterus, causes numerous neurobehavioral abnormalities in children and laboratory animals. Studies have shown that lead crosses the placenta, and total lead content in fetal tissues increases throughout pregnancy. However, little is known about the effects of lead on placental functionality and its consequences on the fetus health. The aim of this study was to evaluate the effects of experimental exposure to lead on placental morphology. **Methods:** Two groups of pregnant BALB/c mice (n=5), on day 8 of gestation, were exposed, in a single parenteral dose, to 0, and 56 mg/kg of lead acetate trihydrate. On gestational day 18, females were euthanized by ether asphyxiation. Placentas were fixed in 4% phosphate buffered formalin and embedded in paraffin. Placentas were sectioned and stained with haematoxylin-eosin. **Results:** results showed no maternal differences in body weight at the day of sacrifice, or in the number of live and dead fetuses between groups; fetal body and placental weight were unaffected by lead exposition. The number of infarctions increased in placentas from exposed group (p=0.01). Morphologic changes included an increase in phagocytic activity of trophoblast giant cells, hemorrhages, and vascular congestion in the labyrinth, with no significant changes in the junctional zone. **Conclusions:** These results suggest that a single dose of lead may condition morphologic changes that could be the result of compensatory mechanisms to maintain the placental functionality.

IMMUNOLOCALIZATION OF COLLAGEN TYPES I, III AND V IN THE MOUSE UTERUS FROM DAYS 6 TO 8 OF PREGNANCY

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Embryo implantation in rodents is marked by the trans-differentiation of endometrial fibroblasts into decidual cells. In the mouse, decidualization is accompanied by extensive remodeling of the endometrial extracellular matrix, resulting in reduction of extracellular spaces and increasing diameter of collagen fibrils. Previous studies indicate that these thick fibrils are formed by the aggregation of thin collagen fibrils. Although collagen types I, III and V are the main fibrilar components in the non-pregnant and pregnant endometria in rodents, and an unusual homotrimeric form of collagen V is expressed in the mouse decidua, the molecular composition of the thick collagen fibrils is unknown. The goal of this study is to identify the collagen types that form thick collagen fibrils in the decidualized and non decidualized endometrial stroma. **Methods:** Non pregnant and pregnant uteri from day 6 to day 8 were collected, fixed in methacarn and embedded in paraplast. Sections were immunostained against collagens I, III and V and visualized with immunofluorescence microscopy. **Results:** Immunoreaction for collagen types I, III and V was strong in the decidua. These collagens were similarly localized around decidual cells, around blood vessels and in the subepithelial region of the uterine lumen. In non decidualized endometria, the immunoreaction for the three types of collagen was very weak. **Conclusions:** Our results suggest that thick collagen fibrils of the decidua are heterotypic and formed by a mixture of these three types of collagen. These thick fibrils may act as an important structural support to the growing pregnant uterus.

EFFECT OF VASOPRESSIN AND DOCA-SALT IN 11β̃HYDROXYSTEROID DESHYDROGENASE TYPE 2 AND MINERALOCORTICOID RECEPTOR EXPRESSION IN CARDIAC TISSUE

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It is known that high plasma mineralocorticoid levels in combination with a high salt diet induce cardiac hypertrophy, fibrosis and heart failure. However, the molecular mechanism explaining the role of high salt is unknown. Cardiac tissue express the mineralocorticoid receptor (MR) and the11β-hydroxysteroid-dehydrogenase type 2 enzyme (11β-HSD2), which confers specificity to aldosterone action on MR. In the present study we tested if vasopressin is involved in 11β-HSD2 cardiac expression. **Methods:** Uninephrectomized rats received deoxycorticosterone (20 mg/Kg) plus 1% NaCl in drinking water, 8 days. Total RNA were extracted from the left ventricle, mRNA of MR and 11β-HSD2 was quantified by real time RT-PCR, normalized to 18S mRNA. Primary cultures of cardiomyocytes and fibroblasts were stimulated with increasing concentrations of vasopressin. Total RNA was extracted from cell cultures. **Results:** Incubation of cardiomyocytes or cardiac fibroblasts with vasopressin (10^{-11}-10^{-9}M) increased 11β-HSD2 mRNA. No changes were observed in MR transcript abundance. Plasma vasopressin levels were significantly increased in DOCA-salt rats (600±4,1% P<0,01). Left ventricle 11β-HSD2 mRNA abundance was also increased after mineralocorticoid treatment (200%). **Conclusions:** Ours results suggest that 11 □-HSD2 expression in cardiac tissue is modulated by elevated vasopressin levels present in DOCA-salt rats. Supported by MED 001-04.

DISTRIBUTION OF COLLAGEN AND PROTEOLGYCANS IN PLACENTAS OF DIABETIC RATS

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Collagen fibrillogenesis is a complex molecular process influenced by a number of intracellular and extracellular factors. Several lines of research have suggested that small leucine rich proteoglycans (SLRPs), as decorin and biglycan, are important regulators of collagen fibril growth. During the establishment of the maternal-fetal units, the differentiation of the trophoblasts cells and remodeling of extracellular matrix occurs. Pathological conditions during the pregnancy are related with the fetal morbidity and abnormal morphology of the placenta. Our aim was determine whether maternal diabetes affects the placental morphology and distribution of collagen and SLRPs during the placental development. **Methods:** Pregnant female Wistar rats of 14 and 20 days-old were used. The placentas were removed and fixed in methacarn solution. Collagens were evaluated by Picrosirius stain and small leucine rich proteoglycans (decorin and biglycan) were evaluated by immunocytochemical techniques. **Results:** In physiological conditions, increase of collagen and proteoglycans were observe on the placental and umbilical cord tissues, when 14 and 20 days-old was compared. The collagen stains diminished in placentas and umbilical cord from diabetics rat, whereas proteoglycans distribution did not change. **Conclusion:** Maternal diabetes affect the collagen distribution in the placental tissues during the establishment of the maternal-fetal units. Supported by DIPUV (University of Valparaíso, Chile). CNpq and FAPESP (Brazil).

APOPTOSIS INDUCED BY PROLONGED EXPOSURE TO ODORANTS IN CULTURED CELLS FROM RAT OLFACTORY EPITHELIA

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Multicellular organisms undergo programmed cell death (PCD) as a mechanism for tissue remodelling during development and tissue renewal throughout adult life. Overdose of some neuronal receptor agonists like glutamate can trigger a PCD process termed excitotoxicity in neurons of the central nervous system. Calcium has an important role in PCD processes, especially in excitotoxicity. Since odour transduction in olfactory receptor neurons (ORNs) involves an increase in $[Ca^{2+}]_i$, we investigated the possibility that long term exposures to odorants could trigger an excitotoxic process in olfactory epithelial cells (OEC). **Methods:** We used single cell $[Ca^{2+}]_i$ determinations and fluorescence microscopy techniques to study the effects of chronic odorant exposures in OEC in primary culture. Induction of PCD was evaluated by three independent criteria: 1) measurements of DNA fragmentation, 2) translocation of phosphathidylserine to the external leaflet of the plasma membrane, and 3) caspase-3 activation. **Results and Conclusion:** Our results support the notion of an odorant-induced PCD in OEC. This odorant-induced PCD was prevented by LY83583, an odorant response inhibitor, suggesting that ORNs are the main epithelial cell population undergoing odorant-induced PCD. Supported by FONDECYT 1990938 & 1020964, MIDEPLAN ICM P99-031-F (Chile). *These authors contributed equally to this work.

DEFECTIVE PLACENTATION IN PERIGESTATIONAL ALCOHOL INGESTION, IN MOUSE. ROLE OF PROSTAGLANDINS AND NITRIC OXIDE PATHWAYS

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Alterations in prostaglandins (PGs) and nitric oxide (NO) levels would be involved in abnormal placentation. The aims were to evaluate the early placentation after maternal alcohol ingestion and the role of PGs and NO in this process. **Methods:** female mice were treated with 10% alcohol 15 days previous to superovulation and during gestation up to day 10 of pregnancy (A). Controls (C) received water. At day 10, the endothelial and inducible nitric oxide synthases (eNOS, iNOS) were immunolocalized in the implantation site (IS). In miometrial (M) and decidual (De) tissues the PGE, PGF_{2alfa} and nitrates/nitrites (Ns) content were determined by RIA and the Griess reaction respectively. M and De were incubated with or without Indomethacin, Meloxicam, L/D-NAME (NOS inhibitor), s-Nonoate (NO donor) and PGF_{2alfa} released assessed by RIA. **Results:** the A-IS had low decidualized Decidua Basalis and Capsularis, low development of villous with collapsed intervillous space in labyrinth and altered trophoblastic zone. The villous and trophoblastic eNOS and iNOS expression increased in A-IS. The PGF_2 and Ns content significantly increased in A-M and De. Both M and De-PGF_2 release were significantly reduced with Indo and Melox in C and A. L-NAME diminished the PGF_2 in A-M and S-Nonoate inhibited the A-De-PGF_2 levels. **Conclusions:** the perigestational alcohol intake produced early resorption and defective placentation due to low decidualization, abnormal villous and trophoblastic zona and high NO-oxidative stress. The increased PGF_2 and altered NO-PGF pathway would lead to deregulation of the maintenance of uterine relaxation and overcome abnormal angiogenesis and placental circulation. Supported by CONICET Grant Resol.Nr.1008 and PIP CONICET 2309 (Argentina).

ADENOSINE TRANSPORT VIA EQUILIBRATIVE NUCLEOSIDE TRANSPORTERS 1 AND 2 ARE NOT MODULATED BY INSULIN IN HUMAN UMBILICAL VEIN ENDOTHELIUM UNDER HYPOXIA

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Adenosine uptake is mediated by Na^+-independent, human Equilibrative Nucleoside Transporters 1 (hENT1) and hENT2 in HUVEC. hENT1-mediated adenosine transport is inhibited by low oxygen tension (hypoxia) in this cell type. The aim of this study was to characterize the effect of insulin on hENT1- and hENT2-mediated adenosine transport in HUVEC under hypoxia. **Methods:** HUVEC were cultured in normoxia (5% O_2, 12 h) and then exposed for 24 h to 5 or 2% O_2 (hypoxia) in a sealed hypoxic chamber. Cells were incubated with 1 nM insulin for the last 8 h of hypoxia incubation period. [^{3}H]Adenosine transport (7.8-500 μM, 4 μCi/ml, 20 s, 22ºC) was measured in absence or presence of 1 μM nitrobenzylthioinosine, 2 mM hypoxanthine, or both. **Results:** Hypoxia reduced hENT1- and hENT2-mediated uptake. Insulin decreased the maximal velocity for hENT1-mediated (2.4-fold), but increased hENT2-mediated (2.7-fold) transport in normoxia, without significant changes in the apparent K_m. However, insulin did not alter hENT1- or hENT2-mediated adenosine transport under hypoxia. **Conclusions:** These results demonstrate that neither hENT1-, nor hENT2-mediated adenosine transport is modulated by insulin under hypoxia in HUVEC. This could be due to reduced insulin sensitivity by this cell type under hypoxic conditions. Supported by FONDECYT 1030781/1030607/7050030 (Chile).

MECHANISM OF PERSISTENT ALTERATIONS AFTER RECOVERY OF ACUTE RENAL FAILURE: CONTRIBUTION OF KALLIKREIN AND CYCLOOXYGENASE-2

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In acute renal failure (ARF) after the tubular necrosis there is a functional recovery at 35 days post-ischemia. Despite of the recovery, long term follow up of patients indicate that they have a susceptibility to develop renal diseases and hypertension. We have recently demonstrated a persistent alteration of the expression of kallikrein in the recovery phase of ARF. Since Cyclooxygenase-2 (COX-2) is regulated by the kallikrein product, bradykinin and both kallikrein and COX-2 contribute to sodium excretion and are renoprotective, we hypothesized a deficiency of COX-2 in this model. **Methods:** ARF was performed by bilateral renal ischemia on male SD rats (200-250gr). At 35 days after ARF rats were divided in two groups (n = 4 each) and were placed on a normal-salt diet (NS; 0,8% NaCl) or a high-salt diet (HS; 6,9% NaCl), and were compared with sham rats. The animals were sacrificed at the 35, 42, 49 and 56 days post ARF. Renal kallikrein and COX-2 were studied by inmunohistochemistry and Western blot. **Results:** In the recovery phase of ARF was observed a decreased expression of both kallikrein and COX-2. HS diet further decreased COX-2 whereas on NS diet COX-2 recovered normal values. Regardless of the diet, kallikrein levels persisted low. **Conclusions:** Since kallikrein and COX-2 are renoprotective and contribute to sodium excretion, our results suggest that the deficiency of both can contribute to the susceptibility to develop renal diseases and hypertension observed after recovery of ARF. Additional work is necessary to test if they will develop salt-sensitive hypertension when placed on a long term high sodium diet. Supported by FONDECYT 1050977.

DIFFERENTIAL FOS EXPRESSION IN NEURONS OF THE ASCENDING AROUSAL SYSTEM IN SEXUAL BEHAVIOR

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The appetitive phase of motivated behaviors is accompanied by behavioral arousal. Behavioral arousal results from the concerted activity of wake-active neurons collectively named the ascending arousal system (AAS: tuberomammillary nucleus, TMN; lateral hypothalamic area, LHA; ventral tegmental area,VTA; dorsal raphe, DR; latero dorsal tegmental nucleus, LDT; locus coeruleus, LC). While the TMN is responsible for the arousal during feeding, it is not known how the different nuclei of the AAS contribute to the arousal in sexual behavior. The aim of this research was to determine whether the different nuclei of the AAS activate simultaneously, or differentially, in anticipation of the copula. **Methods:** Neural activation was studied using Fos-ir in the AAS, and in the medial preoptic area (mPOA), known to be active during sexual behavior. Adult male rats were exposed during 30, 60 and 120 min to receptive (proestrous) or nonreceptive (diestrous) females, preventing the copula. **Results:** Males showed significantly increased approaches and sniffing time of the receptive females compared to nonreceptive females. After 30 min of exposure to receptive females the TMN, DR, LC and orexin neurons of LHA showed significant neural activation. Fos-ir declined after 60 and 120 min, while the mPOA became active. Only the TMN and LC activated when the males were exposed to nonreceptive females. **Conclusions:** Our results suggest that simultaneous activation of the TMN, LHA, DR and LC is responsible for the arousal during the appetitive phase of sexual behavior, contrasting with the single involvement of TMN in feeding-related arousal. Financed by Fondecyt 1020718 and DIPUC.

PLACENTARY HISTOLOGICAL ALTERATIONS IN HIV-1 PATIENTS

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HIV-1 infection is a world health problem. In the last years there has been an increase in the number of women and consequently children infected by the virus. This occurs because vertical transmission is responsible for 90% of the notified cases in children and among them 35% occurs in uterus. Different studies of placenta morphology have given controversial results in relation to the existence or not of morphological alterations. Therefore this study is exceedingly relevant. Our purpose is to study the histology of term placentas of HIV-1 women with CD4 over 200 cells/mm^3 with anti-retroviral therapy (ARVT), comparing them with a control group. **Methods:** we have collected fragments of the foetal and maternal faces of 10 samples of HIV-1 placentae and 10 samples of HIV-1 placentae from women without morbidities at the Gaffré e Guinle Hospital. Samples were stained with hematoxiline-eosine and observed by confocal and optical microscopy. **Results:** We did not observe villites nor corioamniotites. Hofbauer cells revealed hyperplasia and hypertrophy, whereas stroma cells presented a larger quantity of granules. We observed calcifications in the stroma of several corionic villosities and basal membranes were enlarged. There was also an enlargemement of terminal villosities blood vessels. **Conclusions:** Although we found alterations in the placenta morphology, we cannot still define their importance in the vertical transmission and further studies are necessary to clarify such facts.

HISTOLOGICAL STUDY OF THE EFFECTS OF RESPIRATORY EXPOSURE OF WOMEN TO THE CIGARRETTE SMOKE

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Among the drugs which damage the neonate, tobacco is one of the mostly used during pregnancy, being associated not only to the increase on the risk of perinatal death but also associated to prematurity and pregnancies with low weight at birth, causing placentary morphological disorders. We have used in this study ten termly placentae obtained from cesarean section of pregnant women aged up to 30, cigarette smokers, non drinkers, free from infections, pre-eclampsia and diabetes, having been compared to other 10 of non-smokers, which have been coloured with hematoxilline and eosine and observed by means of confocal and optical microscopy. **Results:** The morphological study of the placentae of smoking pregnant women has demonstrated a greater placentary weight, the presence of infarctions and calcification, an enlargement of the villosities capillary ramification and density, and an increase of the trophoblastic volume. A thickening of the basal membranes has been observed. Such alterations have not been found in the control group. **Conclusions:** Cigarrette smoking during pregnancy implies alterations which may compromise placentary foetal circulation. Adaptative angiogenesis tries to compensate the reduction of gases exchange area and nutrients between the mother and the foetus. These alterations are responsible for intra-uterine foetal development and growth deficiency.

EXPRESSION OF KALLIKREIN, B2-BRADYKININ RECEPTOR AND ENDOTHELIAL NITRIC OXIDE SYNTHASE IN GUINEA-PIG UTERO-PLACENTAL UNITS ALONG EARLY, MID AND LATE PREGNANCY

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To study the participation of interrelated vasodilators in key sites for the process of uterine spiral artery transformation, the immuno-histochemical expression of kallikrein, B2-kinin receptor (B2R) and endothelial nitric oxide synthase (eNOS) was evaluated in cytotrophoblasts, syncytial streamers, interstitial and endovascular cytotrophoblast in the guinea-pig utero-placental interface. **Methods:** Utero-placental units were obtained from early (22-26), mid (36-39) and late pregnancy (45-50 days) in 6 guinea pigs. Formalin-paraffin embedded utero-placental sections were immunostained with kallikrein antiserum, anti B2R and anti-eNOS using biotin-streptavidin/DAB. To identify cytotrophoblasts, smooth muscle and endothelial cells we used antibodies against cytokeratins, smooth muscle actin and von Willebrand factor respectively. Controls were done in absence of first antibody. **Results:** In early and mid pregnancy, cytotrophoblasts, and syncytial sprouts expressed kallikrein, B2R and eNOS. Syncytial streamers invading the endometrium in mid pregnancy towards blood vessels expressed kallikrein, B2R and eNOS. In late pregnancy peri and intraarterial trophoblasts were stained for kallikrein, B2R and eNOS. **Conclusions:** The expression and localization of two interrelated paracrine vasodilator systems in the invading trophoblasts support our hypothesis that vasodilator factors participate in trophoblast invasion, in spiral artery vasodilatation and in the subsequent enhancement of uteroplacental blood flow, and thus in the development and maintenance of feto-placental perfusion. Supported by Fondecyt 1050707 (Chile).

T LYMPHOCYTES SUBPOPULATIONS, PROGESTERONE AND PROLACTIN DURING SWINE IMPLANTATION

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It has been shown that progesterone (P_4) and prolactin (Prl) have an immunoregulatory action on T lymphocytes as they allow successful implantation and pregnancy. The aim of this study was to investigate the relation between T lymphocyte subpopulations with P_4 and Prl. This study was carried out on pregnant gilts of 10 and 30 days of gestation and non-pregnant gilts. Blood was collected with and without heparine, to obtain lymphocyte and serum respectively. T lymphocytes subpopulations were examined by flow cytometry using a panel of monoclonal antibodies to pig lymphocytes CD4 and CD8 surface antigens. The serum concentrations of progesterone and prolactin were determined by RIA. $CD8^+$ and $CD4^-CD8^-$ lymphocytes in peripheral blood predominate in porcine compared to other species. During peri-implantational period there is a significant increase in the $CD4^+CD8^+$ subpopulation in peripheral blood in relation to the non-pregnant gilts ($p<0.05$). The $CD4^-CD8^-$subpopulation ($p<0.05$) diminishes significantly after implantation. The P_4 and Prl are significantly high ($p<0.01$) during the peri-implantational. The decrease in double negative cells during post-implantational period occurs together with a decrease in the citolytic activity of such cells at uterine level. P_4 would favour the migration of double negative lymphocytes towards uterine tissue. Prl stimulates the citotoxic activity of NK cells which would show the importance of Prl as a regulatory hormone besides its luteotrophic role. Both hormones, would regulate the immune functions which are crucial for the beginning and development of gestation. Supported by FONCyT and SECyT (Argentina).

$P2Y_1$ AND $P2X_1$ NUCLEOTIDE RECEPTORS ARE DISTRIBUTED DIFFERENTLY ALONG THE HUMAN PLACENTA VESSELS

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Extracellular nucleotides act on P2Y or P2X receptors. We assessed the endothelium/smooth muscle distribution and functional activity of P2Y and P2X receptors along placental vessels. **Methods:** Caesarean placentae were used to obtain umbilical and chorionic vessels. Nucleotide receptor mRNA and protein expression were detected by RT-PCR and immunobloting. **Results:** The mRNAs coding for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$ and the $P2X_1$ receptor were detected by RT-PCR. Immunoblots for $P2Y_1$ revealed similar receptor levels in the endothelium and smooth muscle for umbilical vessels. In vessels distal to the cord, the distribution shifted towards a greater proportion of this receptor in endothelial cells. $P2Y_2$ receptors were detected in all placental vessels. 2-MeSADP and UTP, preferential $P2Y_1$ and $P2Y_2$ receptor ligands respectively, contracted isolated umbilical and chorionic vessels, but relaxed *ex-vivo* perfused cotyledons, indicating that the differential regional localization is related to functional differences in the coupling of the receptor to signalling pathways. In contrast, $P2X_1$ receptors expression is similar in the cord, chorionic vessels and cotyledons with or without endothelium. $P2X_1$ receptor inmunoblots showed a significantly larger expression in chorionic arteries and veins and the umbilical vein closer to the placenta, indicating a gradient of protein expression towards placenta vessels. **Conclusions:** $P2Y_1$ and $P2X_1$ receptors are unevenly distributed along the human placenta vasculature highlighting their putative role in maternal-foetal blood flow distribution. Funded by FONDAP and MIFAB grants.

STUDY OF PLACENTAL MORPHOMETRY AND MATERNAL-FETAL DNA DAMAGES IN DIABETIC RATS

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In the streptozotocin-induced diabetic rats, a status that mimics human type 1 diabetes poorly controlled, the placenta is larger and the fetus is smaller compared with non-diabetic. The hyperglycemia not only generates more reactive oxygen species (ROS) but also attenuates antioxidant system. The aim of this study was to evaluate placental morphometry, and maternal and fetal DNA damage of diabetic rats. **Methods:** Diabetic pregnant rats (>200 mg/dl) were anaesthetized at day 21 of pregnancy, and placentas were collected and processed for morphometrical analysis using computer image analyzer. Maternal and fetal blood samples were used to determine DNA damage using Comet assay. **Results**: Diabetic rats presented increased decidua mean area and decreased labyrinth mean area; increased placental weight (0.62) and index (0.15) compared to non-diabetic rats, respectively. There were no changes in spongiotrophoblast mean area between groups. Maternal and fetal DNA damages were increased in diabetic rats related to non-diabetic group. **Conclusions:** Maternal diabetic status and hyperglycemia-induced ROS caused maternal and fetal DNA alterations and placental morphological changes, which caused placental weight and index disturbances. Supported by FAPESP (Brazil).

ROTTLERIN MODIFICATION OF CFTR Cl^- CHANNEL MATURATION AND FUNCTION SEEMS NOT TO BE RELATED TO PKC NOVEL ISOFORM INHIBITION

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The CFTR Cl^- channel has several consensus sites for phosphorylation by protein kinase A (PKA) and by PKC. The channel is acutely activated by PKA, but its function and expression is modulated by PKC. Our aim was to study the effect of PKC inhibitors on CFTR protein expression, localization and function. **Methods:** HEK293 cells stably expressing CFTR (HEK293-CFTR) were exposed for 16 h to PKC inhibitors bisindolylmaleimide 1 (Bim1, 500 nM, a generic PKC inhibitor), Gö 6976 (1 μM, conventional isoforms), or rottlerin (6 μM, novel PKCδ inhibitor). Total CFTR protein was quantified by western blotting and chemiluminescence. Forskolin-induced CFTR currents were recorded using perforated, whole-cell patch-clamp. CFTR was visualized by confocal microscopy. **Results:** Exposure of HEK293-CFTR cells to rottlerin resulted in a minor reduction of the mature form of CFTR (band C) and in a 50% increase of the immature form (band B). The increase of this partially glycosylated form of CFTR correlated with the appearance of a well-defined, single-spot cytosolic accumulation of CFTR fluorescence label, which did not colocalize with ER or Golgi. Additionally, whole-cell forskolin-activated CFTR Cl- currents were reduced by 40% in rottlerin-treated cells. Remarkably, the effects induced by rottlerin were not reproduced by the broad PKC inhibitor Bim1 or by cPKC inhibitor Gö 6976. **Conclusions:** Our results suggest that in this conditions CFTR expression and function is not modulated by PKC and that rottlerin effects are not mediated by δ isoform of novel PKC. Supported by FONDAP 15010006.

COMPARATIVE TOTAL CURRENTS BETWEEN APICAL SYNCYTIOTROPHOBLAST MEMBRANE IN NORMAL AND PRE-ECLAMPTIC HUMAN PLACENTA TRANSPLANTED TO *Xenopus laevis* OOCYTES.

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Placental transfer involves specific transport mechanisms through both apical and basal plasma membranes. Pre-eclampsia is a pregnancy disease that affects multiple functions, among them transport function, with consistent fetal growth restriction. Our previous results showed that the open probability (P_o) of the Maxi-chloride channel present in microvillous membrane from pre-eclamptic placentas becomes voltage independent, at difference from normal placentas. The aim of this study was to compare total currents of *Xenopus laevis* oocytes transplanted with microvillous membrane from normal (MVM) and pre-eclamptic (MVMpe) placentas. **Methods:** Two electrode voltage clamp technique was performed in *Xenopus* oocytes injected with MVM or MVMpe purified vesicles. Functional incorporation of foreign channels into the oocyte membrane was assessed by recording membrane currents (I) in response to voltage pulses from holding potential -100mV up to +60mV, in 20mV steps in uninjected (control), MVM and MVMpe injected oocytes. **Results:** I/V relationship showed differences between injected oocytes (MVM and MVMpe) and uninjected oocytes. Current intensity at +60mV was 2.5 fold in MVM injected oocytes (n=21), 8 fold in MVMpe injected oocytes (n=44), compared with uninjected oocytes (n=60). **Conclusions:** These results are consistent with those obtained from single channel recordings. Higher currents would be expected for MVMpe injected oocytes due to P_o alterations detected in single chloride channels recordings in pre-eclamptic placentas. Supported by FONDECYT 1040546, Beca AT-403031 CONICYT and Beca PG/48/2004 U. de Chile (Chile).

TISSUE LEVELS OF cGMP ARE DEPENDENT ON THE TENSION OF THE SMOOTH MUSCLE

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Cyclic nucleotides are essential for vascular smooth muscle dilatation; therefore, we assessed the importance of the basal tissue tension, intra and extra cellular calcium sources and gap junction functioning on the tissue content of cGMP in isolated segments of rat aortas. **Methods:** Segments of the thoracic aorta from adult Sprague Dawley rats were mounted in superfusion baths and maintained with Tyrode buffer (37°C, bubbled 95% O_2/5% CO_2) at different tensions. Isometric contraction was recorded; RIA assayed tissue cGMP content. **Results:** The cGMP content was dependent on the tension at which the aortas were artificially maintained. At 0 tension it was 0.067±0.012 pmol/mg tissue; while at 0.7, 1.5, 2, y 4 g tissue tension, the cGMP levels were 0.12±0.02, 0.27±0.07, 0.54±0.02 and 0.25±0.07 pmol/mg tissue respectively. The rise in cGMP tissue content elicited by 2g tension was annulled following endothelium denudation, raising the hypothesis that the signal triggering cGMP production must come from the endothelium. The calcium channel agonist (+)202791 increased concentration-dependently the tissue cGMP content while the calcium channel antagonist (-)202791 or nifedipine, decreased in a concentration-dependent manner the cGMP content in aortas with tension. Thapsigargin, decreased concentration dependently cGMP levels. Gap junction blockade with GAP27 or 18-□-glycerrhetinic acid, reduced concentration-dependently the cGMP level in tissues with 2g tension. **Conclusion:** the aorta cGMP content depends on the tissue tension and on endothelium integrity; calcium movement probably through gap junction is also essential. Funded by FONDAP and MIFAB grants.

PREGNANT RATS TREATED WITH A SEROTONIN PRECURSOR HAVE A REDUCED PLASMA VOLUME EXPANSION

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Fetal growth restriction and preeclampsia are pregnancy complications characterized by high vascular resistance and inadequate plasma volume expansion, probably secondary to an abnormal vasodilator/vasoconstrictor ratio. The aim of the present study was to test the hypothesis that the administration of a serotonin precursor (5-hidroxytryptophan, 5HTP) to pregnant rats would limit volume expansion and fetal growth. **Methods:** At day 13, pregnant Sprague-Dawley rats were randomly assigned to a 5HTP group (100 mg/kg ip, n=10) or to a control group (C, n=9). Both groups were studied at d21, after overnight urinary collection. Additional C and 5HTP rats were treated with ketanserin, a $5HT_2$ receptor antagonist. Statistical analysis was performed by ANOVA and values are expressed as mean ± sem. **Results:** 5HTP rats had lower plasma volume (C=22±1.1, 5HTP=17±0.7 ml, P<0.001), kallikrein activity (C=1297±189, 5-HTP=232±5.1 nmoles/16h, P< 0.001), and creatinine clearance, although blood pressure was only slightly increased. Litter size and fetal weight (C=5.5±0.8, 5-HTP=4.2±0.2 g, P<0.001) were reduced. Renal architecture was grossly altered. All these abnormalities were abolished by the use of Ketanserin. **Conclusions:** Present data indicate that the administration of a serotonin precursor to pregnant rats limits plasma volume expansion and fetal growth via $5HT_2$ receptors, suggesting a possible role for serotonin in abnormal pregnancy.

BRAIN HYPOMETABOLISM AFTER 24h OF HYPOXEMIA IN THE LLAMA FETUS

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The llama (*Lama glama*) is a specie adapted to live in chronic hypoxia. The ability of the llama fetus (FLL) to grow and develop normally under this condition suggests that has adaptive mechanisms to withstand chronic hypoxia. We searched evidence to support that to respond to prolonged hypoxia, the FLL reacts with adaptive brain hypometabolism. Since Na-K-ATPase activity is tightly associated to cell O_2 consumption, we determined its activity and measured brain temperature (T°). Also, we looked for apoptosis in the brain cortex of FLL. **Methods:** Under general anaesthesia, brain and core T° from FLL were determined 1h before, and every hour during 24h of hypoxemia (n=5). A normoxemic group was the control (n=5). After 24h we determined brain cortex Na-K-ATPase activity and ouabain binding. Brain cortex apoptosis was assessed by using the PARP proteolysis. **Results:** We found a mean decrease in brain cortex T° of 0.56°C during prolonged hypoxemia, which was accompanied by a decrease in brain cortex Na-K-ATPase activity of 51%. These changes occurred in absence of apoptosis. **Conclusions:** All these results provide evidence to support that the FLL responds with adaptive brain hypometabolism to prolonged hypoxemia. Supported by FONDECYT 1020599 (Chile).

ASSESSMENT OF CARDIOVASCULAR MONITORING IN EMERGENCY CESAREAN SECTION. UNIVERSITY OF CHILE CLINICAL HOSPITAL

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During labour the fetus is exposed to intrauterine hypoxia. Cardio-fetal monitoring assesses indirectly the respiratory function of the placenta during this period. Cardio-fetal monitoring has an accuracy of 90% during normal states, and a 15% in pathological situations. **Methods:** Forty nine monitoring reports from emergency caesarean section in the Clinical Hospital of the University of Chile were checked, all of them prescribed because of pathological cardio-fetal reports. Variables analyzed were: kind of abnormality in cardio-fetal monitoring reports, presence of meconio, circulars of the umbilical cord, Apgar Score, umbilical cord gases and pH, neonatal reanimation procedures and hospitalisation. **Results:** The most frequent pathological monitoring patterns were: Maintained Bradycardia (34.7% Complicated Desacelerations), Variables (14,2 %) and patterns with a decrease in Variability (14.3%). In 51% of the cases meconio was absent, with a positive predictive value VP(+) of 20.4% related with maintained bradycardia. In 71% of cases did not have umbilical cord circulars, and 83.7% of the new borns did not develop neonatal depression. VP(+) was 16.3% in pathological reports when related with the Apgar Score. A 53% of the new borns had umbilical blood pH >7.15, with a VP(+) of 24.5% when related with pathologic reports, pCO_2 was <60 mmHg in 55.1% and pO_2 was >11 mmHg in 53.1% of the cases. 69.5% had normal rates of excess base (BE). Over 63% of the new borns did not require oxygen and 6.1% were hospitalised. Patterns of cardio-fetal monitoring with Moderated Bradycardia were not related to neonatal depression in the first 5 min of life. **Conclusions:** Pathological cardio-fetal monitoring reports have a VP(+) of 16.3% when related with Apgar scores and a VP(+) of 24.5% regarding the pH of the umbilical cord blood. These findings suggest that this should be a reliable way to assess the respiratory function of the placenta.

EXPRESSION OF *rPer2* IN CEREBRAL CORTEX IN FOOD ENTRAINED RATS

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The circadian rhythms are controlled by a master clock, the suprachiasmatic nucleus (SCN). The molecular mechanism of the circadian activity of single SCN neurons consists of autoregulatory transcriptional and translational feedback loops systems, being the protein PER2 a critical negative component of this feedback loops. Rats under a restricted feeding schedule (RF) show food-anticipatory activity (FAA) characterized by increases in locomotor activity and core temperature. This FAA is controlled by a food entrainable oscillator (FEO) different from the SCN. Per2 expression in the cerebral cortex, but not in the SCN, is sensitive to RF, and lesion of medial prefrontal cortex prevents the anticipatory increase in temperature. The objective was to determine which cortical areas changed their temporal pattern of *rPer2* expression in response to RF. **Methods:** Locomotor activity and body core temperature were analyzed during 12 days under RF by radiotelemetric recording, and *in situ* hybridization for *rPer2* was assessed in rats killed during the hour preceding food arrival. **Results:** We found increased locomotor activity and core temperature in anticipation of meal time, in parallel with increased *rPer2* expression in the prefrontal cortex. **Conclusions:** We propose that the prefrontal cortex is one output of the FEO. Supported by FONDECYT 1020718.

DEFEROXAMINE INCREASES THE EXPRESSION OF KALLIKREIN IN A CELLULAR LINE OF HUMAN CYTOTROPHOBLASTS

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In human placenta, the temporal expression of kallikrein – an enzyme generating vasodilatory kinins - in trophoblast is associated to that of the O_2 changes along gestation. A binding site for Hypoxia Inducible Factor-1 (HIF-1) was identified at 1582-1596 bp in the regulatory sequence of the kallikrein gene with the MatInspector software. With the hypothesis that trophoblast expression of kallikrein is regulated by O_2 tension through HIF-1, we determined the effect of chemically induced hypoxia on the kallikrein expression in cytotrophoblasts. **Methods:** BeWo cytotrophoblasts (ATTC number: CCL98) were cultured in Hanks F-12 medium supplemented with 10% FBS and maintained at 37°C in 95% air-5% CO_2. Twenty four hours previous to the experimental intervention cells were serum deprived, and were then cultured in the presence of deferoxamine (DFO) at 100μM for 0-3-6-12-24 h. The expression of kallikrein was analysed through inmunocytochemistry with biotin-streptavidin/DAB in 3 experiments, and by western blot in 10% polyacrylamide gel using a polyclonal kallikrein antibody (1:1000). Quantitative analysis of the intensity of the immunocytochemical expression per cell was performed with Image ProPlus and ImageJ softwares in 3 monolayer fields x 400 x coverslip. **Results:** DFO treated cells showed a 2.4 increase in kallikrein expression at 3 h ($p< 0.05$ by ANOVA and post hoc Tukey test), while no changes were observed at 6, 12 and 24 h. Western blot showed an increase of kallikrein at 6 h. **Conclusions:** The kallikrein expression in BeWo cells increased during DFO treatment, suggesting a dependence of oxygen, probably mediated by HIF-1. FONDECYT 1050707 (Chile). C. Escudero holds a MECESUP-PhD fellowship.

HYPOXIA-INDUCED HEME OXYGENASE-1 REGULATES THE L-ARGININE/ NITRIC OXIDE PATHWAY IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Hypoxia decreases the activity of L-arginine/nitric oxide (NO) pathway (Arg/NO) and upregulates the heme oxygenase (HO)/carbon monoxide (CO) pathway (HO/CO) in endothelial cells. HO/CO and Arg/NO modulate each others activity. We determined whether the hypoxia-induced HO-1 expression regulates L-arginine transport and NO synthesis in human umbilical vein endothelium (HUVEC). **Methods:** Cells were isolated, cultured in medium 199 containing 20% sera (Ethics Committee approval was obtained) and exposed (3-24 h) to normoxia (5% O_2) or hypoxia (2% O_2). L-Arginine transport (5-1000 μM) was measured in the presence or absence of Zn protoporphyrin IX (ZnPP, 1-100 □M, HO inhibitor). eNOS and HO-1 mRNA were determined by RT-PCR. eNOS activity was measured as L-[^{3}H]citrulline formation from L-[^{3}H]arginine (4 μCi/ml, 30 min, 37°C). HO-1, total and phosphorylated eNOS (p-eNOS, Ser^{1177}) proteins were detected by Western blot. **Results:** In normoxia ZnPP initially (1-3 h) stimulated and later (12-24 h) inhibited L-arginine uptake (25-70%). eNOS mRNA level was significantly up-regulated at short-term (3-6 h), but decreased at long-term (24 h) hypoxia. Hypoxia significantly reduced p-eNOS/total eNOS protein and eNOS activity (92%). Parallel to an increased HO-1 mRNA and protein in hypoxia, a decrease in eNOS activity was observed. **Conclusions:** Hypoxia-induced up-regulation of HO-1 decreases L-arginine/NO pathway suggesting that expression and activity of HO-1 could be involved in the L-arginine transport inhibition in hypoxia. Supported by FONDECYT 1030607/1030781/7050030 (Chile). V.G. holds a PUC-MSc fellowship.

DIFFERENTIAL FEMORAL AND PULMONARY ADRENERGIC VASCULAR REACTIVITY IN HIGH AND LOW ALTITUDE NEWBORN LAMBS

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Chronic hypoxia produces changes in the arterial endothelium and smooth muscle, altering their response to vasoactive agents, among them catecholamines. Moreover, noradrenaline (NA) plasma levels are increased in high altitude (HA) new born lambs (NBL). We hypothesized that HA NBL (3589m) have a higher adrenergic vascular reactivity in femoral and pulmonary beds compared to low altitude (LA) NBL (580m). **Methods:** NBL (5 HA and 5 LA) were euthanized following animal guidelines and ethics committee approval. Small femoral and pulmonary arteries (200-400μm) were dissected and mounted on a wire myograph. Isometric force and concentration response curves were performed for K^+ (6 to 125mM); NA (10^{-10} to 10^{-3}) and phenylephrine (Phe, 10^{-10} to 10^{-3}). **Results:** In femoral arteries, adrenergic responses to NA and Phe were higher in HA NBL (139% and 116% maximum K^+-induced contraction (K^+_{max}), respectively) than in LA (105% and 57.2% K^+_{max}, respectively). In pulmonary arteries, Phe responses were lower in HA NBL (36.9% K^+_{max}) than in LA NBL (56.4% K^+_{max}). In contrast, the responses to NA were similar between HA and LA NBL. **Conclusions:** Femoral arteries from HA NBL have higher adrenergic reactivity compared to LA NBL. This higher adrenergic reactivity could help to maintain a higher femoral vascular resistance in highland NBL. In contrast, the higher pulmonary artery pressure observed in HA NBL is probably not mediated by the adrenergic system. Supported by: FONDECYT 1050479(Chile), Wellcome Trust CRIG 072256 UK.

EXPRESSION OF EXTRACELLULAR MATRIX (ECM) COMPONENTS IN PLACENTAS OF DIABETIC RATS

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Placental development depends directly on extensive ECM remodeling and it is altered in pathological conditions, such as diabetes. Impaired growth potential and increased fetal morbidity observed in this condition may be related to abnormal placentation. Our aim was to determine whether maternal diabetes affects the placental expression of ECM components, such as perlecan, fibronectin and laminin (α1, β1, β2). **Methods:** Diabetes was induced in pregnant female Wistar rats, by a single injection of alloxan (40mg/kg i.v.) in the second day of pregnancy. The diabetic rats were also treated with insulin (1IU s.c., 48/48h). Control animals received vehicle injections. Pregnancy was interrupted at days 14, 17 or 20, and the placentas were removed and frozen in liquid nitrogen. ECM components mRNA expression was evaluated by RT-PCR. **Results:** Maternal weight gain was lower in diabetic rats. Decreased body weight was observed in the offspring from diabetic rats. The number of implantation as well as the rate of no-viable implantation was higher in the diabetic groups. Placental expression of ECM components changed along the pregnancy: increased fibronectin expression was seen at day 17; decreased perlecan and α1 laminin expression was observed at days 17 and 20; β2 laminin expression decreased during pregnancy development whereas β1 laminin expression did not change. The increased fibronectin expression at day 17 as well as the decrease in β1 laminin expression were enhanced in placentas from diabetic rats. **Conclusion**: Maternal diabetes affects the expression of specific ECM components during placentation. Supported by CNPq, FAPESP (Brazil) and DIPUV (University of Valparaíso, Chile).

VASOMOTION IN HUMAN UMBILICAL VEINS: ROLE OF CALCIUM AND GAP JUNCTIONS

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Vasomotion has not been properly characterized in human blood vessels. To assess the role of gap junctions, calcium movements and the underlying ionic basis of vasomotion, we used placental and umbilical cord vessels as a model bioassay. **Methods:** Isolated rings from these vessels were used to record spontaneously isometric oscillatory waves using force displacement transducers. **Results:** Umbilical cord vein rings presented rhythmic contractions occurring with a frequency of 1.5±0.01 min^{-1} (n=135) and 274±2.2 mg in amplitude, which corresponds to 11.1±0.4% of the maximal KCl contracture. Vasomotion waves were recorded for 8 hours; their amplitude and duration was larger in umbilical veins than arteries or chorionic vessels ($p<0.001$). Segments of the umbilical vein closer to the fetus showed the largest amplitude. Gap junction blockers: peptide Gap 27, 18□-glycyrrhetinic acid, and octanol, reduced the amplitude but not the frequency. Regarding the ionic basis, potassium channel blockers and L-type calcium channel blockers reduced wave amplitude but not vasomotion frequency. The role of intracellular calcium stores was evidenced using calcium-free buffer, which reduced oscillation amplitude and basal tension. Cyclopiazonic acid confirmed the role of intracellular calcium by increasing wave amplitude, basal tissue tension and reducing oscillatory frequency. **Conclusions:** We suggest that pacemaker cells in the smooth muscles of the umbilical cord trigger oscillatory waves, which are synchronized and propagated via gap junctions; internal calcium reservoirs and K_{ATP} channels are essential for vasomotion. FONDAP 013980001 and MIFAB Institute grant funds.

IVERMECTIN IS A SELECTIVE MODULATOR OF THE PURINERGIC $P2X_4$ RECEPTOR

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Ivermectin (IVM) is a widely used antiparasitic drug known to act on glutamate and $GABA_A$ gated chloride channels. IVM has also been reported to be an allosteric modulator of chicken and human □$_7$ nicotinic receptors and of the $P2X_4$ purinergic receptor. In order to identify the putative allosteric site of IVM, we now characterize its mechanism of potentiation by comparing it to the modulation by zinc and examined its specificity for the $P2X_4$ receptor among other P2X members. **Methods:** The cDNA of the $P2X_4$ receptor was injected intranuclearly to *X. laevis* oocytes and the currents evoked by ATP and the modulation by IVM were tested with the two-electrode voltage-clamp technique. **Results:** IVM (3nM-10μM) potentiated the currents evoked by ATP on the $P2X_4$ receptor, but not on $P2X_2$ and $P2X_7$ receptors. 3 μM IVM reached a maximal 3-fold potentiation within 2-min of pre-incubation and remained constant after longer pre-exposures. We compared the effect of IVM with the trace metal zinc, another positive modulator of $P2X_4$ receptor. 10 μM zinc potentiated the activity of the receptor about 2.5-fold, similarly to IVM. When zinc and IVM were co-applied together, an additive effect was observed. To address whether IVM and zinc act at a common site, experiments will examine the effect of IVM on $P2X_4$ receptor mutants insensitive to zinc-modulation (C132A). We will also examine the mechanism of IVM potentiation on the ATP by analyzing its effect on ATP concentration-response curves. **Conclusions:** IVM appears to be a specific positive allosteric modulator of the $P2X_4$ receptor which probably acts at a site different from the zinc allosteric site. Supported by FONDAP 13980001 and MIFAB grants.

DETERGENT–RESISTANT MEMBRANE MICRODOMAINS IN APICAL MEMBRANES FROM HUMAN SYNCYTIOTROPHOBLAST

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The human syncytiotrophoblast separates maternal and fetal blood, and may be viewed as an epithelium. Previously, we have described two fractions within the microvillous membrane using differential sucrose density migration: a heavy fraction (H-MVM) and a light fraction (L-MVM). Functional compartments have been described in many cell membranes, characterized by specific lipid and protein composition, and are thought to participate in membrane trafficking and signaling events at the cell surface. Membrane microdomains are characterized by their resistance to detergent extraction combined with the ability to float during the density gradient centrifugation. Our aim was to explore whether detergent resistant microdomains exist in both purified apical membrane fractions. **Methods:** Detergent-resistant membranes (DRMs) from isolated H-MVM and L-MVM were prepared with 1% Triton X-100 on ice, followed by flotation in sucrose gradient (5-35-40%). Samples were collected after centrifugation and tested by Western and Dot blot for PLAP, Annexin 2, and Glycosphingolipid GM1. **Results:** We have obtained DRMs from samples of the 5% and 35% bands of the sucrose gradient in both purified apical fractions. DRMs from L-MVM showed a consistent peak of the markers used. DRMs of H-MVM had no conclusive pattern: PLAP and GM1 were detected as expected, but Annexin 2 was not present in these DRMs. **Conclusions:** We have found two distinguishable DRM subsets from heavy and light purified microvillous membranes. Our results are the first evidence of the presence of DRM microdomains in apical syncytiotrophoblast membranes. Supported by FONDECYT 1040546, Beca AT-403031 CONICYT and Beca PG/48/2004 U. de Chile (Chile).

NAD(P)H-OXIDASE EXPRESSION IN TROPHOBLAST CELLS

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The NAD(P)H-oxidase is an electron donor, plasma membrane-associated enzyme that catalyses the production of superoxide anion, a reactive oxygen species (ROS) involved in oxidative stress processes. It comprises the membrane-bound components gp91-phox and p22-phox, the flavocytochrome b558 and, the cytosolic components p47-phox, p67-phox and p40-phox. Recent studies show that trophoblast cells are able to produce ROS, an activity that in humans is also implied in the gestational disorder, preeclampsia. Here we investigated the expression of NAD(P)H-oxidase components in the mouse trophoblast. **Methods:** NAD(P)H-oxidase expression was assessed by immunohistochemistry and rT-PCR. Trophoblast cells were obtained from ectoplacental cones of mice embryos at gestation day 7.5. The samples were cultivated by 48 h, treated or not with PMA (100ng/L) for 6-12 h and processed for localization of gp91-phox, p47-phox, p40-phox and p67-phox protein and mRNA. **Results:** The subunits p47-phox, p67-phox and gp91-phox were all expressed in trophoblast cells in the presence or absence of PMA. The presence of PMA increased the protein expression of p47-phox, p67-phox and gp91-phox and the gene expression of p67-phox, p40-phox and gp91-phox. **Conclusions:** Trophoblast cells express the proteins p47-phox; p40phox, p67-phox and gp91-phox associated to the enzymatic NAD(P)H-oxidase activity. However the expression of these components seems to be modulated by specific stimulus suggesting NAD(P)H-oxidase may play an adaptive role in the placenta physiology. Supported by CAPES and FAPESP.

DIFFERENTIATION OF THE MITOCHONDRIAL RESPONSE TO BAX IN RAT SPERMATOGENIC CELLS

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Residual bodies (RB), that are cytoplasm droplets released from spermatids and phagocytized by Sertoli cells during mammalian spermatogenesis, present several characteristics of apoptotic bodies. These properties of RBs have led to propose that in mammalian spermatogenesis occurs "cytoplasmic" apoptosis leaving both mitochondria and nuclei unaffected. In this work we have explored the hypothesis that mitochondria in spermatogenesis are undergoing a functional differentiation that makes them refractory to the action of pro-apoptotic stimuli such as Bax. **Methods:** Recombinant Bax tagged with a poly-His tail was generated by amplification in E-coli and purified by Ni^{2+} chromatography. Cytochrome c release was measured by Western blots of supernatants from digitonin-permeabilized spermatogenic cells. Mitochondrial membrane potential was estimated from measurements of excitation ratios of rhodamine 123 fluorescence. **Results:** Pachytene spermatocytes mitochondria respond to Bax with release of cytochrome c and membrane potential depolarization in a dose-dependent fashion. Instead, round spermatids are refractory to the actions of Bax on cytochrome c release and membrane potential depolarization. **Conclusions:** Our results are in agreement with the idea that post-meiotic mitochondria in spermatogenic cells are protected from apoptotic stimuli. Supported by FONDECYT 1020927 and 1040800.

EPITHELIAL SODIUM CHANNEL (ENaC) EXPRESSION IN RENOVASCULAR HYPERTENSION AND POSSIBLE REGULATION BY COX-2

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Prostaglandins (PGs) have a key rol in the regulation in Na balance. PGs are produced by two enzymes: COX-1, expressed constitutively and COX-2, highly regulated in the kidney. Selective inhibition of COX-2 causes Na retention and hypertension. A limiting step in Na reabsorption is ENaC. The role of COX-2 in the regulation of α, β and γ subunits of ENaC has not been defined. We hypothesized that ENaC expression is modified by COX-2 inhibition in hypertension. **Methods:** Male SD rats were used. Left renal artery was clipped using 2K1C method to generate hypertension and sham operation served as controls. Six groups were established (n=6/each): 2K1C rats treated with COX-2 inhibitors; specific: Celecoxib (Cele) (20 mg/kg/day), not specific: Ibuprofen (Ibu) (30 mg/kg/day) or vehicle and three sham-operated groups with the same treatment. Kidney function, ENaC and COX-2 expression were studied after 10 days of treatment. **Results:** COX-2 inhibition in 2K1C did not alter Na excretion (*p=ns*) or blood pressure (188,3 ± 10,6 vs 182,5 ± 14,9 mmHg (*p=NS*)) but creatinine clearance was decreased in this group (0,53 ± 0,07 vs 0,8 ± 0,05 ml/min/100g, $p < 0.05$). WB and RT-PCR of COX-2 showed low expression in the non clipped kidney and an increase in the clipped kidney in 2K1C rats with Cele. In sham rats with Cele the expression of COX-2 increased whereas α and β subunits of ENaC decreased. **Conclusion:** COX-2 inhibition decreases glomerular filtration in 2K1C rats. COX-2 expression in clipped and non clipped kidneys suggest a differential regulation. Increased COX-2 and the low expression of α and β subunits of ENaC in Cele-treated sham rats suggest a common regulatory mechanism. Fondecyt 1050977.

MUSCARINIC CHOLINERGIC RECEPTOR EXPRESSION IN PLACENTA FROM RATS EXPOSED TO METHYL PARATHION

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Decrease in the density of muscarinic cholinergic receptors (mChR) due to exposure to organophosphorus pesticides, such as methyl parathion (MP), has been reported in developing brain. The aim of this study was to determine the effect of prenatal exposure to MP on placental mChR (M1 and M2 subtypes) density by immunohistochemistry. **Methods:** Twenty pregnant rats were divided into control and exposed groups. MP was orally administered at 0.0, 1.0, 1.5, and 2.0 mg/kg. Placentas from each group were fixed in 10% formalin, and embedded in paraffin. Histochemical analysis for M1 and M2 mChR localization was done, using specific monoclonal antibodies. Optical density (OD) of mChR signal in placentas was measured by image analysis using the Image ProPlus software. **Results:** The M1 and M2 mChRs were detected in the labyrinth area, mainly in syncytiotrophoblast, with an 10% predominance of M2 over M1. Placentas exposed to MP (1.0, 1.5, and 2.0 mg/kg) showed a decrease in the labeling of M1 mChR (3.42±0.55, 3.49±0.56 and 2.91±0.56 ODs respectively, (geometric mean ± standard deviation) when compared to control group (4.22 ± 0.55 OD, $p<0.01$). Similar results was observed for M2 mChR; a dose-response effect was not detected. **Conclusion:** Our results demonstrated that MP causes a decrement in density of M1 and M2 mChRs, which could impair the placental cholinergic system and, in consequence, it may have a role in regulating transport of nutrients from maternal to foetal interface. However, the functional significance of these findings is yet unknown. Supported by the Margarita Miranda de Mascareñas Foundation.

COMPARISON OF STYLES OF LEARNING IN STUDENTS OF HEALTH AREA AT UNIVERSITY OF ANTOFAGASTA

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The knowledge acquisition entails a diversity of learning forms that are related to intrinsic processes of the individual. The cognitive approaches of learning process given by Kolb (1984), and Money and Munford (1990), propose the learning in phases; each individual concentrates its preferences in certain phase cycle. These preferences are learning styles whose knowledge has been used by some Universities to know the profile students who enter to first year and to modify the education strategies. The objective is to identify and to compare learning styles of first year students of health area at the University of Antofagasta (UA) and to correlate it to the yield of physiology methods. **Methods:** The Questionnaire of Honey-Alonso (1999) was applied to distinguish learning styles: Active (A), Reflexive (R), Theoretic (T), and Pragmatic (P), to a total of 184 students of the first years of Medicine, Dentistry, Medical Technology, Obstetrics and Kinesiology in 2005. The data were tabs and analyzed by statistical test of differences between proportions ($p\geq0.05$). The academic yield was analyzed as official note acts. **Results:** The order of preference of learning styles distributed by race of greater to minor is: Medicine: A, T, P, R; Medical Technology: P, R, A, T; Obstetrics: T, A, R, P; Dentistry: R, P, T, A; Kinesiology: A, R, P, T. The comparison of learning styles, shows that active style was chosen preferred by Kinesiology (55%) followed by Obstetrics and Medicine. The reflective style was indicated in the first place by Dentistry students. Obstetrics shows its theoretical preference (51.2%) whereas the pragmatic style had its greater preference by the students of Medical Technology (50%) followed by Dentistry and Medicine. **Conclusions:** Ours results suggest that students of first year of the area of the health at University of Antofagasta, exhibit different profiles from learning at the beginning of their university studies, which is possibly related to its atmospheres of learning. (Partially supported by Dpto. Biomédico, FACSA, University of Antofagasta).

EARLY PREGNANCY FACTOR (EPF) NEUTRALIZATION IN PREGNANTS RATS

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EPF is a molecule with immunossupressor and growth factor activities involved in pregnancy. The purpose of this work was evaluate *in-vivo* EPF effects on embryos development by neutralizing its activity through porcine anti-EPF pAb in rats. **Methods:** pAbs were obtained in rabbits immunized with synthetic porcine EPF (KTYKSEIAHRDFKLDGQQLY). Mated rats were passively immunized with 500 ug of anti-EPF pAb (Treatment group-T) at 8, 16, 32 and 40 hs post mating, controls were used (saline solution and nonspecific IgG) and the effect on embryo number, weight and size and corpora lutea number (CL) were determined on 10 d of pregnancy. Samples of embryos and their placenta were fixed in phormol to evaluate the development stage. **Results:** The treatment caused a significant decrease in the average of embryos number and in the Embryo/CL ratio compared with the two controls groups ($p\leq0.05$). Embryonic weights and sizes of T group were also significatively decreased ($p\leq0.05$). Microscopic study revealed that embryos of control groups presented a normal development stage. In contrast, the development stage of T embryos were smaller than controls without somites formation. **Discussion:** These results demonstrate that passive immunization affects the growth of embryos, leading to decreasing embryonic development with subsequent failure to implant. The decrease of embryo/CL demonstrates a lack in reproductive efficiency. In addition, embryonic weights and sizes decreased showed an important role of EPF during the early pregnancy as possible growth factor. Research supported by FONCyT (Argentina).

ROLE OF cGMP IN THE RESPONSE TO ATP IN OVIDUCTAL CILIATED CELLS

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Ciliated cells from the oviduct increase the ciliary beat frequency (CBF) in response to ATP. In the airways, nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway enhances the increase of CBF induced by ATP, however in the oviduct NO has the opposite effect. The aim of this study was to determine the role of NO/cGMP in the ATP effect on CBF in the oviduct. **Methods:** In cultures of rat oviductal ciliated cells, CBF and $[Ca^{2+}]_i$ were measured in response to ATP (1-100μM) with photodensitometry and spectrofluorescence (Fura-2AM) respectively. Experiments were done in the presence of ODQ (10μM), a specific blocker of soluble guanylate cyclase or in the presence of 8-Br-cGMP (100μM) a permeable analogue of cGMP. **Results:** ATP induced an increase in $[Ca^{2+}]_i$, that reaches a plateau at 10μM ATP. In the presence of ODQ, we observed a significant decrease ($p<0.05$) in CBF (44.5% vs 14.8%) and $[Ca^{2+}]_i$ expressed as the area under the fluorescence curve (0.85±0.2 vs 0.34±0.1 a.u.). We also observed that the time constant for $[Ca^{2+}]_i$ decaying (τ) was significantly faster in the presence of ODQ (104.3±21.8 sec vs 27.6±5.9). In the presence of 8 Br-cGMP, the ATP response in $[Ca^{2+}]_i$ significantly ($p<0.05$) decreased the area under the curve from 0.56±0.1 to 0.28±0.06 a.u., and τ decreased from 86.9±28.7 sec. to 40.1±13.1. **Conclusions:** These results indicate that NO pathway participates in the modulation of CBF in the oviduct affecting $[Ca^{2+}]_i$ mobilization. Supported by FONDECYT 1040804.

EFFECT OF HIGH GLUCOSE ON B1 KININ RECEPTOR SIGNALING IN RAT ENDOTHELIAL CELLS

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The endothelium is responsible of multiple functions, such as the regulation of hemodynamics. In Diabetes, endothelial dysfunction is the first step to develop vascular disorders. Bradykinin is a nonapeptide that regulates endothelial function through the activation of B1 and B2 kinin receptors (KR). B1KR, is normally expressed in low levels in vascular tissue, but is induced in inflammation/injury. In addition, B1KR signalling is increased in pathological situations. We postulate that B1KR signalling, activated by des-Arg^9-BK (dABK, a B1KR agonist), is modulated by high glucose. **Methods:** Endothelial cells from rat mesentery, were incubated in 5 or 25 mM glucose and stimulated with dABK for different periods. Nitrite levels were determined in the absence or presence of calcium chelators by Greiss method. Levels of p-ERK 1/2, p-ERK 5, e-NOS and i-NOS were measured by western blot, and proliferation by thymidine incorporation. **Results:** NO levels increased by dABK in 25mM glucose were unaltered by calcium chelators. In addition i-NOS levels increased in cells cultured in 25mM glucose, and in mesentery from diabetic rats. In 25mM glucose, dABK produced a transient increase followed by a decrease in p-ERK 1/2, and a decrease in p-ERK-5 compared to 5 mM glucose. Furthermore, dABK reduced ^{3}H-thymidine incorporation in 5mM but not in 25mM glucose. **Conclusion:** These results suggest that i-NOS could participate in the increase in NO observed in response to dABK in 25mM glucose; in addition the decrease in the activity of kinases involved in mitogenic and proinflammatory pathways could be related to the absence in proliferation observed in 25 mM glucose. Supported by FONDECYT 1040809 & 1040816 (Chile).

MATING ASSOCIATED SIGNALS HAVE PROFOUND EFFECTS ON OVUM TRANSPORT PARAMETERS IN RATS

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Oviductal transport of eggs to the uterus lasts 96h in mated (pregnant) and 72h in non mated (cyclic) rats and in both it is reduced to less than 24h after a single injection of estradiol (E_2), although the signaling routes are different. In non mated rats, acceleration of egg transport by E_2 results from non genomic effects that involve protein phosphorilation, whereas in mated rats it results from genomic effects requiring RNA and protein synthesis. Here we characterized the effect of mating on oviductal transport responses to E_2. **Methods:** We determined: 1) Time latency from subcutaneous injection (sc) of E_2 to onset of acceleration of isthmic transport on day 2 of pregnancy (P2) and cycle (C2). 2) Dose-response curve to sc E_2 on P1 and C1. 3) Sensitivity of oviductal transport to sc E_2 on P1, C1 and day 1 of seudopregnancy (mating with vasectomized male) and to intraoviductal E_2 on P1 and C1. 4) Bioavailability of serum E_2 after sc E_2 on P1 and C1. **Results:** Time latency for the acceleration of isthmic transport is 6h longer in pregnancy than in cycle. Sensitivity of oviduct to sc or local E_2 is 10 times lower in pregnancy than in cycle and for sc E_2 it is similar in cycle and seudopregnancy. Bioavailability of E_2 is similar in pregnancy and cycle. **Conclusions:** Mating profoundly modifies ovum transport responses to exogenous E_2, within the first 48h, provided spermatozoa enter into the genital tract. This unveils an intrinsic change in oviductal responsiveness, induced by mating-associated signals. Supported by FONDECYT 1030315 (Chile).

BASIC FGF AND ITS RECEPTORS IN RELATION TO PROLIFERATIVE ACTIVITY IN THE BUFFALO PLACENTA DURING GESTATION

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The bFGF (basic Fibroblast Growth Factor) is involved in the placental organogenesis as a potent angiogenic molecule and it is related to the proliferative activity of placental cells. The identification of proliferating cells can be made through the detection of Ki-67 nuclear antigen, present in these cells. The aim of this study was to evaluate the space-temporal expression of bFGF, FGFR1 and FGFR2 in buffalo placenta in correlation to proliferative activity. **Methods:** For the localization of bFGF, FGFR1, FGFR2 and Ki-67, 13 buffalo placentas in different gestational phases were collected in slaughterhouses and immunohistochemistry assays were carried out following ABC method and Nova Red cromogen. Placental proliferative activity was evaluated through the expression of Ki-67 antigen. **Results:** Expression of the bFGF and its receptors was detected in the nucleus of buffalo placental cells during gestation. For fetal epithelium, significative correlation ($p<0.05$) was observed between the expression of bFGF and FGFR2 with Ki-67 ($r= 0.313$ e 0.384 respectively) and in fetal stroma for FGFR1 and Ki-67 ($r = 0,358$). High correlation was observed between FGFR1 and Ki-67 in maternal epithelium and stroma ($r = 0.789$, $r = 0,511$ respectively). **Conclusions:** The results suggest that bFGF and its receptor 2 may be involved in the modulation of trophoblast proliferation, whereas FGFR1 may modulate the proliferative activity in the other cells by activation through another proliferative growth factor.

CATION CHANNEL FUNCTION IN THE HUMAN SYNCYTIOTROPHOBLAST IS UNDER CONTROL OF MICROTUBULAR STRUCTURES

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Polycystin-2 (PC2) encoded by *PKD2*, one the genes whose mutations cause polycystic kidney disease is abundantly expressed in the apical domain of term human placenta, the syncytiotrophoblast (hST). PC2, a TRP-type Ca^{2+}-permeable non-selective cation channel is also present in the primary cilium of renal epithelial cells, an important microtubular structure with sensory function. Microtubules are cytoskeletal superstructures, which regulate cell cycle and replication, control vesicular transport in cells, and are present in sensory organelles as cilia and flagella. The aim of the present study was to assess whether PC2 interacts with microtubules in hST. **Methods:** hST apical membranes were prepared by ultra-centrifugation from normal term placenta from vaginal delivery, and inserted into a lipid bilayer reconstitution chamber to assess cation channel activity. The hST vesicles were also fixated and incubated with primary and secondary antibodies for immunofluorescence to detect PC2 and microtubules in the hST membranes. **Results:** Acute addition of the microtubular disrupter colchicine (15 □M) rapidly blocked (<1 min) PC2 channel activity in the hST membranes. This effect was not reversed by further addition of exogenous tubulin and GTP. However, addition of microtubular components stimulated PC2 channel activity in control membranes. Addition of the microtubular stabilizer paclitaxel (taxol, 15 □M) also stimulated PC2 channel activity in hST membranes. We detected the presence and colocalization in hST membranes of PC2 with microtubular components, including tubulin isoforms, and acetylated α-tubulin by Western blot and immunofluorescence analyses. Both, colchicine and taxol modified the pattern of microtubular organization and PC2 redistribution. **Conclusions:** The data provide the first direct demonstration of a microtubular interaction with PC2, which may thus play an important regulatory role in ion transport in human placenta.

ROLE OF OXIDATIVE STRESS IN THE CONTROL OF THE TRPP2 CHANNEL, POLYCYSTIN-2 IN THE HUMAN SYNCYTIOTROPHOBLAST

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Pregnancy is susceptible to oxidative stress (OS) and antioxidant defenses can be altered in response to elevated levels of OS such as gestational diabetes mellitus (GDM), where products of lipid peroxidation may be increased. Growing evidence implicates OS in the pathophysiology of preeclampsia, hydatidiform mole, and free radical-induced birth defects and abortions. TRP channels such as TRPM2, is directly gated by OS agents, including, hydrogen peroxide (H_2O_2), which stimulates TRPM2 channel activity, in a phenomenon which may be associated with NADH to NAD^+ conversion. The human syncytiotrophoblast (hST) expresses abundant polycystin-2 (PC2, TRPP2), a TRP-type Ca^{2+}-permeable non-selective cation channel. Here, we explored the effect of OS in PC2 channel activity of term hST apical membranes. **Methods:** hST apical membranes were prepared by ultra-centrifugation, and reconstituted lipid bilayer assay chamber to assess cation channel activity. **Results:** Addition of H_2O_2 or terbutylic peroxide, rapidly and completely inactivated PC2 channel activity in hST vesicles. To explore whether this effect was mediated by a direct interaction the oxygen reactive species with the channel, or was it mediated by lipid peroxidation, membrane phospholipids were first peroxidized and then channel activity explored in their absence. **Conclusions:** The data are consistent with a regulatory effect by lipid peroxidation of the PC2 channel, suggesting its potential role as a target of OS in such pathological conditions as GDM, known to induce lipid peroxidation. The study further supports the hypothesis that OS in human pregnancy may be linked to dysregulation of Ca^{2+} transport by channels such as PC2. Whether or not increased antioxidant intake can reduce the complications of GDM in both mother and fetus may be required to assess whether PC2 channel function can be restored.

CONTRIBUTIONOFTHECYCLICAMPSECONDMESSENGERPATHWAYTOTHE CONTROL OF CATION CHANNELS IN HUMAN SYNCYTIOTROPHOBLAST

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The cAMP second messenger pathway plays an important role in placental development and physiology, including a contribution to cell differentiation, the release of important metabolites, and the activation of transcription factors. The cAMP pathway is the concerted effort of G protein-dependent receptor induced production of cAMP, cAMP-dependent kinase (PKA) activation, and phosphatase-mediated cAMP degradation. Here, we explored the role of the cAMP pathway on channel function in apical membranes of the term human syncytiotrophoblast (hST). **Methods:** hST apical membranes were prepared by ultra-centrifugation, and reconstituted in lipid bilayer chamber to assess cation channel activity. Addition of the purified enzyme (10 μg/ml) and MgATP (1 mM) assessed the effect of PKA in the hST membranes. Two other maneuvers were also tested. The cAMP analog, 8-Br-cAMP was added to mimic cAMP increase, and vanadate was added to inhibit phosphatase activity. Channel activity was followed with a patch-clamp amplifier and signals were processed for single channel analysis. **Results:** Addition of PKA and ATP, but not ATP alone, induced an increase in K^+ currents. This effect was mimicked in purified polycystin-2 (PC2), the TRP channel associated with this channel activity in hST. Interestingly, direct addition of either 8-Br-cAMP or the phosphatase inhibitor vanadate to the *cis* chamber, also increased PC2 channel activity in hST vesicles. **Conclusions:** The data indicate that PKA phosphorylation directly activates PC2 channel function in apical membranes from hST. The data further suggest that local cAMP production and hydrolysis both help regulate PC2-mediated cation transport and electrical parameters in the hST.

NITRIC OXIDE REGULATES THE ACTIVITY OF MATRIX METALLOPROTEINASES IN THE FETO-PLACENTAL UNIT OF DIABETIC RATS

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Matrix metalloproteinases (MMPs) are enzymes associated with tissue remodeling and growth. Overexpression of MMPs has previously been detected in the feto-placental unit of diabetic rats, an alteration probably involved in non-controlled matrix degradation and incorrect cellular migration. Nitric oxide (NO) levels are increased in fetuses and placenta from diabetic rats. In different cell types, NO induces MMPs activation. The present work evaluated the influence of NO on MMP2 and MMP9 activities in the feto-placental unit of diabetic rats at midgestation. **Methods:** Rats were made diabetics by administration of streptozotocin (90mg/Kg) to neonates, a method that leads to mild hyperglycemia (150-230 mg/dl) in the adult. Fetal and placental tissues (maternal and fetal placental sides) from diabetic rats (D) and controls (C) were evaluated on day 13.5 of pregnancy. NO synthase (NOS) activity was evaluated *in situ* by NADPH-diaphorase (NADPH-d) histochemistry. The effect of NO on MMP2 and MMP9 activities were measured after 1 h incubation without additions or in the presence of either a NO donor (sodium nitroprusside (NP) 600 μM) or a NOS inhibitor (L-NAME 600 μM). **Results:** NADPH-d activity was enhanced in D placenta when compared to C, mostly in the labyrinth zone. In C and D placenta, NP additions increased MMP9 activity (30%, $p<0.05$) in the maternal side while NAME additions reduced MMP9 and MMP2 activities in both maternal (22%, $p<0.05$) and fetal (20%, $p<0.05$) sides. In C and D fetuses, NP additions increased MMP2 activity (30% and 48% respectively, $p<0.01$) while NAME reduced its activity (25% and 40% respectively, $p<0.01$). **Conclusions:** Our results showed that NO positively regulates MMPs activation. In maternal diabetes, overactivation of MMPs in the feto-placental unit may be the result of increased NO levels and may lead to abnormal remodeling processes.

TAURINE TRANSPORT: A NEW ROLE FOR THE MAXI-CHLORIDE CHANNEL FROM HUMAN PLACENTAL SYNCYTIOTROPHOBLAST

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Taurine is the most abundant aminoacid in fetal blood and is necessary for normal fetal growth and neurological development. It is inadequately synthesized by the fetus and therefore must be supplied by the mother. Diverse studies suggest that in addition to carriers, an ionic channel may be a conductive pathway for taurine in human placenta. A molecular candidate for taurine transport in apical syncytiotrophoblast membrane is a Maxi-chloride channel with conductance over 200 pS, voltage-dependent open probability, multiple substates and anionic aminoacid permeation. Our aim was to study whether this channel could be a taurine conductive pathway in the apical membrane. **Methods:** Purified human placental apical membranes were reconstituted in giant liposomes suitable for patch clamp recordings. To detected single-channel currents carried by taurine, solutions containing 500mM taurine at pH 8.2 were used, achieving 120mM of negatively charged taurine (taurine is a zwitterionic molecule at pH 7.4). **Results:** Typical Maxi–chloride channel activity was detected in symmetrical Cl^- solutions at pH 7.4. Similar activity was detected when experiments were performed at pH 8.2. In asymmetric conditions (Cl^- pipette, taurine bath) the reversal potential shifted from 0mV to around -22mV, with a permeability ratio for taurine over Cl^- of 0.4 (n=11). In taurine symmetric conditions, single channel currents were smaller compared to amplitudes using equivalent Cl^- concentrations, with a slope conductance of 60 pS (n=3). **Conclusions:** These data demonstrate that the Maxi-chloride channel from apical syncytiotrophoblast membrane is permeable to taurine. Supported by FONDECYT 1040546(Chile).

LEPTIN EXPRESSION IN PLACENTAL CELLS

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Leptin, the 16 kDa protein product of the obese gene, was originally seen as an adipocyte-derived signaling molecule, but later found to be expressed in other tissues particularly in placenta. It has been suggested to be involved in some functions during pregnacy such as growth, angiogenesis and immunomodulation. The molecular mechanisms involved in the adhesion of the embryos to uterine epithelium and growth are still unknown. Recently, our group observed that, in JEG-3 and BeWo cells, recombinant leptin stimulated cell proliferation and protected placental cells from apoptosis. We also reported that IL-6, IL-1, 17β-estradiol, hCG and pregnenolone stimulated leptin secretion in placental primary cultures. Therefore, we proceeded to study the factors that could regulate leptin expression in placental cells, and this was the aim of the present investigation. **Methods:** JEG-3 and BeWo cells were cultured under standard conditions. Western blot analyses were carried out to detect leptin and leptin receptor proteins. Leptin promoter activity was evaluated by transient transfection with a plasmid containing different promoter regions directing the expression of the reporter gene luc. These results were normalized by the constitutive expression of the β-gal gene. **Results:** We observed a maximum increase in promoter activity of 8.8 and 12.9 times when cells were treated with 0.1 μM 17β-estradiol and 100 UI of hCG/ml, respectively. These effects were dose dependent and not evidenced with a promoter length of less than -1951bp. Similar results were obtained when studying endogenous leptin expression. On the other hand no significant changes were observed when cells were incubated with pregnenolone (0.1-10 μg/ml). **Conclusions:** All these findings put leptin as a possible link between embryo and endometrium communication and, thus, participating in the regulation of the implantation process. Supported by a grant from Universidad de Buenos Aires (UBACYT X-048).

DIFFERENTIAL MOLECULE EXPRESSION IN EUTOPIC SECRETORY ENDOMETRIA FROM WOMEN WITH ENDOMETRIOSIS

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Endometriosis, gynecologic disorder of unknown etiology, is associated to infertility. It is known that progesterone through its receptor isoforms (RP A and B) regulates genes involved in reproductive events. **Objective:** To study some molecules involved in secretory endometrial differentiation in eutopic endometrium from women with (EE) and without (NE) endometriosis. **Methods:** Glycodelin, total PR, LIF (immunohistochemistry), PRA, PRB (immunoblot) and PR(A+B), PRB, matrix metalloproteinase (MMP-26) mRNA (RT-PCR) were assessed in secretory endometrium from NE (36±5 years, n=20) and EE (33±6 years, n=20), approved by Ethical Institutional Committee. Statistics: $p<0.05$ using t Test. **Results:** Glycodelin was detected only in glands and was reduced 40% in EE vs NE ($p<0.05$). PRB protein was augmented 76% in EE ($p<0.05$); PRA mRNA [PR(A+B)-PRB;$p<0.05$] and protein increased 50% in EE vs NE ($p=0.06$); LIF was 45% higher in EE glands ($p<0.05$). No differences were detected in MMP-26 or stromal total PR between groups. **Conclusion:** Although PRB/PRA ratio was not significantly modified, the increase of each PR isoform may affect glycodelin and LIF proteins, molecules involved in embryo reception and implantation. The estrogenic micro-environment described in EE could induce an augmented response to progesterone, which may partially explain the infertility shown by these patients. FONDECYT 1040412.

EFFECT OF MATERNAL SMOKE EXPOSURE IN THE PLACENTAL MORPHOMETRY AND MATERNAL-FETAL DNA DAMAGE

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Maternal smoking during pregnancy is associated with embryofetal and placental growth and development disturbances. The aim was to evaluate placental morphometry and maternal DNA damage of rats exposed to cigarette smoke and to relate with DNA damage in their offspring. **Methods:** Non-pregnant Wistar female rats were exposed to cigarette smoke at a carbon monoxide concentration of 193.50 ppm. for 1h/7 days/week, for 50 days before and during pregnancy. Plasma nicotine and cotinine levels were used as exposure measures. At day 21 of pregnancy, placentas were collected and processed for morphometrical analysis using computer image analyzer. Maternal and fetal blood samples were used to determine DNA damage using Comet assay. **Results:** Maternal smoke produced no alteration in the labyrinth, spongiotrophoblast and decidua mean areas ($p>0.05$) compared to non-exposed rats. Maternal and fetal DNA damages were increased in rats exposed to smoke (M= 0.65; F= 0.99 – $p<0.05$) related to non-exposed group (M= 0.12; F= 0.29). **Conclusions:** Our results suggest that rats exposed to cigarette smoke used in this work presented maternal and fetal DNA alterations, which were not related to placental morphological changes in the experimental conditions analyzed. Supported by FAPESP (Brazil).

ULTRASTRUCTURAL OBSERVATIONS OF THE LLAMA PLACENTA (*Lamaguanicoe glama*)

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The placenta of the llama has been described like epitheliochorial, but existent researches don't study in depth microscopic aspects. In order to detail their ultrastructurals characteristics observations were made by light microscopy and scanning electron microscopy (SEM). **Methods:** Samples of nine uteruses between 28 to 36 weeks of pregnancy were collected in association with fetal membranes. A part was fixed with solution of 4% paraformaldehyde and embedded either in paraffin or in glycol methacrylate resin. Sections of 5µm in thickness were stained with haematoxylin and eosin, Masson's trichrome staining and PAS for observation by light microscope. Another part was fixed with 2.5 % glutaraldehyde and post-fixed in 1% osmium tetroxide and processed for SEM. **Results:** The chorionic projections presented ramifications in number from 4 to 5. Great amount of blood vessels are in the maternofetal interface, between the cells of uterine epithelium and around of the chorionic projections. Collagen fibers are observed in the mesenchymae and inside the chorionic projections. PAS positive reaction was observed in the maternofetal interface. The allantois is structured by an unique layer of different sizes cells, in their surface are circular and polyhedral structures, representing a morphological consideration from this type of function allantois cells. The epidermal membrane is formed by 7 or more layers of cells with one, two or three nucleus. The superficial layer presents more smooth cells. **Conclusions:** The high vascularization of the maternal and fetal faces indicates an optimal interchange of substances between both. The collagen inside the chorionics projections serves as a support skeleton. Suported by CNPq (Brazil).

BLOOD FLOW REDISTRIBUTION IN THE LLAMA FETUS DURING ACUTE HYPOXEMIA: THE ROLE OF NITRIC OXIDE

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The fetal llama responds to hypoxemia with a marked peripheral vasoconstriction. We tested the role of nitric oxide (NO) in blood flow redistribution during hypoxemia in this species. **Methods:** Ten fetal llamas were catheterized under general anesthesia. Five fetuses received the NO synthase blocker, L-NAME (bolus 20 mg Kg^{-1} + infusion of 0,5 mg Kg^{-1} min^{-1}), whilst the other group (n=5) had 0.9% NaCl infusion (control). The fetuses were submitted to 3h protocol: 1h normoxemia, 1h of acute hypoxemia (PaO_2 12±1 mmHg) and 1h of recovery. The infusion started at 45min of normoxemia and lasted all the hypoxemic period. Systemic arterial pressure and heart rate were registered continuously. Combined cardiac output and its distribution to the different organs, were measured with radiolabeled microspheres. Total (TVR) and organ vascular resistances (VR) were calculated. **Results:** TVR, umbilical, cerebral, heart, intestinal, renal and carcass VR increased during L-NAME infusion during normoxemia. During hypoxemia plus LNAME the VR was higher than control in all territories, except in heart and gut. **Conclusions:** In the llama fetus NO has an important role in maintaining a vasodilator tone during normoxemia and hypoxemia to counterbalance the strong vasoconstrictor mechanisms extremely active in this species. The Wellcome Trust CRIG 072256 (UK).

PROTONS AND CALCIUM MODULATE THE OPEN PROBABILITY OF A NON SELECTIVE CATION CHANNEL IN EPITHELIAL CELLS

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Early events in necrotic cell death involve an increase in intracellular calcium (Ca^{2+}_i) and a decrease in pH. Previous results in our laboratory suggested that a non-selective cationic channel (NSCC) present in HTC cells (rat hepatoma-derived cell line) implicated in the necrotic volume increase is activated by $[Ca^{2+}]_i$. However, the effect of protons on the open probability is unknown. Therefore, the aim of this study was to explore the pH dependence of the open probability (P_o) and single conductance of this channel at different $[Ca^{2+}]_i$. **Methods:** HTC cells were grown at 37°C in a 5% CO_2-95% air atmosphere in DMEM supplemented with 5% FBS, 2 mM L-glutamine, 50 U/ml penicillin-streptomycin, and 2.5 µg/ml amphotericin. Inside-out patch recordings were performed at room temperature at different intracellular H^+ concentrations (pH 5.8 to 7.8) and different $[Ca^{2+}]_i$ (0.15, 0.3 and 0.9 mM). Free $[Ca^{2+}]$ were calculated using WinMaxc. **Results:** P_o was found to have a bell-shaped dependence respect to intracellular pH with a maximum at pH 6.8-7.0 at 0.3 mM Ca^{2+}. At a $[Ca^{2+}]_i$ of 0.15 mM complete inhibition of channel activity was found at pH 6.2, while at a $[Ca^{2+}]_i$ of 0.9 mM) inhibition was shifted to a lower pH (5.8). Single conductance of the channel was unaffected by pH at any $[Ca^{2+}]_i$ tested. **Conclusions:** Intracellular pH modulates the P_o of this epithelial NSCC channel in a $[Ca^{2+}]_i$–dependent fashion. Supported by FONDAP 15010006.

DIFFERENTIAL PROSTAGLANDINS (PGs) ROLE IN THE PULMONARY CIRCULATION IN HIGH AND LOW ALTITUDE NEWBORN LAMBS DURING HYPOXEMIA

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PGs are involved in the regulation of the pulmonary arterial pressure (PAP) during the transition from fetus to neonate. The aim of this study was to determine PGs role in regulating PAP during hypoxemia (*H*) in newborn lambs (NBL) born at high altitude (HA NBL; 3589m) and low altitude (LA NBL; 580m). We hypothesized that the increased in PAP during *H* in NBL is partially regulated by PGs. **Methods.** We placed a Swan Ganz catheter in the pulmonary artery to measure PAP in 5 HA NBL and 6 LA NBL. Both groups were submitted to 3 hours protocol: 60 min of *N*, 60 min of *H* and 60 min of recovery. In HA and LA controls (C), an infusion of 0.9% NaCl was administrated at min 45 of *N* and *H* period. In HA and LA treated lambs, indomethacin (I) (COX inhibitor) (1.5mg Kg^{-1}) was administered as a bolus in min 45 of *N*, followed by an infusion (25 ug min Kg^{-1}) during *H* period. **Results.** The I-treated HA NBL had a marked increase in PAP during H compared to C HA NBL. In contrast, the I-treated LA NBL had a significant decrease in PAP during H compared to C LA NBL. **Conclusions.** These results suggest that in HA NBL, the increase in PAP during H is partially regulated by a vasodilator PG, for example prostacyclin. On the contrary, in LA NBL, this increase could be regulated by a vasoconstrictor PG, such as thromboxane. FONDECYT 1050479(Chile); The Wellcome Trust CRIG 072256(UK).

IFN-GAMMA UP-REGULATES THE EXPRESSION OF 35 GENES IN MICE TROPHOBLAST CELLS

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IFN-gamma is an important immune regulatory cytokine in biological defense's processes that co-exists at the maternal-fetal interface. In spite of that, in several circumstances IFN-gamma can act as an abortion inductor factor. **Methods:** In this study, we ascertain *in vitro* the direct effect of IFN-gamma on trophoblast cells through cDNA macroarray assays and semi-quantitative rT-PCR. **Results:** IFN-gamma (100 U/mL) did not induce modifications in the morphology and proliferation and cell death rates in trophoblast cells, but led to a differential expression in 42 genes, suggesting a functional role for this cytokine in the fetal placenta. Part of the more expressed genes was related to immune response processes or IFN-gamma-mediated cellular activation. **Conclusion:** Our data suggest that in low doses IFN-gamma may participate of the maternal-fetal interface's physiology, activating multiple regulatory pathways in trophoblast cells. Financial support: FAPESP and CNPq.

INHIBIN PRODUCTION BY RAT EARLY ANTRAL FOLLICLES IN CULTURE

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Development and inhibin production by antral follicles is mainly dependent on FSH, which is a polymorphic hormone. Follicular synthesis of inhibin *in vitro* has not been reported. The aim of this study was to determine the effect of FSH glycosylated variants on production of inhibin, by rat early antral follicles (EAF) in culture. **Methods:** FSH variants bearing biantennary and truncated (WB) or high mannose and hybrid-type oligosaccharides (FB) were obtained after Con-A chromatography of recombinant human FSH (rhFSH). EAF (~350µm) were dissected from ovaries of Sprague-Dawley rats (24-26 day-old) treated with diethylstilbestrol. Follicles were cultured during 24 h in absence or presence of 25 ng/mL rhFSH or molecular variants. Following incubation, dimeric inhibin A and B and Pro-αC were assayed in the medium by ELISA. **Results:** Under basal conditions, follicles produced 4-fold more inhibin B than Inhibin A (A/B ratio = 0.32 ± 0.07). Inhibin A levels increased in response to rhFSH, WB and FB ($P<0.05$), although response to FB was significantly lower in comparison to WB ($P<0.01$). Addition of FSH at a dose of 25ng/mL failed to stimulate inhibin B production. Pro-□C levels were increased after treatment with WB and FB ($P<0.05$). **Conclusions:** These results show that rat EAF in culture produce and secrete inhibins, in similar proportion as observed in serum. Inhibin production was differentially stimulated by FSH variants. Supported by PROGRESAR.

CARDIAC EXPRESSION OF DEGENERIN/EPITHELIAL SODIUM CHANNEL PROTEINS AND POTENTIAL MODULATION BY MINERALOCORTICOIDS

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The epithelial sodium channel (ENaC) is formed by three subunits (α, β and γ). The channel activity in epithelia is modulated by aldosterone (ALDO) affecting ENaC subunits gene expression. Since the heart is a target tissue for ALDO, we studied the cardiac expression of ENaC protein subunits and the potential in vivo modulatory effect of mineralocorticoids. **Methods:** total RNA was isolated from rat left ventricle for RT. To detect ENaC subunits mRNA, PCR experiments were performed with specific primers. The presence of ENaC proteins was detected by Western Blot in rat ventricular homogenates. Immunoprecipitation experiments were performed to evidence ENaC subunits physical association. The potential modulatory role of mineralocortocoids was tested by Western Blot in ventricular protein homogenates obtained from adrenalectomized rats (ADX), ADX rats with mineralocorticoid replacement therapy (ADX-DOCA) and control rats. **Results:** In RT-PCR experiments we obtained products of the expected size using rat α, β and γ subunits specific primers. Western Blot studies with anti α, β and γ ENaC demonstrated the presence of specific immunoreactive bands of 96, 102 and 92 kDa respectively. Immunoprecipitation experiments with anti-β ENaC co-precipitated α and γ ENaC; α and β ENaC were co-precipitated with anti-γ ENaC. Adrenalectomy decreased ventricular γ ENaC abundance to 81,4±5,3% of control levels (n=8, $P<0.05$), an effect prevented by DOCA. No significant changes for α or β subunits were found. **Conclusions:** Our results demonstrate for first time cardiac ENaC subunits expression, probably forming a heteromeric channel regulated by ALDO. FONDECYT 1050690.

STICHOLYSIN II LYTIC ACTIVITY IN PEROXYL-OXIDIZED ERYTHROCYTES

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Oxidation of membrane constituents has been extensively described in erythrocytes exposed to AAPH derived peroxyl radicals. The aim of this study is to evaluate the lytic response of membrane damage cells to StII, a cytolysin purified from *Stichodactyla helianthus*. Colloidal osmotic lysis provoked by StII is influence both by K^+ efflux, that precedes and retards cell rupture and, by membrane viscoelastic properties. **Methods:** Erythrocytes are exposed to peroxyl radicals derived from 2,2'-azobis(2- amidinopropane) (AAPH) and, after AAPH removal, time profile of erythrocytes lysis and K+ efflux were evaluated simultaneously. **Results:** Peroxyl damaged erythrocytes showed an increase in the rate of lysis due to StII ; t_{50} decreased from 17.9 ± 5.7 min to 8.1 ± 2,5 min, n=6, (p<0,001). Also, osmotic fragility was slightly increased in treated cells. As erythrocytes lost K^+ after treatment with AAPH, the increase in lysis rate can be due to a decrease in the protection afforded by K^+ efflux. This may be explained both to its smaller intracellular concentration and to the reduced rate of K^+ efflux (t_{50} of K^+ efflux increases from (t_{50} increased from 4,6±1,0 min to 7,8±2,6 min, n=6, p=0,006). Protection afforded by the presence of Trolox during AAPH treatment, confirmed that alterations in the response of treated cells to StII are a consequence of membrane oxidative damage. **Conclusions:** Red cells pre-oxidation increases lytic action of St II. This effect is probably related to a diminished protection afforded by K^+ efflux.Support from FONDECYT 1030033.

HEMOXYGENASE EXPRESSION IN LUNG TISSUE FROM HIGH AND LOW ALTITUDE NEWBORN LAMBS

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Haemoxygenases 1 and 2 (HO1 and HO2) catalyze the conversion of haem groups into carbon monoxyde (CO), biliverdin and iron. CO has vasodilator effects through activation of soluble guanylate cyclase and K_{Ca} channels. As chronic hypoxia promotes a strong increase in pulmonary vascular tone, we studied whether this response to hypoxia could correlate with HO1 and HO2 expression changes by comparing both enzyme isoform expression between lambs gestated and born at high altitude (HA) and low altitude (LA). **Methods:** Newborn lambs between 4-15 days old were used in the study. Semiquantitative RT-PCR from lung total RNA of LA (580m) and HA (3600 m) animals was performed with specific primers to amplify HO1, HO2 and 18s rRNA as housekeeping gene. The amplicons were analyzed by densitometry after electrophoresis on ethydium bromide agarose gels. **Results:** HO1 and HO2 mRNAs were significantly higher in HA than LA animals (LA newborns: HO1/18s = 1.69 $\pm$ 0.12, n = 4, HO2/18s = 0.82 $\pm$ 0.12, n = 5; HA newborns: HO1/18s = 4.17 $\pm$ 0.61, n = 7, HO2/18s = 1.75 $\pm$ 0.31, n = 7; p<0.05, LA vs HA. **Conclusions:** Both haemoxygenase mRNAs were induced by chronic hypoxia suggesting that the transcription of HO1 and HO2 genes is up-regulated with this stimulus. This response could be necessary to counteract the strong increase in pulmonary vascular contractility developed under hypoxic conditions. The Wellcome Trust 072256 and FONDECYT 1050479.

ASSOCIATION OF POLYMORPHISM IN TOLL RECEPTOR 4 (TLR4) WITH INFLAMMATORY PATHOLOGIES IN TERM PREGNANCIES

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Genetic variations like Asp299Gly TLR4 polymorphism (A299G), associates with elevated risk of diverse diseases. The role of TLR 4 in inflammatory process related to pregnancy is poorly known. Our aims were to analyze if A299G associates with pathologies related to inflammation at term in pregancies and to investigate the presence of TLR4 in human amniotic epithelium at term in normal and pathological pregancies. **Methods:** DNA from umbilical cords of 70 term newborns was analyzed by PCR using mist matching primers. Amnion from 5 normal pregancies placentas were analized by immunocytochemistry and western blotting. **Results:** 34/70 pregnancies had one or more of the following pathologies: premature rupture of membranes, toxemia, intrauterine growth restriction, urinary infection, hemorrhage, and fetal distress. A229G was found in 15 % of pathological pregancies and only in 5.5 % of normal pregnancies (odds ratio 2.93). Immune positive reaction to TLR4 was observed in the epithelium near to the site of rupture of the choriamnios in all of the analyzed tissues. **Conclusions:** These differences between both groups suggest a tendecy of A299G to be present in patients with obstetric pathologies related to inflammatory process. More cases and additional studies are necessary to confirm this hypothesis. The presence of TLR4 in the amnion suggests its role in the inflammatory mechanisms that leads to delivery.

THE ET-1 SYSTEM IN THE PULMONARY CIRCULATION FROM NEWBORN LAMBS SUBMITTED TO CHRONIC HYPOXIA

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ET-1 is involved in the regulation of the pulmonary arterial pressure (PAP) during the transition from fetal to neonatal life, acting through ET_A and ET_B receptors eliciting vasoconstriction and vasodilatation respectively. The aim of this study was to determine the role of ET-1 and its receptors in the increased PAP observed in chronically hypoxic newborn lambs gestated and born at high altitude (HANBL, 3589m) compared to low altitude newborn lambs (LANBL, 580m). We hypothesized that an increased vasoconstrictor response to ET-1 may contribute to the elevated PAP in HANBL. **Methods.** We placed a Swan Ganz cathether in the pulmonary artery from 5 HANBL and 6 LANBL, and we measured basal PAP during 45 min and the effect of an infusion of the ET_A receptor blocker BQ123 for 30 min. We also performed semiquantitative RT-PCR from total lung RNA to assess ET_A and ET_B receptor expression, using a 18s rRNA as housekeeping gene. **Results:** BQ123 infusion did not change PAP either in LANBL or in HANBL. We did not observe significant differences in the ET_A or ET_B receptor mRNA expression between both groups of lambs (LANBL: ET_A/18s = 0.59 $\pm$ 0.11; ET_B/18s = 0.51 $\pm$ 0.13. HANBL: ET_A/18s = 0.83 $\pm$ 0.05; ET_B/18s = 0.36 $\pm$ 0.11). **Conclusion:** These results suggest that ET-1 and its receptor system do not play a major role in the increased PAP in the chronically hypoxic newborn lamb. The Wellcome Trust 072256; FONDECYT 1050479.

ROL OF CHRONICALLY INGESTED COPPER IN LONG-TERM POTENTIATION (LTP) OF RAT HIPPOCAMPUS

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The objective of our study was to find evidence of copper interaction in long-term potentiation (LTP), motivated by copper involvement in neurodegenerative illness, like Parkinson, Alzheimer and Amyotrophic Lateral Sclerosis, and we initiated the study of this element in the LTP. **Methods:** For this purpose we used hippocampus slices of rats chronically consuming copper dissolved in water (CuDR), (n=26), and non copper-consuming rats (CR)(n=20). The CuDR rats received 8 to10 mg/day during 20 to 25 days. **Results:** Electrophysiological tests showed absence of LTP in CuDR slices, contrary to CR slices. The stimulus-response test applied before and after LTP showed a significant increase of synaptic potential in the CR group. This did not occur in the CuDR group, except for the initial values, which probably seem associated to an early action of copper. The paired-pulse (PP) test, applied to CR and CuDR prior to tetanic stimulation, showed a significant reduction in PP, for the 20, 30 and 50 ms intervals in CuDR. At the end of the experiments copper concentration was 54.2 times higher in CuDR slices, compared to the concentration present in CR slices. **Conclusions:** Our results show that copper reduces synaptic sensibility and also the facilitation capability. These effects represent a significant disturbance in the plasticity phenomenon associated with learning and memory.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF RABBIT PETROSAL AND NODOSE GANGLION NEURONS

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The petrosal (PG) and nodose (NG) ganglia provide sensory innervations to the respiratory, circulatory and digestive tract. It has been suggested that electrical properties of these neurons are related to their sensory modality. We studied neuronal electrical properties in the ganglia to determine the existence of population inhomogeneities. **Methods:** NGs and PGs from 6 anesthetized male rabbits were obtained with ketamin/xylazine and placed in Hanks´ solution buffered with 5mM HEPES (pH 7.43; 22°C). Neurons were recorded with glass microelectrodes filled with 3M KCl. We measured the resting membrane potential (MP), amplitude and duration of action potential (AP) components, and input resistance (R_{in}) and capacitance (C_{in}). **Results:** Neurons were classified as "humped" and "non-humped", according to an inflection in the repolarizing phase of the AP. 81.2% of NG cells were "humped" (NG_H) and 18.8% "non-humped" (NG_{NH}), whereas 26.7% of PG neurons were "humped" (PG_H) and 73.3% were "non-humped" (PG_{NH}). AP duration was significantly longer in "humped" than in "non-humped" neurons and in NG than in PG neurons of the same category (ANOVA, $p< 0.05$). In response to long depolarizing pulses 56.3% of NG_H, 83.3% of NG_{NH}, 37.5% of PG_H, and a 45.5% of PG_{NH} discharged multiple APs. R_{in} was significantly different only between "humped" neurons (Student test, $p< 0.05$). MP, AP amplitude and C_{in} were not significantly different between groups. **Conclusions:** Our results indicate that electrical properties are not homogeneous in these ganglia, and –at least- four categories can be discerned on each ganglion that may reflect different sensory modalities. Supported by FONDECYT 1040638 (Chile).

MITOCHONDRIAL DYNAMICS AND CYTOSKELETAL ASSOCIATION IN RAT PACHYTENE SPERMATOCYTES AND ROUND SPERMATIDS

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Several morphological and subcellular distribution changes of mitochondria take place in mammalian spermatogenesis. An even distribution of mitochondria in the cytoplasm is observed in primary spermatocytes. Instead, these organelles are distributed close to the plasma membrane in round spermatids. This cell differentiation-related peculiar distribution of mitochondria allows asking whether this it can be associated to cytoskeleton components, and if this affects the self-association and dynamics of these organelles in these cells. **Methods:** Mitochondrial labeling was accomplished vitally by incubating the cells with MitoTracker Red CM-H2XRos or after fixation by immune labeling with anti-cytochrome c antibodies. To modify the mitochondrial association with the cytoskeleton, we utilized actin (latrunculin B) and tubulin (nocodazol) depolymerizing drugs. Mitochondria and immune-labeled actin and tubulin were observed under confocal microscopy. **Results:** Spermatocyte and spermatid mitochondria mainly co-localized with actin in these cells. The mitochondrial distribution was altered by latrunculin B and nocodazol in spermatocytes and spermatids, respectively. Mitochondria associate with each other forming linear aggregates in spermatids, and non-linear groups in spermatocytes. The rates of movement of individual mitochondria in spermatocytes and spermatids do not differ significantly from each other. **Conclusions:** Our results suggest that mitochondria in spermatogenic cells interact with cytoskeleton components in these cells. These interactions appear to be more important with actin in spermatocytes, but also influenced by tubulin in round spermatids. (Supported by FONDECYT 1040800)

PROTECTIVE EFFECT OF *UGNI MOLINAE* TURCZ ("MYRTACEAE") IN THE L-ARGININE TRANSPORT IN THE PRESENCE OF oxLDL IN HUVEC

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Atherosclerosis is characterized by endothelial dysfunction associate to an alteration in the L-arginine/nitric oxide pathway in the presence of low density lipoprotein (LDL) modified by oxidation (oxLDL). Studies in the native species *Ugni molinae* Turcz ("Myrtaceae") demonstrate compounds such as phenolic acids, able to inhibit oxidation of LDL *in vitro*. The aim of our study was to demonstrate the protective effect of a methanolic extract of *Ugni molinae* in the inhibition of the L-arginine transport in the presence of oxLDL in human umbilical vein endothelial cells (HUVEC). **Methods:** The endothelial cells were obtained from normal pregnancies. The native LDL was oxidatively modified by cupper ions (0,5 mg of protein ml^{-1}; 10 µM $CuSO_4$, 8h). The HUVEC viability was evaluated by trypan blue exclusion in presence of Methanolic Extract (EMEb) for different period of time (0-24 h). The EMEb concentration was expressed as Gallic acid equivalent (0,1-50-100 nM, GAE) The L-arginine transport was assayed at the same conditions. eNOS protein levels was determined by western blott. **Results:** The extracts did not show cytotoxic effects on HUVEC at concentrations smaller than 50 nM (GAE) up to 24 hrs. OxLDL diminished the L- arginine maximal transport capacity (V_{max}/K_m) and EMEb 0,1 and 50 nM (GAE) showed protective effects concentrations dependent manner. The eNOS protein levels showed an increase at 4 hours of incubation in presence of EMEb 0,1 and 50 nM (GAE). **Conclusions:** These results allow postulating that Myrtaceae would be a potential protective agent of endothelial cells functions exposed to oxLDL. Support: DIUC 204074037-1.

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN THE MOUSE UTERUS DURING EARLY PREGNANCY

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Vascular Endothelial Growth Factor (VEGF) is a potent mitogen for endothelial cells and a key regulatory growth factor for vasculogenesis and angiogenesis. New uterine blood vessels that originate from preexisting vessels are a crucial event for the success of pregnancy. The aim of this study was to investigate the expression of VEGF in the mouse uterus. **Methods:** Expression of VEGF was determined by immunohistochemistry assayed between days 5.5 and 7.5 of pregnancy in the mouse uterus. **Results:** On day 5.5, VEGF was present in mesometrial and antimesometrial decidual cells surrounding the implantation chamber. On day 6.5, VEGF expression was observed on mesometrial decidual cells, mesometrial and anti-mesometrial predecidual cells, uterine natural killer cells, and the uterine glands. On day 7.5, the staining pattern observed on the stroma was similar to those observed on previous days, excepting from the positive staining on the ectoplacental cone cells. **Conclusions:** These evidences suggest that the mesometrial decidual and predecidual cells, uterine gland cells, and mainly uterine natural killer cells, might be strongly involved in the VEGF synthesis in the pregnant uterus during early pregnancy. *Financial support from FAPESP 01/09019-9.

OZONE-INDUCED LUNG DAMAGE IN POSTNATAL PERIOD IN RATS IS DELAYED BY PRENATAL EXPOSURE DURING THE 2nd HALF OF THEIR GESTATION

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Ozone (O_3) a photochemical air pollutant, may induce lung damage. O_3 response is more severe in younger experimental animals. However, its effects in newborns from mothers exposed to O_3 during the 2nd half of their gestation have been scarcely studied. Our aim was to evaluate the eventual protection provided by prenatal 0.5 ppm O_3 exposure on postnatal period to the same O_3 dose: 0.5 ppm, 5 h daily, 5 days a week. **Methods:** Pregnant Sprague-Dawley rats and their newborns were exposed to O_3 from 11th day of gestation up to 14 (n=6), 30 (n=6) and 60 (n=6) days after birth.Controls breathed room air (n =18) or O_3 exclusively (n=18) in their postnatal life for the same periods of time. Histopathology was done in the left lung. Total proteins, total phospholipids and γ- glutamyl-transpeptidase (GGT) activity were determined in the right lung bronchoalveolar lavage fluid (BALF). **Results:** Rats subjected to O_3 exposure in the immediate postnatal period showed: alveolar hemorrhage and edema (14 day-old); increased GGT activity in BALF (30 day-old) and slight lung inflammation and increased proteins in BALF (60 day-old). Rats subjected to pre and postnatal O_3 exposure delayed the appearance of detectable lung injury up to 60 days-old, presenting mild inflamation. **Conclusions:** Rats exposed to O_3 exclusively in postnatal period presented early lung damage. In contrast, antenatal O_3 exposure seems to protect the lung from its nocive effects, since rats exposed in both antenatal and postnatal periods did not show early histological lung damage. Supported by FONDECYT 1981127(Chile).

ALANO PATHWAY IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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L-Arginine transport and nitric oxide (NO) synthesis (L-arginine/NO pathway) are altered in gestational diabetes or intrauterine growth restriction. L-Arginine/NO pathway is up-regulated by the endogenous nucleoside adenosine in primary cultures of human umbilical vein endothelial cells (HUVEC). This study summarizes findings of molecular mechanisms accounting for modulation of L-arginine/NO pathway by adenosine in HUVEC. **Methods:** Cells were exposed (0.001-10 μM) to adenosine or CGS-21680 (A_{2a} agonist) in absence or presence of ZM-241385 (100 nM, A_{2a} antagonist). L-Arginine (2 μCi/ml, 37°C, 1 min) and adenosine (4 μCi/ml, 22°C, 20 s) transport was measured in absence or presence of nitrobenzylthioinosine (NBMPR, 0.001-10 μM, nucleoside uptake inhibitor). hCAT-1 (human Cationic Amino acid Transporter), hENT1 (human Equilibrative Nucleoside Transporter) and endothelial NO synthase (eNOS) mRNA levels were quantified by RT-PCR. L-[^{3}H]Citrulline formation from L-[^{3}H]arginine (4 μCi/ml, 37°C) in absence or presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) was measured. hENT1 and total and Ser^{1177}-phosphorylated eNOS protein levels were estimated by Western blot. **Results:** Adenosine, CGS-21680 and NBMPR increased L-arginine transport (2.7-fold) and eNOS (1.9-fold) activity, as well as hCAT-1 (1.8-fold) and eNOS (2.1-fold) mRNA levels. However, hENT1 protein abundance was reduced. Activation of A_{2a} purinoceptors reduced *SLC29A1* (for hENT1) promoter activity. **Conclusions:** Reduced adenosine transport due to lower hENT1 expression is a starting point for stimulation of L-arginine transport via hCAT-1 and eNOS due to activation of A_{2a} purinoceptors, a functional relationship characterized as 'ALANO' (Adenosine/L-Arginine/NO) pathway in HUVEC. Supported by FONDECYT 1030781/1030607/7050030 (Chile).

REGULATION OF THE ENDOCANNABINOID SYSTEM DURING EARLY PREGNANCY IN THE RAT

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Anandamide (AEA) is synthesized by the murine uterus during early pregnancy. High AEA is toxic for the embryo and the implantation process. It is well known that estradiol (E) peaks prior to implantation. We have previously observed that AEA synthesis is highest during estrous in the rat uterus. Thus, the aims of the present work were to characterize AEA synthesis during early pregnancy and if it is regulated by progesterone (P) and E. **Methods:** Wistar control rats were sacrificed on days 1 to 6 of gestation. Another group of Wistar rats were ovariectomized on day 4 of gestation, treated with vehicle, P (2 mg/kg) or E (0.1 mg/kg) and sacrificed on day 8 of pregnancy (delayed implantation model). The uterine tissue was obtained. AEA synthesis was determined by the conversion of [^{14}C]-arachidonic acid into [^{14}C]-AEA. **Results:** During early pregnancy, AEA production remained constant until day 5 of gestation (1.40±0.13 nmoles AEA/mg prot/h), when the peak of E has been reported, and diminished towards day 6 ($p<0.01$). On day 6, implantation sites showed lower AEA production compared to inter-implantation sites ($p<0.05$). In the delayed implantation model, P that maintains the uterus in a refractory state to implantation, stimulated AEA synthesis (0.22±0.01 vs 0.35±0.03 nmoles AEA/mg prot/h), while E, that turns the uterus receptive to the blastocysts, did not modify it. **Conclusions:** These results indicate that in the rat uterus, AEA production is selectively modulated by P and E, suggesting that these ovarian hormones could be regulating the level of AEA during implantation, a crucial step in early gestation and successful pregnancies. Supported by PICT 10901 (Argentina).

HEMICHANNELS FORMED BY CONNEXIN43 ARE SENSITIVE TO CHANGES IN CELLULAR REDOX POTENTIAL

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In astrocytes under metabolic inhibition (MI) opening of hemichannels constituted of connexin43 (Cx43-HCs) is greatly increased. Protein oxidation has been proposed to open Cx43-HCs. We studied the effect of changes in cellular redox potential on Cx43-HCs. **Methods:** Neonatal rat cortical astrocytes were used. Cells were subjected to MI (100 µM iodoacetic acid and 5 ng/ml antimycin A) and then exposed to 10 mM dithiothreitol (DTT). Cx43-HCs on the plasma membrane were labeled with sulfo-NHS-SS-biotin and isolated with neutravidin beads. Cx43-HCs levels, nitrosylation and phosphorylation state were analyzed by Western blot and their openings were evaluated through ethidium bromide (EtdBr) uptake. **Results:** Cx43-HCs of control astrocytes were mainly phosphorylated. MI increased Cx43-HCs on the cell surface and induced their nitrosylation and dephosphorylation. Application of 10 mM DTT at 40 min of MI, inhibited the EtdBr uptake and nitrosylation but did not prevent Cx43-HCs dephosphorylation. Nitric oxide (NO) donors mimicked the IM effect on S-nitrosylation state and activity of Cx43-HCs. **Conclusions:** Our data indicate that the changes in redox potential affect directly the Cx43-HCs activity. Supported by FONDECYT 10310945 and CONICYT 403088.

SPACE-TIME DISTRIBUTION OF 3H-ESTRADIOL IN DIFFERENT CELL TYPES OF MOUSE ENDOMETRIUM DURING EARLY PREGNANCY

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Estradiol plays an important role in the proliferation, differentiation and function of uterine cells during early pregnancy. Estrogen action is mediated by its nuclear receptor. In this study, we followed the space-time distribution of estradiol receptor in the mice uterine stroma during early pregnancy. **Methods:** Females Swiss albino mice at 1½ to 7 ½ days of pregnancy were used. ^{3}H-Estradiol was injected into the tail vein. Mice were killed 1 h afterwards. Uterine horns were cut in a cryostat and thaw-mounted on emulsion-coated slides. The mounted slides were stored and the end of exposure, slides were fixed and then photographically processed. **Results:** On day 1.5 the nuclear concentration of ^{3}H-estradiol in both sub-epithelial and deep endometrium was homogeneously distributed. However, on day 2.5 and 3.5 the nuclear retention of 3H-estradiol was higher in the subluminal antimesometrial area than in the deep antimesometrial endometrium. On day 4.5 to day 7.5, decidual cells show a weak signal for 3H-estradiol. On the contrary, intense labeling was observed in predecidual cells and non-decidualized stroma. High and homogenous 3H-estradiol retention was observed in stromal cells of the mesometrial area. **Conclusions:** Our results show that during the early pregnancy different cell populations of the uterus exhibit different degrees of binding of the 3H-estradiol. Grants from FAPESP (00/00098-1).

INSULIN-LIKE GROWTH FACTOR-I AND -II AND IGF-I RECEPTOR mRNA EXPRESSION IN COLOMBIAN WOMEN WITH HYDATIDIFORM MOLE AND SPONTANEOUS ABORTION

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Hydatidiform mole (HM) is a form of gestational trophoblastic disease (GTD) characterized by abnormal proliferation and increased invasiveness of trophoblastic tissue. Involvement of insulin-like growth factors (IGFs) in regulation of cell proliferation and differentiation is well established. The aim of this study was to investigate the role of IGF system in the development of HM. **Methods:** 32 patients with HM in gestational weeks 8 through 20 with clinical, echosonographic and gonadotropin (hGC) diagnosis and 15 patients with spontaneous abortions (SA) during weeks 8 through 18 were analysed (patients were selected from hospitals in Bogotá, Colombia, with written consent). Tissue and serum total IGF-I/-II were measured by radioimmunoassay and tissue mRNA levels of IGF-I/-II and the IGF-I and GH receptors were analysed by RT-PCR, using GAPDH as housekeeping gene and IGFBP-1 as control of residual decidual tissue. Data were analyzed by Kruskall-Wallis non-parametric test and Dunn test for significant differences with SAS program. $P<0.05$ was considered as significant. **Results:** We found lower IGF-I levels in serum and tissue in HM compared to SA, although no significance was achieved, but was consistent with the lower IGF-I mRNA expression levels observed in HM compared to SA. In contrast, serum IGF-II levels in MH were higher than in SA. IGF-II and IGF-IR mRNA expression was significantly higher in HM than in SA, and no differences were observed in the GH receptor mRNA levels. **Conclusions:** The increased expression of IGF-II and IGF-IR in HM suggest an association between IGFs and the development of the disease via autocrine and/or paracrine way. Support: Colciencias (Colombia).

DIFFERENT EFFECTS OF MELATONIN ON PER2 EXPRESSION IN THE ADRENAL GLAND OF FETAL AND ADULT CAPUCHIN MONKEY

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The adult and fetal primate adrenal gland show circadian production of cortisol, respond to melatonin (Mel) in culture, and express clock genes. We investigated whether Mel affects clock gene expression in these glands. **Methods:** Adrenal gland explants from fetuses (90% gestation, n=3) and adult animals (n=4) were incubated in DMEM (control) and DMEM + 100 nM Mel. Mel was added for 12 hrs at daytime (fetal and adult adrenal) and nighttime (fetal adrenal). The ratio Per2/18S-rRNA was measured by RT-PCR. **Results:** In control fetal adrenal gland explants, Per2 showed low expression. Daytime Mel induced a modest increase in Per2 whereas nighttime Mel induced a marked increase ($P<0.05$, ANOVA). In contrast, in control adult adrenal gland explants Per2 showed a peak at 14h that was abolished by Mel treatment. **Conclusions:** Our data show a time of day dependent response to Melatonin in the fetal adrenal gland. In addition it shows different effects of melatonin on Per2 expression in the fetal and adult adrenal gland. We speculate that in the fetal adrenal melatonin may increase degradation of Per2 protein (PER2), since PER2 accumulation inhibits its own gene expression whereas in the adult adrenal melatonin may inhibit transcription of Per2, possibly by decreasing CREB phosphorylation. Supported by FONDECYT 1030425.

MELATONIN RESTRAINS CORTISOL PRODUCTION IN SHEEP FETAL ADRENAL IN LATE GESTATION

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The increase in fetal cortisol is a key factor for fetal maturation and the onset of labor in sheep. Thus, restraining fetal cortisol production during most of gestation is important to prevent premature organ maturation and preterm delivery. We propose that melatonin (Mel) restrains sheep fetal adrenal function by inhibiting ACTH stimulated cortisol production and/or by increasing intra-adrenal inactivation of cortisol to cortisone. **Methods:** Fetal adrenal explants (n=6, 90% of gestation) were incubated 18h with: DMEM (control), 10nM ACTH, ACTH + 1-10nM Mel and ACTH + Mel and 1μM luzindole (antagonist of Mel receptors mt1 and mt2). Cortisol and cortisone production (RIA) was normalized by mg of explant. **Results:** Mel inhibited ACTH-induced cortisol production, effect that was reverted by luzindole. Control fetal adrenal explants produce more cortisone than cortisol (1.0±0.2 vs 0.4±0.1 ng/mg tissue, respectively; $P<0.05$, Student t test). ACTH increased cortisol/cortisone ratio, Mel treatment had no effect on this ratio. **Conclusions:** Mel restrained ACTH stimulated cortisol production and did not augment intra-adrenal inactivation of cortisol. The reversion of melatonin action by luzindole suggests that melatonin acts through mt1 and/or mt2 receptor in sheep fetal adrenal. Wellcome Trust CRIG 072256(UK). MECESUP PUC-0211.

DIFFERENTIAL EXPRESSION OF THE CLOCK GENE CRY2 IN CAPUCHIN MONKEY FETAL TISSUES

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In adults, transcription/translation feedback loops of clock genes Bmal1, Clock, Per2 and Cry2 are found in most tissues of the body. In contrast to fetuses of rats and hamsters, in the capuchin monkey, Bmal1 and Per2 oscillate in suprachiasmatic nucleus (main circadian clock) and in adrenal gland, a peripheral organ very active in the primate fetus. To asses whether clock gene expression relates to organ function, we studied expression of clock genes and measured Cry2 in pituitary, thyroid and brown adipose tissue (BAT), active during fetal life and in the pineal gland that starts functioning after birth. **Methods:** We obtained pineal (n=6), thyroid (n=3), BAT (n=4) and pituitary (n=8) from 90% of gestation fetuses. We identified Per2, Clock, Bmal1 and Cry2 and measured the ratio Cry2/18S-rRNA per ng RNA by RT-PCR. **Results:** The 4 clock genes were detected in pineal, thyroid, BAT and pituitary. The expression of Cry2 was higher in the pineal, intermediate in the thyroid and lower in BAT and pituitary (1.76±0.41, 0.8±0.14, 0.08±0.04 and 0.09±0.06, respectively, $P<0.05$, ANOVA, Bonferroni). **Conclusions:** The observation that Cry2 expression is higher in inactive fetal tissues like pineal, than in active tissues like pituitary and BAT, suggests a functional correlation between clock gene expression and organ function. FONDECYT 1030425 (Chile).

PRODUCTION OF REACTIVE OXYGEN SPECIES INDUCED BY LIPOPOLYSACCHARIDE IN ENDOTHELIAL CELLS

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Binding of lipopolysaccharide (LPS) to Toll-like receptor-4 (TLR4) evokes reactive oxygen species (ROS) production earlier than cytokine expression and release. This time course suggests hat ROS generation is independent of cytokine effect. Furthermore, the direct interaction of TLR4 with NAD(P)H oxidase could be responsible for ROS generation. However, the way by which NAD(P)H oxidase is activated is no completely understood. We intended to study the mechanism(s) of LPS-induced ROS production. **Methods:** EA hy926 cell line were cultured at 37ºC in DMEM (10% FBS). ROS production was detected by DCF fluorescence. Cells were incubated with 10 μM DCFH-DA for 30 min at RT and then exposed to LPS or PBS for 30 min. **Results:** LPS treatment (1 and 10 μg/ml) increased intracellular DCF fluorescence (160% and 220%, respectively). When the cells were pre-incubated with the PLC inhibitor (U73122 5 μM), PI3-K inhibitor (Ly204002 10 μM), PKC inhibitor (BIM-I 0.5 μM) and conventional PKCs inhibitor (Gö 9676 10 μM), LPS failed to evoke any significant change in DCF fluorescence over the control level. In addition, pre-incubation with NAD(P)H oxidase inhibitor (DPI 5 μM) decreased LPS-induced DCF fluorescence. However, the inactive PLC inhibitor (U73343 5 μM) has no effect in LPS-induced DCF fluorescence. In addition, no changes in DCF fluorescence were detected in control cells. **Conclusions:** our results suggest that LPS-induced ROS production in endothelial cells is dependent on NAD(P)H oxidase, possibly by PLC, PI3K, and conventional PKC activation. Supported by UNAB DI 32-04 and UNAB DI 60-04.

IGF-I AND IGF-II DETERMINATION IN SERUM AND CONDITIONED MEDIUM PORCINE DURING PREGNANCY

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The presence of IGF-I and II in the uterus particularly during pregnancy, indicates the involvement of the IGF system in endometrial growth and development of the conceptuses and placenta by way of endocrine, autocrine and paracrine actions. The present study was undertaken to investigate the relationships of IGF-I and II in pig pregnancy at different gestational days. The study was carried out on 30 pregnant gilts. Porcine Placenta Conditioned Medium (PPCM) were obtained at 30, 60, 90 days of pregnancy and at term. Serums from the same gilts were obtained at 10, 30, 60, 90 days of pregnancy, and at term. In these samples, IGF I and II were measured by ELISA (Quantikine R&D System). We found a significant increase of IGF-I at seric level at the 10 day of gestation with a marked decrease at 60 day which remained constant up to the moment of parturition. Placental IGF-I had a secretion profile opposite to the serum concentrations. The highest values were obtained at day 90 of gestation. Serum IGF-II had a secretion profile opposite to IGF-I with the highest value at day 60 of gestation ($p<0,05$) and a hundred times higher than the concentration of serum IGF-I. The highest concentration of placental IGF-II was observed at day 30 of pregnancy ($p<0,05$) with a marked decrease at day 60. The result indicate the existence of an equilibrium between IGF-I and IGF-II, both factors being crucial to placental and fetal development due to their presence along the gestational period. Research funded by FONCyT and SECyT (Argentina).

PREDICTIVE VALUE OF QUANTITATIVE B-HCG AT THE TIME OF INITIAL INTRAUTERINE GESTATIONAL SAC VISUALIZATION

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Determination of B-hCG plasmatic levels is the earliest sign of human gestation. In fact, several curves have been published correlating mean values at implantation and two weeks after conception. However, there is little information about the levels reached at the time of initial gestational sac visualization. The purpose of this study was to correlate the B-hCG levels three weeks post ovulation with ultrasound findings and the viability of singleton pregnancies. **Methods:** 37 infertile patients who became pregnant with intrauterine insemination or timing of intercourse were prospectively followed by weekly transvaginal ultrasound starting on the third week after ovulation. Plasmatic levels for B-hCG were obtained two and three weeks post ovulation and correlated with pregnancy viability or abortion. **Results:** Thirty-two viable pregnancies and 5 abortions were obtained (13.5% abortion rate). The mean diameter of gestational sac was 4.8±1.2 mm (range 3-7) while the mean B-hCG at the second and third week after ovulation was 429±314 and 5216±3397 mIU/ml, respectively. There was a significant difference ($p< 0.05$) between mean values at third week for viable pregnancies (5718±3377 mIU/ml) compared to abortions (1995±705 mIU/ml). Using 3000 mIU/ml as a cut point gave a sensibility of 0.89, a specificity of 0.92, a positive predictive value of 0.98, a negative predictive value of 0.61 and a positive likelihood ratio of 10.73 for viable pregnancies. **Conclusions:** Although a well-known wide dispersion is observed in B-hCG values as pregnancy progresses, it is still a useful tool for predicting viable pregnancies, even at third week after conception.

MODULATION OF ADENOSINE TRANSPORT BY INSULIN IN HUMAN FOETAL ENDOTHELIUM

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Adenosine transport is mediated by human Equilibrative Nucleoside Transporters 1 (ENT1) and hENT2, which differentiate according to their inhibition with nitrobenzylthioinosine (NBMPR), in human umbilical vein endothelial cells (HUVEC). High D-glucose (25 mM) reduces overall adenosine transport in HUVEC. In this study we investigated whether insulin alters the effect of high D-glucose on adenosine transport via hENT1 or hENT2 in HUVEC. **Methods:** Cells exposed (24 h) to 5 or 25 mM D-glucose were incubated with 1 nM insulin (last 8 h of D-glucose incubation period). [^{3}H]Adenosine transport (7.8-500 μM, 4 μCi/ml, 20 s, 22ºC) was measured in absence or presence of 1 μM NBMPR, 2 mM hypoxanthine, or both. hENT1 and hENT2 mRNA number of copies was quantified by Real Time RT-PCR. **Results:** hENT1-mediated adenosine transport was reduced (~60%), however hENT2-mediated transport was unaltered by 25 mM D-glucose. Insulin increased (2.1-fold) overall adenosine transport in 5 mM D-glucose, associated with higher V_{max} for hENT2 (1.7-fold), but decreased V_{max} for hENT1 (45%). In cells exposed to 25 mM D-glucose overall adenosine transport was increased (2.3-fold) paralleled by increased V_{max} for hENT2-mediated transport (2.4-fold). However, hENT1-mediated transport was unaltered by insulin under this condition. Changes induced by insulin in adenosine transport were paralleled by increased mRNA levels for hENT1 (1.7-fold) and hENT2 (2.4-fold). **Conclusions:** These results demonstrate that insulin reverses the effect of D-glucose, an effect most likely due to increased hENT2 activity and expression in HUVEC. Supported by FONDECYT 1030781/1030607/7050030 (Chile).

EFFECT OF DIABETES ON THE SIGNALING PATHWAYS ACTIVATED BY BRADYKININ: CONTRIBUTION OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS

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Diabetes can induce endothelial dysfunction (ED). Bradykinin (BK), a vasoactive peptide, participates in the regulation of arterial tone. Angiotensin converting enzyme (ACE) degrades BK, and ACE inhibitors (ACEI) can prevent ED. We propose that cells respond differently to BK in a diabetic compared to a normal state, and that ACEI revert this effect. **Methods:** Sprague Dawley rats were made diabetic with streptozotocin (IP), and at 4 weeks were sacrificed. One week before sacrifice captopril (an ACEI) was administered on the water to a control and a diabetic group. One day before sacrifice, urine and blood samples were collected to measure creatinine clearance (CC). Kidneys were excised and used for protein and immunocytochemistry assays. Mesenteric vessels (MV) were perfused with BK and the endothelial response, enzymatic activities and protein content were measured. **Results:** CC showed no change in any of the 4 groups. ERK 1/2 phosphorylation was decreased while ERK 5 phosphorylation was increased in MV from diabetic rats. Captopril partly reverted these effects. Endothelial NOS was detected in MV from normal but not from diabetic rats, whereas inducible NOS was observed in diabetic but not in normal rats. In addition, NO bioavailability in response to BK was decreased in diabetic rats. In kidneys from diabetic rats, catalase activity was increased but B2 immunoreactivity was decreased. **Conclusions:** Our results suggest that in diabetic rats, BK can activate signaling pathways that are not changed in control animals, and that captopril can partly revert this response to that observed in physiological conditions. Supported by FONDECYT 1040809 & 1040816 (Chile).

DEFICIENT LIF PRODUCTION BY ENDOMETRIAL INFILTRATED CD3 T-CELLS COULD BE ASSOCIATED WITH REPRODUCTIVE FAILURE

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Leukemia inhibitory factor (LIF) was postulated as an essential factor for embryo implantation. We investigated the expression of LIF in edometrial and peripheral T cells from patients with recurrent spontaneous abortion (RSA) and patients with recurrent failure to in-vitro fertilization; and its modulation following alloimmunotherapy. **Methods:** Endometrial samples and peripheral blood obtained from these patients and from fertile women, were processed for LIF expression by Western Blot and flow cytometry. Specific detection of LIF in CD3+ cells was performed using mAbs for surface CD3 staining and intracellular LIF staining. **Results:** RSA and FIV-failure-patients did not expressed LIF when samples were studied either by Western Blot or by flow cytometry. In particular, flow cytometry allowed to detect the absence of intracellularly LIF in CD3+ endometrial cells in RSA (n=5) and in FIV-failures (n=5) patients. This lack of expression contrasted with the expression of LIF observed in fertile women (9% positive). In addition, endometrial CD3 cells from RSA patients increased intracellular LIF production after treatment with paternal leukocyte (5% positive). **Conclusions:** The defective production of LIF by infiltrated endometrial T-cells may contribute to the development of recurrent spontaneous abortions and in vitro FIV failure. This phenomenon might be modulated by treatment with paternal leukocytes which results in increasing LIF production and successful pregnancy rate. Supported by PROEGRE, Sociedad Argentina de Ginecología Reproductiva y Endocrinología.

DISTRIBUTION OF DECORIN AND BIGLYCAN IN THE MOUSE UTERUS DURING THE ESTROUS CYCLE

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The female organism is typically cyclic and controlled by precise levels of the ovarian hormones estrogen and progesterone. These regulators may influence synthesis and secretion of extracellular matrix (ECM) components, such as glycoproteins and proteoglycans. Decorin and biglycan are small leucine-rich proteoglycans (SLRPs), which share structural and functional similarities. The aim of this study is to analyze the differential expression of decorin and biglycan in the mouse uterus during the estrous and diestrous phases of the estrous cycle. **Methods:** The estrous cycle phases were determined through vaginal smear and the uteri were collected, fixed in methacarn and embedded in paraplast. Sections were submitted to immunocytochemistry, treated with anti-decorin and anti-biglycan antibodies and visualized with DAB. **Results:** In estrous, positive reaction against decorin was observed in the whole stroma, where swollen glands were shown. In the deep stroma, the reaction was scarcer due to the apparent edema. Biglycan was absent in the stroma, being restricted to the myometrium. In diestrous, intense reaction against decorin was seen in the deep stroma, weak around endometrial glands and absent under the lumen. Positive reaction for biglycan was moderate in the stroma, yet more intense close to the myometrium. However, it was absent in the subepithelial region. **Conclusions:** SLRPs participate in collagen fibrillogenesis and in the assembly of the ECM during growth and development. Our results suggest that production and distribution of decorin and biglycan may be influenced by ovarian hormones. To confirm this hypothesis, studies with castrated animals are ongoing. Supported by FAPESP.

2-METHOXYESTRADIOL INDUCES APOPTOSIS IN OVARIAN CANCER CELLS BUT NOT IN NORMAL CELLS

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2-Methoxyestradiol (2ME) is an endogenous metabolite of estrogen with antitumoral, antiangiogenic and apoptotic effects both *in vitro* and *in vivo*. Advanced ovarian carcinoma is the main cause of death from genital neoplasms in women. Despite different therapies the majority of them will die from their disease. The objective was to determine if 2ME would induce apoptosis in both ovarian cancer cells (A2780 cells and primary tissue cultures from carcinomatous ascitis) and normal primary tissue cultures established from reproductive tissues (ovary, endometrium, fallopian tube and cervix). **Methods:** To address the effect of 2ME in ovarian cancer cells and normal cells, dose curves for 2ME exposure were built. To assess the cytotoxicity, MTS assays were performed. To confirm the effect was due to apoptosis PARP cleavage, *in vitro* caspase-3 activity assays and DNA laddering were made. **Results:** Upon 2ME exposure, cell viability decreases significantly in dose-dependent manner (0.5-5uM) in ovarian cancer cells but not in normal cells. The cytotoxicity was due to apoptosis as confirmed by all the methods previously described. In contrast, in normal cells, no apoptosis was detected. **Conclusions:** 2ME induces apoptosis in ovarian cancer cells but not in normal cells. 2ME could be an attractive new reagent in the treatment of ovarian carcinoma with no toxicity in normal reproductive tissues. Supported by Fondecyt 1050744 and 1020715.

FIBRILLIN-1 IN MOUSE ENDOMETRIUM DURING EARLY PREGNANCY

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Mouse embryo implantation leads to the formation of decidua, which is crucial for embryo development and the success of gestation. Decidualization comprises an intense remodeling of the endometrial stroma, including a decrease of extracellular matrix (ECM), thickness of collagen fibrils, and a temporospatial differential expression of various ECM molecules. Fibrillin-1 is an ECM glycoprotein that participates in cell proliferation, migration and differentiation. Fibrillin-1 is conjectured to be related to TGF-β and may have a function on uterine expansion throughout pregnancy. Our goal is to analyze the spatial-temporal distribution of Fibrillin-1 during pre and post-implantation periods. **Methods:** Implantation sites from 2nd and 5th days of pregnancy (dop) were collected, fixed in Methacarn and embedded in Paraplast. Sections were immunostained with anti-Fibrillin-1 antibody. **Results:** On 2nd dop, Fibrillin-1, although widely distributed throughout the uterus, was concentrated in the region of basement membranes of both uterine lumen and glands. In the stroma, it was distributed as a network of thin fibrils. After implantation, the network-like positive fibrils were restricted to the non-decidualized endometrium. Immunoreaction irradiated from the embryo implantation crypt, towards the peripheral stroma of the antimesometrial region. **Conclusions:** The distribution of Fibrillin-1 on mouse endometrium changes according to the differentiation and proliferation state of endometrial fibroblasts. This differential expression could be under the control of ovarian hormones, growth factors or other ECM components, such as proteoglycans. Supported by CAPES (Brazil).

EFFECT OF EMBRYONIC CULTURE MEDIA (ECM) ON THE EXPRESSION OF MARKER MOLECULES OF ENDOMETRIAL FUNCTION

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Paracrine interactions between epithelial (EEC) and stromal (ESC) cells, as well as, embryonic secretion products are necessary to achieve the receptive phenotype of the human endometrium. The aim of this study was to evaluate the action of human embryo secretion products on mRNA levels of endometrial markers in a EEC/ESC co-culture model (CC). **Methods:** The ECM were derived from two groups: (a) hCG-beta(+), (b) hCG-beta(-); as control, culture media alone were used (CM). CC system was developed using endometrial biopsies from healthy women obtained during the mid secretory phase. EEC/ESC were treated with 10(-8)M estradiol and 10(-6)M progesterone for 48 h; then, ECM were added only to EEC. Expression of Prolactin (PRL), progesterone receptor (PR) isoforms and Mucin-1 (MUC-1) was assessed by RT-PCR. **Results:** In EEC, ECM-beta(+) decreased PR levels (55%) and MUC-1 (20%). No changes in MUC-1 were observed with ECM-beta(-). The paracrine action of ECM-beta(+) in ESC increase PR-A (36%, $p<0.05$), isoform which is related to the regulation of implantation genes. Also, increase of PRL mRNA levels (15%), which indicates decidual reaction, was observed. No effects were obtained for ECM-beta(-). **Conclusions:** The data suggest that embryonic secretion products may act through paracrine mechanisms to induce changes in ESC, which are compatible with the endometrial receptive phenotype. Supported by FONDECYT 1010821-1050098 (Chile).

RECURRENT SPONTANEOUS ABORTION: PREVALENCE OF ALOIMMUNE AND THROMBOPHILIC CAUSES

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We present a series of recurrent spontaneous abortion (RSA) patients submitted to immunological and thrombophilic evaluation after a previous negative common investigation. **Methods:** Retrospective study of 86 patients with 3 or more RSA was done, looking for thrombophilia and disruption of aloimmune response. Aloimmune cause was defined as a negative serological cross-match against husband lymphocytes. Flow cytometry quantification of Natural Killer cells (NK)(CD16+56+) in blood and endometrium was performed. **Results:** Patients had 3,6 ± 1,0 previous miscarriages. Thrombophilia ocurred in 40,8% (31/76) with 23,7% (18) auto-immune, 11,8% (9) genetic and 5,3% (4) both. Auto-antibodies detected included: anticardiolipine (18,6%), antiphosphatidilserine (17,7%), anti-B2glicoprotein (12,8%), antinuclear antibody (12,1%) and lupic anticoagulant (1,9%). The most common genetic thrombophilias were: protein deficiency (20%) and Leiden mutation (14,7%). Crossmatch was negative in 3 patients (3,9%). The mean NK rate was 11,0 ± 5,6% in blood and 34,2. ± 15,8% in endometrium. There was 39,5% (n=34) of the cases with aloimmune factor, 24,4% (n=21) with negative investigation, 24,4% (n=21) with thrombophilic plus auto-immune causes and 11,6% with isolated trombophilia. **Conclusions:** We advise additional investigations for RSA patients since there is a potentially treatable cause in a majority of them: a third part has thrombophilia and more than that might have an alloimune disorder.

TREATMENT OF ALOIMMUNE AND THROMBOPHILIC CAUSES IN RECURRENT SPONTANEOUS ABORTION

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Treatment of immunological causes of recurrent spontaneous abortion (RSA) continues to be a matter of debate. We present a series of 86 patients with 3 or more RSA and a previous negative investigation for usual causes. **Methods:** Auto-immune and genetic thrombophilias were searched, and the recommend treatment was AAS and/or unfractioned or low molecular weight heparine. The alloimune factor was defined as a negative serological crossmatch against the husband lymphocytes and quantification of Natural Killer cells (NK) (CD16+56+) in blood and on endometrium by flow cytometry. Alloimune treatment was alloimunization with husband lymphocytes and/or intravenous immunoglobuline. P values were significant when ≤0,05. **Results:** There was 64% (55/86) prevalence of aloimmune factor and 40,8% (31/76) of thrombophilic causes detected. Patients with aloimmune factor specificly treated increased the pregnancy (5x57%, P=0,00, OR=0,08 [0,01-0,6]) and birth rate (5x43%, P=0,00; OR=0,11 [1,7-118,6]). Thrombophilia was associated in 38% (21/55) of these patients, and its treatment resulted in more pregnancies (30x67%, P=0,04, OR=0,45 [0,24-0,83]) and non-significant more births (26x42%, P=0,30). In the exclusively thrombophilic group (n=10) none untreated patient got pregnant, and there were 4 pregnancies and 2 births among the treated ones. **Conclusion:** It seems that RSA patients with an aloimmune factor benefit from a specific treatment, improving the birth rate.

ESTRADIOL INCREASES CELL COUPLING AND GAP-JUNCTION-DEPENDENT CALCIUM WAVES IN OVIDUCT SMOOTH MUSCLE CELLS

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Ovum and embryo transport through the oviduct depends in part on coordinated contraction of the smooth muscle layer of the oviduct. Intercellular communication through gap junctions (GJs) facilitates the intercellular propagation of calcium waves (ICWs) that are important to coordinate cellular functions. In the oviduct, connexin 43 levels are upregulated by estradiol (E_2). The purpose of this study was to determine the effect of E_2 on cell coupling and ICW propagation in smooth muscle cells (SMCs). **Methods:** Primary cultures of SMCs were prepared from rat oviducts. SMCs were incubated with E_2 (3nM) or vehicle (6 or 15 h). Junctional communication was determined using the dye coupling technique (lucifer yellow). Calcium fluorescence images were obtained in SMCs loaded with Fura2AM. After stimulation of a single cell with a glass microprobe, propagation velocity of ICWs was calculated. **Results:** At 15h after E_2 application both cellular coupling through GJ and propagation velocity of ICWs were significantly increased. ICWs were abolished with 35 µM 18-beta-glycyrrhetinic acid, a GJ blocker, but not with apyrase, an ATP phosphatase, indicating that propagation of ICWs occured via GJs. **Conclusions:** E_2 enhances cell coupling and propagation velocity of ICWs in oviductal SMCs, suggesting a possible involvement of the latter in coordinating contractions of the rat oviduct smooth muscle. Supported by FONDECYT 1040804.

SIGNS OF DEATH IN ESPERMATOCYTES OF TESTIS RAT, GLUCOSE POSSIBLE MODULATOR

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The spermatogenesis is the process by which diploid spermatogonia becomes haploid spermazotoa. During this differentiation the apoptosis support the homeostasis of each cellular type. The objective of this study was to determine the effect of glucose in germ cell survival in vitro. **Methods:** Primary culture of isolated spermatocytes from 25 days old rats were cultured between 0-30 h in KH-medium with or without 10 mM glucose. Cell viability was evaluated by LDH activity and trypan blue exclusion. Fas, p53 levels were evaluated by western blot in testis of 25 days old rat. Co-localization of Fas and TUNEL was made by immunofluorescence in 25 days old rats. **Results:** We found that Fas positive cells colocalized with TUNEL in 25 days old rat. According with their localization in the seminiferous tubules, they were spermatocytes. Isolated Fas-positive spermatocytes showed an increased level of Fas and their transcriptional activator p53. In vitro studies with isolated spermatocytes showed that after 24 hrs of culture 60% of viable cells were found in glucose-containing medium, but 80% in medium with lactate and without glucose. Immunofluorescence showed increase of the level of Fas in cells incubated with glucose. **Conclusion:** Our results indicate that cells undergoing apoptosis are spermatocytes, and that glucose could be a modulator of germ cell survival. Supported by FONDECYT 1040800.

DIFFERENTIAL REGULATION OF ADENOSINE TRANSPORT BY ACTIVATION OF A_{2a} AND A_1 PURINOCEPTORS IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Human umbilical vein endothelial cells (HUVEC) take up adenosine via equilibrative nucleoside transporters 1 (hENT1) and hENT2, a process involved in maintaining physiological plasma adenosine concentrations. Adenosine activates A_{2a} purinoceptors increasing L-arginine transport and nitric oxide synthesis in HUVEC, suggesting that changes in adenosine membrane transport are crucial for the modulation of adenosine vascular effects. We studied whether adenosine alters hENT1 expression and activity in HUVEC. **Methods:** Cells were cultured in medium 199 containing 20% sera. Overall adenosine transport (0-500 μM, 6 μCi/ml, 20 s, 22°C) was measured in cell monolayers preincubated (30 min) with CGS-21680 (0.001-10 μM, A_{2a} agonist), 5`-*N*-ethyl-carboxamidoadenosine (NECA, 0.001-10 μM, nonselective adenosine agonist) and ZM-241385 (100 nM, A_{2a} antagonist). hENT1-mediated transport was assessed using 1 μM nitrobenzilthioinosine (NBMPR) and 2 mM hypoxanthine. Protein abundance was determined by Western blot. **Results:** The maximal velocity (V_{max}) for hENT1-adenosine transport was reduced (~27%) by 30 nM CGS-21680, but was increased (2.5-fold) by 40 nM NECA. Only CGS-21680 effect was blocked by ZM-241385. CGS-21680 (4 h) increased (1.3-fold) hENT1 protein abundance. **Conclusions:** Activation of A_{2a} purinoceptors downregulates hENT1-mediated adenosine transport. However, A_1 purinoceptors activation could be responsible for activation of hENT1-mediated adenosine transport in HUVEC. Supported by FONDECYT 1030781/1030607/7050030 (Chile).

POST-TRANSLATIONAL MODULATION OF EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 1 BY D-GLUCOSE IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Removal of nucleoside from the extracellular space is mainly mediated by human equilibrative nucleoside transporters 1 (hENT1) in human umbilical vein endothelial cells (HUVEC). HUVEC exposed to elevated extracellular D-glucose exhibit reduced adenosine transport and a lower number of transporters for nucleosides in the plasma membrane. We studied whether hENT1 mRNA and protein stability were affected by high D-glucose. **Methods:** HUVEC isolated by collagenase (0.2 mg/ml) digestion were cultured (37°C, 5% CO_2) in medium 199 containing 5 mM D-glucose and 20% sera. HUVEC pre-incubated for 24 h with 5 or 25 mM D-glucose were exposed to actinomycin-D (1.5 μM, 0-24 h). hENT1 mRNA was quantified by RT-PCR and hENT1 protein abundance was determined by Western blot. **Results:** hENT1 mRNA level was unaltered for the first 12 h, but it was similarly decreased after 18 h (~68%) and 24 h (~51%) of actinomycin-D treatment in 5 or 25 mM D-glucose. Protein abundance was unaltered in 5 mM D-glucose, but decreased significantly at 12 h (~40%), 18 h (~70%) and 24 h (~80%) after actinomycin-D treatment in cells exposed to 25 mM D-glucose. **Conclusions:** An environment where D-glucose concentration is abnormally elevated does not alter hENT1 mRNA degradation kinetics in HUVEC. However, hENT1 protein stability is reduced suggesting post-translational modulation of hENT1 by D-glucose in this cell type. In addition, these findings could explain, in part, the D-glucose-reduced adenosine transport detected in HUVEC. Supported by FONDECYT 1030781/1030607/7050030 (Chile).

LOCALIZATION CDK2/CYCLIN E AFTER FERTILIZATION IN ZYGOTES TREATED WITH 3 AMINO-BENZAMIDE (3 ABA)

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We have previously shown that the inhibition of poly(ADP-ribose)polymerase by 3 ABA blocks the increased poly(ADP-ribosylation) of CS histone variants which occurs at the entrance of S phase and cause a severe decrease of DNA replication at the initial cell cycle in sea urchin embryos. To investigate if this S phase inhibition is related to the lack of cell cycle control, we have compared the localization of CDK2 and cyclin E in zygotes treated with 3 ABA with that observed in controls. CDK2 and cyclin E were immunolocalized in zygotes harvested at different times after fertilization by specific antibodies against these proteins. The fusion of both pronucleus was not affected by the treatment with 3 ABA and both proteins, CDK2 and cyclin E, were found to be present in the nucleus of normal zygotes, as well as, in those treated with 3 ABA at a time when the first S phase was initiated. We have also investigated the effect of 3 ABA treatment on the first mitosis by immuno staining the mitotic furrow with antibodies against □-tubuline. We found that the microtubules were polymerized in the zygotes treated with 3 ABA in a manner reminiscent of the normal bipolarized mitotic furrow observed in normal embryos. We postulate that the inhibitory effect of 3 ABA on the initial S phase does not depend on an alteration of the G1/S transition checkpoint, affecting the elongation of DNA replication. Grants: FONDECYT 1050100, DIUC 204.037.001.1.0, PICS-CNRS/CONICYT.

HYPOXIA INCREASES EQUILIBRATIVE NUCLEOSIDE TRANSPORTER 2 ACTIVITY BY A TRANSCRIPTIONAL INDEPENDENT MECHANISM IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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Low oxygen tension (hypoxia) reduces adenosine transport in several types of mammalian cells. Adenosine transport is mediated by human equilibrative nucleoside transporter 1 (hENT1) and hENT2 in human umbilical vein endothelium (HUVEC), a fetal cell type that grows under 5% O_2 (ie. normoxia for this cell type). We studied whether hypoxia alters hENT2 expression and activity in HUVEC. **Methods:** Cells were cultured (0-24 h) in 5% or 2% O_2 (hypoxia), and [^{3}H]adenosine uptake (125 and 500 μM, 4 μCi/ml, 20 s, 37°C) was measured in absence or presence of 100 nM nitrobenzylthioinosine (NBMPR, hENT1 inhibitor). hENT2 mRNA was quantified by real time RT-PCR, and protein abundance was determined by Western blot. *SLC29A2* (for hENT2) promoter activity was measured following transfection (electroporation, 320 V, 30 ms) with pGL3 basic plasmid (*firefly*/*renilla* luciferase reporter gene) carrying -1477 bp and -587 bp of the promoter sequence. **Results:** Hypoxia reduced hENT2 mRNA expression (~55%), and promoter activity (~50%), but did not alter hENT2 protein abundance. Adenosine uptake via hENT2 was increased (2-fold) in hypoxia. **Conclusions:** Adenosine uptake via hENT2 may be modulated by post-translational mechanisms in hypoxia in HUVEC. Supported by FONDECYT 1030781/1030607/7050030. A Torres holds a School of Medicine research fellowship, and M Farías holds a CONICYT-PhD fellowship.

EFFECT OF INSULIN ON L-ARGININE TRANSPORT THROUGH SYSTEM y^+L IN HUMAN UMBILICAL VEIN ENDOTHELIUM

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L-Arginine transport is mainly mediated by systems y^+L and y^+/CATs in human umbilical vein endothelial cells (HUVEC). Insulin increases system y^+/CATs activity in this cell type, but the effect of this hormone on system y^+L has not been studied. We determined whether insulin regulates mRNA levels for *SLC3A2/4F2hc* (4F2hc), *SLC7A7/4F2-lc2* (y^+-LAT1) and *SLC7A6/4F2-lc3* (y^+-LAT2) and transport activity of system y^+L in HUVEC. **Methods:** L-[^{3}H]Arginine transport (0.6-20 μM, 2 μCi/ml, 1 min, 37ºC) was measured in absence or presence of insulin (0.1 nM, 8 h), Na^+, *N*-ethylmaleimide (200 μM, system y^+ inhibitor) or L-leucine (100 μM). mRNA levels of *SLC3A2/4F2hc, SLC7A7/4F2-lc2* and *SLC7A6/4F2-lc3* genes for system y^+L were analyzed by RT-PCR. **Results:** Insulin increased (2.4-fold) the maximal velocity (V_{max}) for L-arginine transport, without significant changes in the apparent K_m for system y^+L. However, insulin reduced *SLC3A2/4F2hc* (~62%), *SLC7A7/4F2-lc2* (~38%) and *SLC7A6/4F2-lc3* (~16%) mRNA levels. **Conclusions:** Our results suggest that insulin increases system y^+L activity possibly through post-translational mechanisms in human umbilical vein endothelium. We speculate that reduced expression of genes coding for system y^+L could be a mechanism to compensate increased L-arginine transport in response to insulin. Supported by FONDECYT 1030781/1030607/7050030 (Chile). J Varas holds a School of Medicine research fellowship, and M Farías holds a CONICYT-PhD fellowship.

EFFECTS OF SEROTONIN AND DOPAMINE ON RABBIT PETROSAL GANGLION ISOLATED NEURONS

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The petrosal ganglion (PG) contains chemosensory neurons that project peripherally to the carotid body (CB) through the carotid sinus nerve. It is generally accepted that the chemosensory activity is initiated by transmitters released from the CB receptor (glomus) cells, as a result of the transduction process. Dopamine (DA) and serotonin (5-HT) have been proposed to mediate this response, but the receptor type involved is still uncertain. In this work we characterized the type of receptor involved in the responses evoked by DA and 5-HT on rabbit isolated PG neurons. **Methods:** PGs obtained from adult rabbits, anesthetized with ketamine/xylazine (75/7.5 mg/kg), were enzimatically dissociated under constant agitation, plated on poly-L-lysine coated 35 mm Petri dishes, and maintained for 3-16 days at 37-38°C, in water-saturated atmosphere. PG neurons were cultured in F-12 medium and 5% CO_2 in air atmosphere for DA experiments, and in L-15 medium and air atmosphere for 5-HT experiments. Using the "whole-cell" patch clamp technique we measured the currents evoked by DA and 5-HT specific agonists and antagonist. **Results:** At a holding potential of -60 mV, DA and 5-HT evoked dose-dependent inward currents in more than 70% of the recorded neurons. Currents evoked by DA and 5-HT were blocked with 10 nM tropisetron, a 5-HT_3-type receptor antagonist, but the currents evoked by DA were insensitive to 10 nM domperidone, a D2 receptor antagonist. **Conclusions:** Our results suggest that in the rabbit petrosal ganglion isolated neurons both transmitters induce inward currents activating serotoninergic 5-HT_3-type receptors. Supported by FONDECYT 1040638.

EXPRESSION OF COMPLEMENT COMPONENT INHIBITORS IS INCREASED IN THE ENDOMETRIUM DURING THE RECEPTIVE PERIOD AND DECREASED IN INFERTILE WOMEN WITH IMPLANTATION FAILURE

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Regulation of the complement pathway in the endometrium is believed to be important for embryo implantation. The aim of this study was to compare mRNA levels of the complement components C1 inhibitor, C4 binding protein (C4BPα), clusterin (CLU) and the citotoxicity inhibitor Serpin B9 (PI9) in human endometrium, in pre-receptive and receptive period and in the endometrium of women with repeated implantation failure. **Methods:** Endometrial biopsies were obtained on LH+3 and LH+7 of the same cycle from 8 fertile women and on day 7 of progesterone administration in hormonally induced cycles of 5 infertile women with repeated implantation failure. mRNA level was determined by semi-quantitative real time RT-PCR. Protein expression was determined by immunochemistry. **Results:** All genes evaluated showed upregulation during the receptive period while C4BPα, Clusterin and PI9 showed decreased expression in women with implantation failure. The expression of the corresponding proteins, immunolocalized in the epitelial and stromal cells, was correlated with RT-PCR results. **Conclusions:** These results suggest strong inhibition of the complement cascade in the human endometrium during the receptive period. Subnormal expression of complement component inhibitors in women with repeated implantation failure suggests they play an important role in the immune environment required for successful embryo implantation. Supported by FONDECYT 1030004, DICYT-USACH (Chile) and CONRAD CIG -02-83 (USA).

D-GLUCOSE STIMULATES NITRIC OXIDE SYNTHASE VIA A MECHANISM THAT INVOLVES TGF-β1 IN HUMAN FOETAL ENDOTHELIUM

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Gestational diabetes and D-glucose increase endothelial nitric oxide synthase (eNOS) activity in human umbilical vein endothelium (HUVEC). Transforming Growth Factor β1 (TGF-β1) level is increased in diabetic patients and by D-glucose. We studied TGF-β1 involvement in D-glucose stimulated nitric oxide (NO) synthesis. **Methods:** HUVEC were exposed (6 or 24 h) to 5 or 25 mM D-glucose in absence or presence of TGF-β1 (2 ng/ml). eNOS activity was estimated by L-[^{3}H]citrulline formation from L-[^{3}H]arginine (4 μCi/ml, 37°C) in absence or presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM). Total and Ser^{1177}-phosphorylated eNOS (P~eNOS), and total and P~Smad2, a TGF-β1 associated signaling molecule, protein levels were determined by Western blot. **Results:** eNOS protein abundance was increased (1.3 to 1.5-fold) by TGF-β1, 25 mM D-glucose, or 25 mM D-glucose + TGF-β1 at 6 and 24 h of incubation. eNOS activity was also increased by these molecules (1.5 to 3.3-fold). At 6 h, P~eNOS was increased by TGF-β1, 25 mM D-glucose, or 25 mM D-glucose + TGF-β1 (2.2 to 3.4-fold). However, at 24 h, P~eNOS was reduced by TGF-β1 (~30%), but was increased by 25 mM D-glucose (1.6-fold). P~Smad2 was increased (3- to 8-fold) by TGF-β1, 25 mM D-glucose, or 25 mM D-glucose + TGF-β1 at 6 and 24 h. **Conclusions:** High D-glucose–increased NO synthesis could result from a mechanism involving TGF-β1 via Smad2 activation in HUVEC. D-Glucose effect on eNOS activity seems fully dependent on TGF-β1 at short times, but only partially dependent at longer times of incubation. Supported by FONDECYT 1030781/1030607/7050030 (Chile). R Vásquez holds a DIPUC-PhD fellowship.

INHIBIN PRODUCTION BY RAT EARLY ANTRAL FOLLICLES IN CULTURE.
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Development and inhibin production by antral follicles is mainly dependent on FSH, which is a polymorphic hormone. Follicular synthesis of inhibin *in vitro* has not been reported. The aim of this study was to determine the effect of FSH glycosylated variants on production of inhibin, by rat early antral follicles (EAF) in culture. **Methods:** FSH variants bearing biantennary and truncated (WB) or high mannose and hybrid-type oligosaccharides (FB) were obtained after Con-A chromatography of recombinant human FSH (rhFSH). EAF (~350μm) were dissected from ovaries of Sprague-Dawley rats (24-26 day-old) treated with diethylstilbestrol. Follicles were cultured during 24 h in absence or presence of 25 ng/mL rhFSH or molecular variants. Following incubation, dimeric inhibin A and B and Pro-αC were assayed in the medium by ELISA. **Results:** Under basal conditions, follicles produced 4-fold more inhibin B than Inhibin A (A/B ratio = 0.32 ± 0.07). Inhibin A levels increased in response to rhFSH, WB and FB ($P<0.05$), although response to FB was significantly lower in comparison to WB ($P<0.01$). Addition of FSH at a dose of 25ng/mL failed to stimulate inhibin B production. Pro-□C levels were increased after treatment with WB and FB ($P<0.05$). **Conclusions:** These results show that rat EAF in culture produce and secrete inhibins, in similar proportion as observed in serum. Inhibin production was differentially stimulated by FSH variants. Supported by PROGRESAR.

REMODELATION OF THE MOUSE PUBIC SYMPHYSIS DURING PREGNANCY
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A finely tuned tissue remodeling takes place in the mouse pubic joint during late pregnancy. At this time, the pubic joint becomes separated by a flexible and elastic interpubic ligament, which at parturition maybe 5 or 6mm long. Following labor, the ligament undergoes rapid involution on the 3 rd or 4 th day and got back together almost completely. It is known that relaxin is involved in reproductive tract physiology, changing the regulation of biochemical processes involved in remodelation of extracellular matrix (ECM) components. Even though there are almost no reports that recognize the specific role of metalloproteinases (MMP) in the remodelation of the mouse pubic symphysis or ligament; it is well known that MMPs are endopeptidases that degrade ECM gelatins in the pregnant uterus and cervix. Methods: Therefore, virgin and pregnant swiss mouse were used for the detection of the MMPs. The MMPs were evaluated by tissue homogenate zymography to distinguish the existence of potential gelatinase activity and tissue immunohistochemical expressions of MMP2 and MMP9 were detected by specific primary antibodies. Results: Zymography detected gelatinases MMP2 and 9 as both proenzyme and mature forms in the symphysis and interpubic ligament. Immunohistochemistry showed the expression of MMP2 and 9 in the reproductive tissues. Conclusions: Our results support the hypothesis that MMPs can facilitate growth and remodeling of the interpubic symphysis by intrinsic processes. The extent of interpubic ligament changes provides evidence for a link between estrogen, relaxin in the pregnancy and MMPs activities for ECM remodelation. Supported by: FAPESP/CNPq.

SPERM CHROMATIN REMODELING AFTER FERTILIZATION IN SEA URCHINS
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Sperm chromatin remodeling involves the replacement of sperm histones (SpH) for CS histone variants recruited from maternal pools. We had postulated a model for degradation of SpH concomitant to male chromatin remodeling involving a cystein protease(SpH protease). In this model it is uncertain yet if this protease may catalyze the degradation of SpH organized as nucleosomes, or if the SpH should be disassembled from DNA prior to their degradation. We had developed an *in vitro* assay in which this protease was incubated with isolated sperm nucleosomes and the SpH remaining were analyzed by Western blot. SpH were not degraded when they are organized as nucleosomes, while the complete set of SpH are readly degraded when they are unbound from DNA. We postulated that the removal of SpH from DNA by histones chaperones or chromatin remodeling complexes is a prerequisite for their degradation. This SpH nucleosome remodeling activity was further investigated by an *in vitro* assay in which disassembly of isolated sperm nucleosomes was investigated. This activities were found in zygotes during the time of male pronucleus formation, declining afterwards and were absent in unfertilized eggs. Furthermore, we had also found that free DNA inhibits this protease. We postulate that this nucleosome remodeling activity participates in male pronucleus formation by releasing the SpH from chromatin and then they are degraded by the SpH cystein-protease. GRANT: FONDECYT 1050100.