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C1.

SORTING OF THE ALZHEIMER'S DISEASE AMYLOID PRECURSOR PROTEIN MEDIATED BY THE AP-4 ADAPTOR

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Sorting of transmembrane proteins to post-Golgi compartments of the endomembrane system such as endosomes, lysosomes, lysosome-related organelles (LROs) and the basolateral surface of polarized epithelial cells is mediated by interaction of (i) signals in the cytosolic domains of the proteins with (ii) adaptors that are components of protein coats. Two types of signal referred to as "tyrosine-based" and "dileucine-based" are recognized by the clathrin-associated, heterotetrameric adaptor protein (AP) complexes, AP-1, AP-2 and AP-3. Recent studies identified a fourth AP complex, AP-4, whose signal-recognition specificity and function remained to be established. We have found that the mu4 subunit of AP-4 recognizes a new type of signal in the cytosolic tail of the Alzheimer's disease amyloid precursor protein (APP), resulting in sorting of this protein from the trans-Golgi network to endosomes. Disruption of the APP-AP-4 interaction decreases localization of APP to endosomes and enhances gamma-secretase-catalyzed cleavage of APP to the pathogenic amyloid-beta peptide. These findings identify AP-4 as a novel regulator of APP traffic and indicate that production of the amyloid-beta peptide occurs in the late secretory pathway rather than in endosomes. Defects in AP-4 should therefore be considered a potential risk factor for Alzheimer's disease.

C2.

THE DIFFERENTIAL ENDOCYTOSIS DYNAMICS OF THE INSULIN RECEPTOR (IR) SPLICE VARIANTS REGULATES MITOGENIC AND METABOLIC SIGNALING

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Insulin signalling comprises a complex cascade of events playing a key role in the regulation of glucose metabolism and cellular growth. Failures in its function lead to diabetes and disregulations of these pathways were described in many cancer types. The IR is a tetrameric receptor tyrosine kinase. Two splice variants exist in mammalian cells: IR-A lacking exon 11, and the full length IR-B.
The main goal of this work is the study of the dynamics of the activation and internalization of the IR. We generated recombinant IR fused to different visible fluorescent proteins without affecting functionality. Biotinylated insulin in combination with fluorescent nanoparticles (streptavidin-quantum dots) allowed us to study IR dynamics after insulin binding by microscopy and flow citometry. These showed that IR-A is internalized more rapidly than IR-B. These differences correlated with higher and sustained activation of IR-A in response to insulin and distinctive ERK 1/2 activation profiles and gene transcription regulation. On the other hand IR-B triggered a higher AKT signaling which is involved in metabolism regulation. These results support a model of diferential localization of the IR signaling: while internalized receptors regulate mitogenic activity, surface receptors are responsible of the metabolic balance regulation.receptors are responsible of the metabolic balance regulation.

C3.

SERENDIPITY, OR HOW WORKING WITH TRYPANOSOMATIDS CHANGED MY LIFE (WITH A LITTLE HELP FROM MY FRIENDS)

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The unexpected *in vivo* finding that unlike what happens in other eukaryotes, glycans transferred from dolichol-P-P derivatives to proteins in trypanosomatids lacked glucose units but displayed those residues once linked to proteins indicated that direct glucosylation of protein-linked glycans could occur in nature. Further work showed that the same reaction occurred in mammalian, plant and fungal cells. Although the reaction could be reproduced in cell-free assays using microsomes as enzyme source and endogenous glycoproteins as acceptors, addition of exogenous glycoproteins did not enhance glucose transfer from UDP-Glc. By chance and after many failed attempts it was found that only glycoproteins displaying non native conformations served as glucose acceptors. This property lead to the proposal of an ER glycoprotein folding quality control mechanism involving a glucosyltransferase, a glucosidase and two lectins (calnexin and calreticulin) behaving as unconventional chaperones. This mechanism prevents exit to the Golgi of misfolded glycoproteins or of folding intermediates and results in an enhancement of folding efficiency. The glucosyltransferase is large two domain single polypetide that recognizes hydrophobic patches in molten globule like conformers, whereas the glucosidase is a heterodimer composed of catalytic (GIIa) and regulatory (GIIb) subunits. This last subunit displays a mannose receptor homologous (MRH) domain at its C-terminus that modulates GIIa activity. As a result of this modulation, ER demannosylation of misfolded/slow folding glycoproteins leads to a prolonged existence of glycans recognized by calnexin/calreticulin.

C4.

FUNCTIONAL CHARACTERIZATION OF NEW GENES IDENTIFIED IN PITUITARY TUMORS

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Several genes and signaling pathways have been identified to play a role in the development of pituitary tumors, but still there is not a clear picture about genes involved. We approached this search by using the mRNA differential display technique comparing tumor and normal pituitary cells. Two genes have been identified to be involved in pathogenesis process: a) in prolactinomas obtained from Dopamine D2R knockout female mice, we have found differential expression of noggin and BPMP-4; b) in GH3 cell line clones overexpressing the IL-6 signal transducer gp130, which have enhanced tumorigenecity in nude mice, we found the expression of a new gene that we cloned, RSUME.

BMP-4 has been found to be augmented and noggin decreased during prolactinoma development and have a crucial role in cell proliferation. On the contrary, in corticotrophinomas BMP-4 has an inhibitory role. RSUME expression was induced under hypoxic conditions, increases VEGF expression, which correlates with increased angiogenic potential of the lactosomatotrophic gp130 clones, and has a potential role during vascularization. Acting on different processes of tumor development these findings may provide new interesting targets for inhibiting steps involved in pituitary tumorigenesis.

C5.

INTEGRATING TOLEROGENIC CIRCUITS AT THE FRONTIERS OF IMMUNOLOGY AND GLYCOBIOLOGY

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Recent efforts toward decoding the glycosylation signature of immune cell processes have revealed dramatic changes in *N*- and *O*-glycan structures during T cell activation and differentiation. These alterations have also been detected during the course of dendritic cell (DC) differentiation and maturation, suggesting that protein-glycan interactions may have a decisive role in the control of immune cell responsiveness and tolerance. The responsibility of deciphering these glycosylation changes is assigned in part to endogenous glycan-binding proteins. Galectin-1, an endogenous soluble lectin which recognizes multiple galactose-β1-4-N-acetylglucosamine (poly-LacNAc) units present on the branches of *N*- or *O*-linked glycans, elicits a broad spectrum of anti-inflammatory and immunomodulatory effects. Secretion of this glycan-binding protein substantially contributes to the immunosuppressive activities of human and mouse cancer cells. Blockade of galectin-1 expression in tumor tissue results in heightened T cell-mediated tumor rejection and increased secretion of T helper type-1 (T_H1) cytokines. Moreover, galectin-1-deficient (*Lgals1*$^{-/-}$) mice exhibit augmented T_H1 and T_H-17 responses and are considerably more susceptible to immune-mediated fetal rejection and autoimmune disease than their wild-type counterparts. In a search for potential mechanisms underlying the broad immunosuppressive activities of galectin-1, we conducted an integrated study of the impact of this protein on T cell and DC physiology. Establishment of galectin-1-glycan lattices selectively blunted T_H1 and T_H-17, but not Th2-differentiated cells consistent with the differential repertoire of cell surface glycans ('glycome') expressed by these cells. Moreover, we have identified an immunomodulatory circuit, based on the differential glycosylation of DCs which links galectin-1 signaling, differentiation of tolerogenic dendritic cells and expansion of regulatory T cells which contributes to the resolution of autoimmune inflammation and modulates T cell responses in antigen-specific and neoplastic settings. Establishment of galectin-1-glycan lattices endowed DCs with the capacity to blunt T_H-17 and T_H1 responses and suppress autoimmune inflammation through mechanisms involving IL-27 and IL-10. Strategies to manipulate this circuit in either direction (stimulation or blockade) may be capable of influencing immune tolerance versus activation, a critical decision with broad therapeutic implications in immunopathology.

S1.

MOLECULAR BIOLOGY OF HUMAN PLACENTAL DEVELOPMENT

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Placental cytotrophoblasts (CTBs) proliferate and differentiate, by fusion, to form a syncytiotrophoblast (STB), event that starts with modifications of the plasma membranes of both partners such as expression of syncytin, connexin 43 and enrichment of phosphatidylserine on the STB surface. We have reported the cloning and characterization of a new member of the START family, involved in the intracellular lipid transport, up-regulated in the choriocarcinoma JEG-3 cell line, denominated StarD7. Elisa assays revealed that StarD7 binds cardiolipin, phosphatidylserine and phosphatidylcholine. In addition, an increased StarD7 protein expression and subcellular relocalization were observed in *in vitro* differentiating cytotrophoblast. Furthermore, we have shown that β-catenin activates human StarD7 expression. By other side, StarD7 silencing led to a marked decrease of Twist1, Cnx43, MBD2, ABCG2 and TGFβRII mRNA levels. In contrast, knocking down StarD7 increased the expression of syncytial formation markers, such as b-hCG protein production and secretion, β-hCG mRNA levels, as well as GCM1, a transcriptional factor required for syncitialization. In addition, a reduction of intercellular desmosomes between adjacent JEG-3 cells after ablation of StarD7 expression was observed, suggesting that StarD7 play a key role in the gene expression control relevant to normal development of trophoblast differentiation.

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S2.

EFFECTS OF HYPO AND HYPERTHYROIDISM ON PREGNANCY AND LUTEAL FUNCTION

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Thyroid pathologies are are a frequent cause of infertility, spontaneous abortion, pregnancy disorders, premature delivery and deficitary lactation. Thyroid hormones (HTs) can disturb fertility indirectly, through interference with the hypothalamo-pituitary-gonadal axis, or directly at the ovarian level, since their receptors (TRs) are present in most of the cell types of the axis. HTs have actions on ovarian steroidogenesis, but stimulate follicular growth. Hypothyroidism (HypoT) produces hyperprolactinemia, that in turn, induces irregular cycles and, in the rat, pseudopregnancy. Hyperthyroidism (HyperT) advances delivery while HypoT delays it and both cause lactation deficit through a partial blockade of the suckling induced hormone release. HTs modify luteal function modulating the balance between luteotrophic (PGE2) and luteolytic factors (PGF2α) in favor of the latter, which in turn determine the moment of induction of 20α-HSD, the key luteolytic enzyme, causing an advance in luteolysis in hyperT rats and a delay in hypoT ones. HTs also modify the expression of hormone receptors and of members of the PRL signaling pathway at luteal and hypothalamic level, compromising the regulation of PRL secretion and its functions at luteal level. HTs have non-negligible direct effects on luteal functions and on the regulation of PRL release. These actions may play a role in the disorders of fertility and pregnancy associated with thyroid pathologies.

S3.

FLUORINE EFFECT ON INSULIN SECRETION AND BONE REMODELING

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Fluorine is a natural element with affinity for calcified tissues. Beneficial effects on enamel have been demonstrated. However, the effects on bone mass are still matter of discussion. Fluorine as sodium fluoride (NaF) or monofluorophosphate (MFP) is administered to treat bone mass loss, specially in osteoporosis. Fluoride contribute to maintain the phosphorylated state of growth factor receptors in the osteoblast. As a consequence, fluoride increase growth factor action and bone formation. Simultaneously, hidroxiapatite is changed into fluoroapatatie in the presence of fluoride, with lower solubility. MFP could interact with differentiation mechanism of osteoblasts that involves Wnt proteins. The beneficial effect of both drugs is opposite to side effects that appears with similar doses. After an oral dose of NaF, a transitory and reversible inhibition of insulin secretion has been demonstrated. This effect involves signaling pathways related to cAMP, diacylglicerol and Ca, and is associated with hyperglicemia and hypoinsulinemia. In addition, when fluoremia return to basal levels a resistance to insulin has been demonstrated. This situation is associated to hyperglicemia and hyperinsulinemia. These effects are not present or are less important in treatments with MFP, because MFP binds to plasma proteins and the levels of plasma fluoride are lower than in treatments with NaF.

S4.

YACON: A POTENTIAL NATURAL PRODUCT FOR DIABETES TREATMENT

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Medicinal plants have long been an excellent source of pharmaceutical agents. Smallanthus sonchifolius (yacon) is an Andean crop used for centuries by prehispanic population in traditional medicine. During the last few years our laboratory has carried out the study of biological effects of the yacon leaves and roots in normal and streptozotocin-induced diabetic rats. We demonstrated that yacón leaves decoction produced a fast and significant decrease in plasma glucose levels of diabetic rats. Moreover, yacon leaves water extract is a protective agent against renal damage in diabetic nephropathy, decreasing TGF-β/Smads signals. The bioactivity screening of organic extracts of yacón leaves revealed that butanol and ethyl acetate are the most effective fractions to reduce postprandial glucose levels. Phytochemical analysis of butanol extract showed the presence of caffeic, chlorogenic and dicaffeoilquinic. Enhydrin was the major sesquiterpene lactone in the ethyl acetate fraction and had significant hypoglycemic action estimated by an inhibitory activity on á-glucosidase. Yacon roots contain low polymerization degree oligosaccharides (FOS) as the main storage saccharides. Oral administration of yacon roots in diabetic rats improve glucose tolerance at post-prandial state and reduced slightly food intake. In addition, yacon roots improve the lipid metabolism in diabetic animals. Increased GLP-1 levels in blood and caecum homogenates accompanied with higher levels of GLP-1 receptor mRNA in pancreatic tissue was observed after yacon treatment. In summary, evidence is presented supporting the antidiabetic potential of the leaves and roots of yacon.

S5.
NEW INSIGHTS IN MOUSE SPERM ACROSOMAL EXOCYTOSIS
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Mammalian sperm must undergo a process termed capacitation to become competent to fertilize an egg. This event takes place in the female reproductive tract. Capacitation renders the sperm competent by priming the cells to undergo a rapid exocytotic event called acrosomal exocytosis that is stimulated by the zona pellucida. During the acrosomal exocytosis, the plasma membrane and the outer acrosomal membrane fuse at multiple points. The acrosome reaction is a regulated exocytosis but with especial characteristics when compared to other types of exocytosis. During capacitation, sperm progress through intermediate stages of acrosomal exocytosis that lead to the incremental exposure and eventual release of selected acrosomal components. This phenomenon is coincident with the stabilization of the structures that are involve in the exocytic process, in order to undergo exocytosis only in the presence of the appropriate stimuli. Over the years, several biochemical events have been associated with the capacitation process; however, the question that has remained unanswered in investigations of capacitation is: *What is the underlying reaction or set of reactions that transform the sperm cell from a state unresponsive to ZP or progesterone-stimulated acrosomal exocytosis (non- capacitated) to the state primed to respond to these stimuli (capacitated)?*

S6.
SIGNAL TRANSDUCTION SPACIO-TEMPORAL DYNAMICS
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Ligand induced tyrosine kinase receptor activation triggers different signal transduction pathways. Although these pathways are well characterized up to date, little is known on the mechanisms ensuring signal specificity in response to a given stimulus. The insulin receptor (IR) is an ideal model to study these mechanism. Ligand induced activation of the IR by insulin or insulin-like growth factors (IGFs) leads to different signaling pathways governing different cellular processes. In certain tissues, insulin activates the metabolic pathway, controlling homeostasis and glucose metabolism. On the other hand, insulin and IGFs are mitogenic, inducing gene expression, differentiation and cell cycle. Failures on insulin responses lead to diabetes, while upregulated IR activity occurs in many cancers. Two splice variants of the IR exist in mammalian cells: IR-A lacking exon 11, and full length IR-B. Using a combination of visible fluorescent proteins, quantum dot-functionalized ligands and specific labeling of cell surface proteins we are studding the dynamics of the differential activation of both isoforms in response to their different ligands. This strategy allows us to visualized in single cells IR activation, internalization and downstream effects in order to dissect the mechanisms ensuring signal specificity.

S7.
ROLE OF GLYPICAN 3 (GPC3) AS METASTASIS SUPPRESSOR IN BREAST CANCER
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Metastasis is the leading cause of death in breast cancer patients. It has been identified a new class of molecules, called **metastasis suppressor**s, able to reduce the ability to develop metastases. These proteins regulate multiple steps of the metastatic cascade and they represent important therapeutic targets.
Previously, we demonstrated that the reexpression of GPC3, a proteoglycan downregulated in breast tumors, induces an inhibition of the *in vivo* metastatic capacity of murine mammary adenocarcinoma LM3, suggesting its role as a metastasis suppressor. We also demonstrated that GPC3 is able to promote a reversion of epithelial-mesenchymal transition.
The GPC3 signaling mechanism is unclear. Although was initially speculated that it regulates IGF signaling, this theory was discarded. Several reports indicate that GPC3 would be a selective modulator of Wnt pathway. In this regard, we recently demonstrated that GPC3 regulates the Wnt pathway in LM3 cells, inhibiting canonical signals involved in proliferation and survival, and activating the non-canonical pathway that controls morphology and migration. We also informed that GPC3 is able to inhibit the Akt pathway while it stimulates the p38 cascade.
We believe in the clinical potential of GPC3. So, we are currently developing and characterizing an *in vivo / in vitro* model of human cells to confirm the results obtained in the murine model. Similarly, we are validating our preclinical studies in human biopsies.

1.

DIFFERENTIAL EFFECTS OF ANANDAMIDE ON PROSTAGLANDINS SYNTHESIS IN HUMAN PLACENTA

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The endocannabinoid system is present in human placenta, and the plasma levels of anandamide (AEA), one of the main endocannabinoids, increase in women at labor. Our objective was to analyze the effects of AEA on prostaglandins (PGs) synthesis in human placenta. Explants from chorionic villous of women without pregnancy complications, were obtained after delivery at term non-labor (TNL) or term with labor (TL, 38-42 weeks of gestation) and were cultured with AEA (10^{-9}-10^{-5}M).
We observed by radioimmunoassay, that AEA diminished PGE2 and PGF2 alpha synthesis in TNL placentas; while it increased the production in TL placentas.
We analyzed by western blot the expression of CB1 and CB2 (endocannabinoids receptors) and FAAH, the enzyme that hydrolises AEA. We observed that CB1 and FAAH were highly expressed in TNL placentas; while CB2 was present with higher expression in TL placentas.
The results obtained suggest that AEA is able to regulate differentialy the synthesis of PGs in term human placenta.

2.

INVOLVEMENT OF ANGIOPOIETIN/TIE 2 (ANGPT1/TIE2) SYSTEM IN THE HIPERSTIMULATION OVARIAN SYNDROME (OHSS)

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Introduction: OHSS is a complication of ovarian induction protocols. It is characterized by an increased vascular permeability due to an increase in the levels of VEGF. However, it is not known if other angiogenic factors are involved in this pathology. Objective: To analyze the involvement of ANGPTs/Tie2 system in OHSS. Methodology: Sprague Dawley rats were injected with eCG (10 UI) and 48h later with hCG (10 UI) (control group). The OHSS group was injected with eCG (50 UI) daily for 4 days and 24h later with hCG (25 UI). Rats were sacrificed 48 h after hCG. Ovaries were removed and the corpora lutea (CL) were isolated for protein extraction for western blot. On the other hand, ANGPT1 and soluble Tie2 were measured by ELISA in follicular fluids (FF) of OHSS and control patients. Results: We found an increase in ANGPT1/ANGPT2 ratio and in Tie2 levels in OHSS group 48h after hCG. In OHSS patients, we found an increase in ANGPT1 and no changes in soluble Tie2 in FF. Conclusion: ANGPT1/Tie2 system may be involved in OHSS and would be useful as a predictive marker of this pathology.

3.

ENVIROMENTAL IMPACT OF A MOTORDROME CONSTRUCTION IN THE SURROUNDING AREA OF THE POTRERO DE LOS FUNES´ LAKE, SAN LUIS, ARGENTINA

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In order to promote tourism in 2007 the San Luis government began with the construction of a motordrome in the surrounding area of the Potrero de los Funes´ lake. Actions including intensive logging, vertical cuts of the mountains and flattened of the ground have been developed. These initial activities alter the ecosystem, affecting soil, watersheds and native wildlife. We proposed to make an environmental impact asessment to reflect the consequences of the motordrome construction. In particular, we aimed to analyse changes on the vegetation cover and to determine the effect on the watersheds. For the environmental impact asessment we performed a Leopold matrix. Additionally we conducted field surveys of the area and used Google Earth's satellite images from 2004. We created two maps through stereoscopy. Our results indicate that the activities that generate the greatest impact are logging and vertical cuts of the field. Furthermore, logging decreased 27.18% of the vegetal cover and altered 69% of the watersheds. We consider necessary to perform future actions as an attempt to mitigate the impact. These actions involve the construction of retaining walls, reforestation with native vegetation and a strict control of tourists during the events conducted on the circuit.

4.

SOCIAL STATUS ASCENT OPPORTUNITY: PREVIOUS BEHAVIORAL AND PHYSIOLOGICAL ADEQUATIONS

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The South-American cichlid fish Cichlasoma dimerus presents a social hierarchy that determines access to reproduction. This hierarchy is maintained between non-reproductive individuals although these did not show a reproductive inhibition at the gonadal level suggesting these would be able to reproduce if the social context changes. Three hypotheses were tested for both sexes independently: a) social hierarchy is determined by size, b) individuals upper in the social hierarchy accede to reproduction when social ascent opportunity is given, and c) the hierarchy is correlated at the behavioral and physiological level. A reproductive pair and 3 individuals of the sex under study were placed in an aquarium. After social hierarchy was established, one of the reproductive individuals was removed. Behavior and physiology related to dominance-reproduction were registered on non reproductive individuals before social ascent opportunity. Males showed a linear dominance and the uppermost of the non reproductive individuals' social hierarchy always ascended in social status (not in females). Physiological parameters were correlated with dominance on both sexes.

5.

INFLUENCE OF CUMULUS AND GONADOTROPINS ON PORCINE OOCYTE *IN VITRO* MATURATION AND THEIR RELATIONSHIP WITH THE METABOLIC PROFILE OF CUMULUS-OOCYTE COMPLEXES

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The effect of cumulus-oocyte complex (COC) metabolism on *in vitro* maturation process remains unknown in the porcine. The aim was to evaluate the influence of cumulus and gonadotropins on oocyte maturational competence and their relationship with the metabolic profile of COCs. Different COC classes were matured during 48 h in medium 199 with and without gonadotropins. *In vitro* fertilization was carried out in mTBM with fresh semen. Nuclear and cytoplasmic maturation percentages were evaluated in relation to glycolytic activity, and amino acid and lipid metabolism of COCs. Nuclear maturation rates were unaffected by cumulus types or gonadotropins, however cytoplasmic maturation was modified by both studied factors ($P<0.05$). Cumulus type sorrounding the oocyte showed a relationship between cytoplasmic maturation and glycolytic activity, protein synthesis and amino acid catabolism during COC *in vitro* maturation. The differences observed in COC response to hormonal stimulation would be dependent on different types of cumulus. The decrease in oocyte lipid content seems not to be related to cumulus features or hormonal effect on COCs.

6.

MORPHOLOGIC STUDY OF BOVINE PARS TUBERALIS

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Little is known about the Pars Tuberalis (PT), which is a part of the adenohypophyseal gland. The aim of the present work was to study the histological structure of the bovine PT. Samples were fixed in Bouin and Zamboni during 48 h and were routinely processed for optic and electronic microscopy. PT is formed by a multicellular layer with 40 or more cells surrounding the Median Eminence (ME) being both areas separated by a thin layer of connective tissue. Cells are located forming a follicle which is surrounded by a thin net of connective tissue consisted basically of reticular fibers and the follicle is in a close relationship with blood vessels. Each follicle is formed by 10-15 cells tightly joined. It is possible to observe secretory cells (specific of the PT and those belonging to the *Pars distalis*) and follicular cells with secretory granules or absent of them. Also, each follicle presents a central area with unknown contents. Portal vessels which lie throughout the PT are large and numerous and they exhibit branches to the PT and ME. The next cell types can be observed in the PT: Specific PT cells with granules with diameters of 250 nm (Type I cells), TSH secretory cells with granules with diameters of 120 nm (Type II cells) and LH secretory cells with granules of 350 nm of diameter (Type IV cells). Very few cells with granules of 190 nm (Type V cells) and 450 nm of diameter (Type VI cells) can be detected.

7.

SYNDROME OF THE CAVERNOUS SINUS IN DOGS, ANATOMICAL DESCRIPTION OF THE INVOLVED STRUCTURES

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Cranial nerves III, IV, VI and the branches ophthalmic and maxilar of the cranial V discourse, next to sympathetic posganglionares fibers that goes whit the internal carotid artery, in close association with the cavernous sinus. This venous system surrounds the pituitary fosse in the base by skull. From the close contact with the mentioned structures, as result those alterations of the sinus unleash a group of symptoms, well-known like syndrome of the cavernous sinus (SSC).

The relations of each one of the anatomical elements are described in detail, which can be seen implied in the syndrome. For such aim three heads of canine were dissected, injected by venous and arterial with colored latex.

The cavernous sinus contacts dorsally with the cranial pairs III and IV, by lateral with the ophthalmic branches and maxilar of the pair V. Cranial pair VI, carotid internal artery and the ophthalmic intern artery crosses the interior of the sinus. As a result of the dissections we set up the correlation structures – symptom that characterize to the syndrome, like ophthalmoparesia/plegia internal and/or external depending on the involved cranial pairs. Diminution of the sensitivity of the cornea and face region nasofacialis are consequences of the affection of the V cranial nerve.

8.

STRUCTURES THAT PREVENT THE HYPEREXTENSION OF THE CARPUS IN THE LLAMA *(LAMA GLAMA)*

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Some domestic ungulate own a system made up of fascia, muscles and/or ligaments destined to limit the hyperextension of the joint of the carpus, preventing that the same collapse and that the distal components move dorsally. There is a system or similar apparatus in the llama? Each one of the implied anatomical elements in limiting the dorsal displacement of the carpus and therefore of the distals joints are described him. Six forelimbs of llamas were dissected, with normal angulation and without pathologies in their locomotive apparatus.

As a result of the dissections it was observed that fascia deep in this species confuses its fibers with the tendons of flexors muscles, mainly with the carpi ulnaris flexor and with the digitalis superficial flexor. In addition, the carpi ulnaris extensor muscle and the carpi ulnaris flexor, owns one steady insertion in the accessory bone of the carpus and derive fibers towards the medial ligament collateral from the joint, in intimate fusion with deep fascia. Previously to join to the principal tendon, the ulnar head of the deep digital flexor, it forming a ring dense and strong that surrounds it. Finally deep fascia unites firmly to the digital tendons, that share a interflexor tendon.

We conclude that the mentioned elements work against the hyperextension of the carpus in this species.

9.

GALECTIN-1 ROLE IN ENDOMETRIOSIS PATHOPHYSIOLOGY

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Endometriosis (EDT) is characterized by the ectopically occurrence of endometrial tissue reassembled in peritoneal or ovarian lesions. EDT is a common benign illness that affects 10% of women in reproductive age and its pathogenesis still remains unknown. Galectin-1 belongs to a family of endogenous lectins and has binding-affinity for multiple N-acetyllactosamine residues comprised on N- and O- glycans. This lectin is a key immunoregulatory, proangiogenic and growth-promoting factor in tumors. Data have not been reported on the role of Gal-1 in EDT etiology yet. Our hypothesis is that Gal-1 expression in endometriotic lesions improves endometrial cell survival and growth at the ectopic sites. In this study, we induced endometriotic lesions in female C57BL/6 wild-type and Gal-1 knock-out (*Lgals1*$^{-/-}$) mice. Four weeks later the number and size of lesions was recorded and the cell proliferation and % vascularized area in the lesions were evaluated by immunohystochemistry for PCNA and CD34 markers respectively. Results show a significant decrease of lesion size ($p<0.05$), proliferating cell-rate ($p<0.05$) and vascularized area ($p<0.01$) in Gal-1 null mice. These results suggest that Gal-1 favours EDT development promoting ectopic endometrial cell proliferation and lesion vasculature expansion.

10.

MICROSTRUCTURE OF UPPER AND LOWER HUMAN ENAMEL PREMOLARS: BIOMECHANIC FUNCTION

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The study of tooth enamel is important to anthropological level because of its sustainability and for being tooth tissue exposed to the oral cavity. The aim of this research was to identify the microstructure of the enamel of premolars and link them with biomechanics. Ten upper and lower premolars were sectioned in longitudinal plane and were included in acrylic resin. Each sample was grinded, polished, etched and prepared for being observed with SEM. The micrographs occurred in the free faces and in outer and internal third part of cusps zones, next to the amelodentinal boundary. Results indicated enamel with bands in the free faces of upper premolars occupying its thickest portion and radial enamel to the external surface. On the other side, in lower premolars where identified irregular enamel, with changes in prisms direction with no bands-like aspects in outer and internal radial enamel. In the vestibular and lingual zones of both dental groups were found internal irregular enamel and outer radial enamel. The pattern present in the studied groups, with the location of the radial enamel in the outer zone and the irregular enamel or bands in the internal zone could constitute specializations of the microstructure of enamel as a biomechanics response to the masticatory wears and fracture.

11.

PARTICIPATION OF TIROSINE KINASE SRC FAMILY IN RAT SPERM CAPACITATION

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Sperm capacitation relies on an increase in protein tyrosine phosphorylation mediated by PKA activation. Recent studies have suggested that Src tyrosine kinase family (SKF) is involved either direct or indirectly in capacitation-associated protein tyrosine phosphorylation. In order to elucidate between these two possibilities, we investigated whether SFK is involved in the signaling events leading to rat sperm capacitation. Results showed that sperm capacitated in the presence of a SFK inhibitor (SU6656), exhibited low levels of both tyrosine and PKA- substrates phosphorylation. Based on the described inhibition of ser/thr phosphatases by SFK, sperm were exposed to a Ser/Thr phosphatases inhibitor (okadaic acid, OA) observing a reversion of the impaired-phosphorylation produced by SU6656. However, OA was unable to induce tyrosine phosphorylation in both uncapacitated sperm and sperm capacitated in the presence of a PKA inhibitor. Finally, addition of both a cAMP agonist and a phosphodiesterase inhibitor did not overcome the inhibition produced by SU6656. Altogether, these results show the indirect involvement of SFK in rat sperm tyrosine phosphorylation supporting the existence of two parallel pathways leading to capacitation: one requiring PKA activation, and the other involving the inactivation of ser/thr phosphatases.

12.

ANANDAMIDE MODULATES NITRIC OXIDE SYNTHASE ACTIVITY DURING THE PROCESS OF IMPLANTATION

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Introduction: it is well known that both anandamide (AEA) and nitric oxide synthase (NOS) activity should be strictly regulated during implantation.
Aim: to study the effect of AEA on NOS activity during the process of implantation in the rat uterus.
Results: AEA production and NOS activity were negatively correlated at the sites of implantation. On day 5 of gestation, when the blastocyst apposites over the endometrium, AEA did not affected NOS activity. Once invasion begins, both in the implantation and in the interimplantaion sites from day 6 of pregnancy, AEA regulated NOS activity through cannabinoid receptors CB1 and CB2. In the implantation sites, AEA inhibited NOS via CB1 only. However, in the interimplantation sites, AEA presented a dual effect: it inhibited NOS activity through CB1, while it increased NOS via CB2.
Discussion: during implantation, AEA differentially regulates NOS activity through cannabinoid receptors. This effect seems to depend on the state of activation of the blastocyst.

13.

EFFECT OF ENDOMETRIOSIS (EDT) ON FERTILITY IN A MURINE MODEL.

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Introduction: One of the most intriguing aspects of EDT is its association with infertility when there is no mechanical alteration of the reproductive tract. Traditionally, pregnancy has been considered to be beneficial for patients with EDT leading to the atrophy of the endometritotic implants. However other authors have reported only symptomatic relief of the disease with pregnancy. Objective: Evaluate the effect of EDT on fertility in a murine model of EDT. Methodology: EDT was surgically induced in female C57BL mice. Four weeks after surgery animals were mated until the presence of vaginal plug. Animals were sacrificed on day 18 of pregnancy. After sacrifice, EDT-like lesions were counted and their size measured. The number of pups, pups weight, and number of implantation sites were evaluated. Results: The time from mating until the presence of vaginal plug was similar between animals with EDT and sham animals. However, the pregnancy rate in mice with EDT was reduced. There were no significant differences between animals with EDT and sham animals in the number of pups per mouse and in the pups weight. There were no significant differences in the number of lesions per mouse in pregnant compared to non-pregnant mice. However, the lesions size was significantly increased in pregnant mice compared to non-pregnant ones. Discussion: These results suggest that EDT affects fertility in mice and support the use of this murine model to evaluate EDT associated infertility.

14.

NUTRITIONAL EVALUATION OF AUTUMN -WINTER FOOD TWO FLOCKS OF GOATS IN THE TUCUMAN EAST

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Parasitic diseases cause losses in animal production systems of tropical and subtropical regions of the world. Animals with low nutritional profile are more likely to suffer parasitic infestation, did not happen in well-nourished animals.
The aim of this study was to assess the nutritional value of foods used to goats during the fall and winter.We worked with two groups of adult animals of average weight ± 40 kg. A group of INTA Leales to 52 km southeast of SM de Tucuman, with rains between October and March, temp.average of 25ºC (January) and 13°C (July). The other group belongs to a producer of Taco Ralo, 155 km South of SM de Tucuman, with low rainfall in summer and a dry winter, temp. average of 17ºC in winter and 37ºC in summer. We assessed the food base for both groups: INTA Leales: Wheat Pellet (Triticum spp) Bermuda grass (Cynodon dactylon) and Taco Ralo: Mistol (Ziziphus mistol) and Atamisqui (Caparis atamisquea). Its determined dry matter (DM) crude protein (CP) by AOAC method and fiber (NDF) by Van Soest. The average results ± standard desviation obtained were: Pellet: %DM:94,39; %CP:12,0; %NDF:39,27; Bermuda Grass: % DM: 94, %CP:9;2; %NDF: 76,58; Mistol: %DM: 91.67, %CP: 14.5, % NDF:36.55; Atamisqui;% DM: 95.6, % CP: 8.1, % NDF: 62.70. The maintenance protein req. for adult goats were 44.38 g / day. The nutritional basis only covers 9.1% (INTA) of. daily protein. req., the other (Taco Ralo) only covers 10.52% of req.protein.

15.

EVIDENCE FOR MORE THAN ONE PATHWAY TO IMPORT UDP-GLC INTO THE ENDOPLASMIC RETICULUM IN S. POMBE

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The UDP-Glc:glycoprotein glucosyltransferase (GT) serves as a glycoprotein folding sensor, labeling incompletely folded glycoproteins in the endoplasmic reticulum (ER) lumen with a glucose (Glc) tag. Its donor substrate, UDP-Glc, is synthesized in the cytosol. From six putative nucleotide sugar transporter (NST) homologues identified in the genome of the fission yeast *Schizosaccharomyces pombe*, only *hut1+* and *yea4+* bear the ER retention signal. We disrupted both genes in *S. pombe* Δ*alg5* background. This allows assignation of protein-linked $Glc_1Man_9GlcNAc_2$ formation to GT activity and thus entrance of UDP-Glc into the ER lumen. ER vesicles purified from Δ*alg5*Δ*hut1* mutants showed a 50% decrease in UDP-Glc transport compared with that of Δ*alg5*, suggesting that *hut1+* is involved in UDP-Glc transport into the ER. However, *in vivo* labeling of *S.p* Δ*alg5*Δ*hut1* (and also of Δ*alg5*Δ*yea4* and Δ*alg5*Δ*hut1*Δ*yea4 mutants)* resulted in $Glc_1Man_9GlcNAc_2$ synthesis. This suggests that there is at least another pathway for importing UDP-Glc into the ER: either through a NST not bearing the classic ER retention signal or by an novel mechanism. The parasite *Giardia lamblia* provides a new system for testing heterologous UDP-Glc transporters as its membranes only transport UDP-GlcNAc. We expressed *S.p hut1+* and *S.p. yea4+* in the parasite and preliminary results confirmed that *hut1+* is a UDP-Glc transporter. We are currently expressing the rest of the NSTs in *Giardia* to determine their substrate transport specificity.

16.

EFFECT OF OLIGONUCLEOTIDE (ODN) IMT504 IN A TYPE I DIABETES MODEL IN MICE

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We have previously shown that the oligonucleotide IMT504 induces a marked recovery of streptozotocin (STZ)-induced diabetes in male rats that correlates with early production of progenitor cell markers *(Diabetologia (2010) 53:1184)*. Here we studied the effect of IMT504 on a type I diabetes model induced by multiple low doses of STZ. 6-8 week-old male BalbC mice were injected with STZ (ip, 40mg/kg) daily for 5 consecutive days or with saline (control, C). Normal glycemia (Glyc) is 149±13 mg/dl. Animals with Glyc ≥ 250 mg/dl were considered diabetics and injected with daily IMT doses (20mg/kg/day, sc) for 10 days (STZ-IMT) or saline as control (STZ) (day 1). C mice were injected with the same IMT doses (C-IMT) too. Animals were submitted to another 5 doses of IMT starting on 21 and 36 days. Body weight and glycemia were recorded. At the end of the experiment, glucose tolerance tests (GTT) were performed (2g/kg BW glucose was injected ip, day 59). IMT treatment induced a marked Glyc decrease [day 66= C: 130±9 vs STZ-IMT: 278±46, $p<0.01$, STZ-IMT vs STZ: 557±20, $p<0.01$]. GTT showed a partial recovery in the STZ-IMT responsiveness [0 min= C: 117±11 vs STZ-IMT: 164±9, ns, C vs STZ: 309±53, $p<0.05$; 30 min= C: 292±51 vs STZ-IMT: 342±33, ns, C vs STZ: 488±36, $p<0.05$; 120 min C: 133±15 vs STZ-IMT: 352±28, $p<0.05$, C vs STZ: 472±52, $p<0.05$]. IMT promoted a transient body weight decrease in diabetic animals. IMT504 could improve the diabetic condition in this model of type I diabetes.
CONICET-ANPCYT-UBA.

17.

ENERGETICAL DISTRIBUTION OF COTILEDONAL RESERVES IN SEEDS OF DIFFERENT SIZES OF *CUPHEA GLUTINOSA* CHAM ET SCHLTDL. (LYTHRACEAE)

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The seed of *Cuphea glutinosa* accumulates important amounts of lipid and proteins, stored as reserves in oil bodies (OB) and protein bodies (PB) in cotiledonal tissues. This species showed changes in the distribution and size of these organelles in seeds of different sizes The objective of this work was to determine the accumulation and distribution strategies of reserves and energy in seeds, whose weight of 1000 big seeds was 1.011 ± 0,003586 g and of 1000 small seeds was 0,557 ± 0,002698 g, and to determine the energetical load for the whole seed and for each particular tissue. Based on the number and distribution of OB and OP, it was determined that in the big seeds 62% of its energy comes from proteins and 38% from lípids, while in small seeds it was 42% and 58% respectively. The bigger proportion of lipidic reserves present in small seeds determined an energetical load of 1,36 Kcal/g, while in big seeds it was 1,12 Kcal/g. This strategy of small seeds is similar to that developed for migratory birds, concentrating a big amount of energy in a minimal weight. The important accumulation of energetic load assures its germination and emergence despite of its little size.

18.

DESCRIPTION OF CELLULAR STRUCTURES AND BIOMINERALIZATIONS IN SEED COVERS OF *CUPHEA GLUTINOSA* CHAM ET SCHLTDL. (LYTHRACEAE)

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The seeds of *C. glutinosa* shows a notorious external tegument, hard, smooth and without visible protrusions. There is a lack of specific information about histological aspects of external teguments in this species. The objective of this work was to determine the tegument characteristics of seeds grown in calcium rich soils with low hydrical retention. The material were seeds collected in Sierra de los Padres, Buenos Aires Province, during the months of February and March of 2009. It was processed with the paraffin inclusion technical and the observations were made with optical and polarized light microscopes, on cuts obtained with rotatory microtome. The testa showed rolled hairs which protrude when seed is hydrated. Other peripheral cells shows birefringent crystals with prismatic morphology, which are indicative of calcium biomineralizations. These seem to play a central role in some plants as regulation of calcium levels, protection against herbivores and detoxification of heavy metals. *C. glutinosa* grows normally in soils of the *sierras* rich in calcium with little hydrical retention. Biomineralizations can be related with water retention as a strategy to increase the resistance to the frequent desiccation to which the species is exposed.

19.

BOVINE OVARY GRANULOSA ESTABLISHED CELL LINE (BGC-1) AND APOPTOSIS

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Several works demonstrate GnRH receptor (GnRHR) presence on granulosa cells (GC) on different species ovaries and its relationship with gonadal function through apoptosis induction "*in vitro*" and "*in vivo*" with various GnRH agonists. We have observed, through morphological and biochemical assays (TUNEL, Caspase 3 activity and Anexine-V binding) that the agonist Leuprolide acetate (LA) induces dose-dependent apoptosis on BGC-1 treated for 24 and 48 hs with 1, 10 y 100 nM LA, being partially inhibited by pretreatment with the antagonist Antide. We concluded that BGC-1 was an adequate model for study, so we proceeded to analyze intracellular pathways of apoptosis regulation. To accomplish this objective, we assessed Bcl-2 family protein expresión (Bax, Bcl-2), by Western Blot and Phosfolipase D activity (PLD). Two asays by triplicate were statistically analizad by a two way ANOVA analysis and two-sided Dunnett's Multiple Comparisons with a control. A significant increment on Bax ($p = 0,067$) and Bax/Bcl-2 ($p = 0,1$) expresión was observed on BGC-1 treated for 48 hs with 100 nM LA. This effect was inhibited by preincubación whith 100 nM Antide. No change was observed on Bcl-2 expression. PLD activity was significantly inhibited by all treatments: 1, 10 and 100 nM LA and 100 nM Antide ($p = 0,0079$; $n = 6$) compared to control level. Therefore LA induces apoptosis through the activation of Bax expression and inhibits the protective apoptosis pathway through inhibition of PLD activity. Antide antagonizes the mitochondrial pathway, showing agonist function on the membrane pathway through the inhibition of PLD activity.

20.

TRYPANOSOMA CRUZI: MOLECULAR MECHANISMS OF NUTRIENT UPTAKE

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Transport processes are particularly relevant in protozoan parasites, since in their evolution have replaced many biosynthetic pathways by transport systems. Our group has identified a new multigene family of amino acid transporters of Trypanosoma cruzi (TcAAP), which is completely absent in mammals. In this work we identified and functionally validated several members of this family using a complementation system in yeast. TcAAP7 proved to be a monospecific and high affinity lysine permease. Over-expressed in epimastigotes, TcAAP7 showed a lysine transport rate 50-times higher than controls. In transfected and wild-type cultures, no other amino acid displaced lysine in competition assays. TcAAP7 fused to GFP was localized in the flagellar pocket and associated structures. While the first TcAAP7 allele could be replaced with a selection marker, all attempts to replace the second allele failed, suggesting that this gene is essential for the parasite survival. Finally, three other TcAAP family members were characterized as permeases of proline, methionine and aspartate/glutamate. Amino acids in trypanosomatids are essential, not only in protein synthesis, but also as carbon sources, energy reservoirs and metacyclogenesis mediators. Hence, these permeases could be therapeutic targets or specific gateways for the entry of trypanocidal drugs.

21.

CHANGES IN SYNCYTIOTROPHOBLAST (hST) MEMBRANE LIPID COMPOSITION AFTER INSULIN TREATMENT

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We previously demonstrated that preeclamptic hST is more rigid than normal hST. Since we observed high insulin serum levels in preeclampsia, we hypothesized that insulin may be implicated in these changes. Our aim was to evaluate if the insulin may alter the lipid composition of hST. Explants from normal term placentas were cultured with different concentrations of insulin during 24 h. Apical (MVM) and basal (BM) membrane vesicles were prepared by differential centrifugation. Lipids were extracted by Bligh-Dyer method, phospholipids (PhLs) separated by TLC and quantified by Fiske-Subarrow. Cholesterol (Chol) was determined by enzymatic method. Insulin treatment produced no changes in the total PhLs concentration and Chol in BM. However, in MVM it showed an increase until reaching a constant value at 100 µUI/mL insulin, which was correlated to an increase in sphingomyelin and phosphatidylcholine content. No changes were found in other PhL species and Chol. Our results suggest that insulin may alter lipid composition and in addition membrane fluidity. Further work is needed to clarify the molecular mechanisms implicated in these changes and its role in the pathogenesis of preeclampsia.

22.

HETEROCHROMATIN CHARACTERIZATION OF *BELOSTOMA DENTATUM* (MAYR) (HEMIPTERA: BELOSTOMATIDAE)

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Previous cytogenetic data in Belostomatidae allow us to propose an ancestral male karyotype 2n=26+XY, from which the karyotypes with multiple sex systems (2n=26+X_1X_2Y) and those with a low 2n (6+XY, 14+XY) have originated by fragmentation of the ancestral X chromosome and autosomal fusions, respectively. To study the mechanisms of karyotype evolution in *Belostoma*, the male meiosis of *B. dentatum* was analyzed by means of fluorescent banding (DAPI/CMA_3) and fluorescent *in situ* hybridization with an rDNA probe. All chromosomes were stained homogenously with both fluorochromes, except for one of the medium-sized autosomal bivalents, which showed a DAPI dull/CMA_3-bright band on terminal position. The nucleolar organizer region (NOR) was located in one of the terminal regions of a medium-sized autosomal bivalent. The CMA_3-bright band is enriched in GC base pairs, and probably corresponds to the NOR region. The present results support the hypothesis that a fragmentation of the X chromosome in the ancestral karyotype gave rise multiple X chromosomes (2n=26+X_1X_2Y), while keeping the ancestral NOR-autosome pair, and a fusion of the ancestral sex chromosome pair with the autosomes carrying the NOR led to the reduction in diploid number (2n=8, 16) and also to the presence of the NORs in both the X and Y chromosomes.

23.

ECOLOGICAL COMPARISON BETWEEN SOCIAL FORMS OF *SOLENOPSIS INVICTA* BUREN (HYMENOPTERA: FORMICIDAE)

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Several populations of *Solenopsis invicta* fire ant possess two alternative social forms (Monogyny and Polygyny). The number of queens establishes how reproduction is divided among members of the colony and the number of individuals produced. Besides, the social form affects the genotype and phenotype of queens and workers. Percent density, volume and foraging area of nests, and number, proportion and mean size of workers of the colony were tested to characterize both social forms of *S. invicta*. Monogyne nests had bigger foraging areas than polygynes and occupied 75% of sampled places (N = 6). A trade-off between workers' size and number by social form was observed. Monogyne ants were larger than polygynes by had higher percent of major, large and medium workers and lower percent of minors. Polygyne nests showed higher reproductive rate due to a queens' bigger production of smaller ants occupying the same volume than monogynes do. These differential reproductive strategies demonstrate how each social form allocates its reserves to reside in a determined territory by regulating the workers' biomass produced.

24.

cAMP-DEPENDENT ACTIVATION OF STEROIDOGENESIS INVOLVES THE ACTION OF THE TYROSINE PHOSPHATASE SHP2

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Protein tyrosine phosphatases (PTPs) play significat roles in many biological processes. PTP activation is a crucial step in the signal transduction cascade that leads to the activation of steroidogenesis triggered by ACTH and LH in adrenal cortex and Leydig cells respectively. However, the identity of the PTP involved in steroidogenesis is still unknown. We used plasmid-mediated gene transfer and RNAi-mediated gene silencing of SHP2 in order to study whether SHP2 is, at least, one of the PTPs involved in steroidogenesis. Overexpression of SHP2 increases cAMP-dependent progesterone production, while overexpression of an inactive form of SHP2 does not. The need for SHP2 expression and function to stimulate steroid synthesis was further demostrated by the inhibition of SHP2 expression. Inhibition of SHP2 expression reduced both StAR induction and progesterone synthesis.
Thus, we provide evidence for the first time that SHP2 participates in the cAMP-dependent signal transduction pathway that activates steroid production.

25.

PROGESTERONE ATTENUATES NEUROPATHIC PAIN AND GLIAL ACTIVATION AFTER SPINAL CORD INJURY

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Glial cell activation and the production of proinflammatory cytokines play a key role in neuropathic pain mechanisms by inducing neuronal activation after spinal cord injury (SCI). Progesterone (PG), a neuroprotective steroid, may offer a promising perspective in pain modulation. This study evaluated the effect of PG administration on pain behaviors and spinal glial activation after SCI. Mechanical and cold allodynia were assessed in injured male rats treated with daily injections of PG or vehicle. One or twenty eight days after injury, the number of cells exhibiting the astrocytic marker GFAP or the microglial marker OX42, was determined in the dorsal horn. PG administration prevented the development of mechanical allodynia and reduced the number of painful responses to cold stimulation. In correlation with the attenuation of pain behaviors, the steroid attenuated the injury-induced increase in the number of GFAP- and OX42- positive cells ($p<0.01$). In addition, PG prevented the upregulation of the mRNA levels of interleukin-1 beta (IL-1β, $p<0.001$) and its receptor IL-1RI ($p<0.01$), found in neurons, and decreased the number of neuronal profiles expressing the active, phosphorylated form of the NR1 subunit of NMDA receptor, thus contributing to a reduction in pain neurotransmission. These results show that PG, by targeting spinal mechanisms, reduces allodynia and thus may be useful in the treatment of chronic pain.

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26.

STUDIES ON THE EXPOSURE OF PHOSPHATIDYLSERINE IN MOUSE FERTILIZED EGGS

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We have recently shown that sperm entry induces in eggs a transient exposure of phosphatidylserine (PS), a phospholipid asimatrically distributed to the inner leaflet of the lipid bilayer. The balance of PS in the plasma membrane is maintained by two enzymes: scramblases which tranlocate phospholipids in both directions allowing the exposure of PS, and flipasses which maintain the asymmetry by actively internalizing PS. In the present work, we have characterized the PS-exposure kinetic in fertilized eggs, and analyzed the expression in eggs of the different enzymes involved in PS mobilization. Quantification of the labeling for fluoresceinated Anexin-5, protein that specifically binds to PS, in the whole egg surface, showed that the highest fluorescensce was observed 1 h post-insemination and then decreased progressively. However, when the intensity was measured only in the labelled regions of the egg, we observed that it did not change along time. RT-PCR analysis showed the expression in eggs of two scramblases PLSCR1 and PLSCR3 and two flipasses (ATP8A1 y ATP8A2. However, the incubation of eggs with the flipasses inhibitor VO_4^{2-} prior to insemination, did not affect the PS exposure patterns. Altogether, these results indicate the presence of scramblases and flipasses in the oocyte, suggesting that these enzymes could regulate the kinetics of PS exposure in fertilized eggs.

27.

LIVER DAMAGE MARKERS IN AN ACUTE POPHYRIA MODEL

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The acute porphyrias are inherited human liver diseases caused by alterations in the synthesis of heme, and the production of oxidative stress (OS) has been reported in relation with them. We have modelled this disease in our laboratory by the administration of 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1,4-dihydrocollidine to Wistar rats. In this study we have evaluated liver damage of these animals, by measuring plasma enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and gamma glutamyl transferase (GGT). We also measured the activity of 5-aminolevulinic acid synthase (ALA-S), parameter indicator of porphyria, which product ALA has been reported as an important generator of reactive oxygen species (ROS). Damage produced by ROS, especially in the liver cell membranes was detected with thiobarbituric acid (TBARS). The plasmatic enzymes were measured using commercial kits. For all of them, increased values were observed with respect to the control. For AST and ALT, these were 40% and 35% respectively; for ALP and LDH were 70% and 50% respectively and the observed increase in GGT was 30%. For TBARS was observed an increase approx. 3 times and the same increase was also observed for the enzyme ALA-S. These results suggest that the increase in plasma releasing tissue enzymes should be due, in part, to the OS damaged membranes.

28.

RESPONSES TO KISSPEPTIN ARE ALTERED IN HYPOTHALAMIC EXPLANTS OF ADULT GABAB1KO MICE

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Adult GABAB1KO mice (KO) show a deregulation of GnRH compared to wild-types (WT), especially in females (AJP-EM.2010;298(3):E683-96). We studied the responses to Kisspeptin in GnRH secretion (RIA) and expression (qRT-PCR) and GAD67 (GAD) expression in hypothalamic explants (HTE) of adult mice (both sexes and genotypes), incubated with Kiss-10 (1.10^{-8}M) or buffer (control) for 150 min. Kiss-10 increased GnRH secretion in all groups (interactions ns, treatment $p<0.001$). However, in the Kiss-10 group, GnRH secretion was lower in KO compared to WT males, without genotype differences in females. GnRH expression was affected by sex, genotype and treatment (triple interaction $p<0.03$). In basal conditions, KO males had lower GnRH expression than KO females and WT males, without sexual differences in WTs. Kiss-10 increased GnRH expression only in KO males, without differences between Kiss-10 groups. GnRH expression was higher in basal than Kiss-10 females. GAD expression was affected by sex, genotype and treatment (triple interaction $p<0.05$). Basal GAD was higher in males than females and higher in KOs than WTs. GAD in Kiss-10 males was similar to basal males, keeping their genotype differences. GAD increased in Kiss-10 WT females compared to basal females, without differences in KO females. Responses to Kisspeptin are sex-specifically altered in HTE of KO mice.

CONICET-ANPCYT-UBA.

29.

PLACENTAL LESIONS AND EMBRYONIC DEATH GENERATED BY ACUTE CADMIUM INTOXICATION IN RATS AT 15 DAYS OF PREGNANCY

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Cadmium accumulation in the placenta alters its structure and affects prenatal development. In order to analyze uterine, placental and embryonic parameters Wistar rats were injected with 10 mg Cd^{+2}/kg of body weight on 4 (G4), 7 (G7) or 10 (G10) days of pregnancy and sacrificed at 15 days of gestation. The weight of the embryonic vesicles, embryos and placentas was recorded. Macroscopic observations were performed to determine resorptions and uterine and fetal alterations. Some samples of placentas were processed for lectinhistochemestry. Only the weight of the placentas from G7 and G10 females was lower compared with controls. All intoxicated groups showed significantly higher number of resorptions than controls. Placentas from Cd-treated dams showed hemorrhage, congestion and necrosis. The carbohydrate pattern showed positive labeling for PNA, UEAI, and SBA lectins only in the intoxicated females. We confirmed the deleterious cadmium effect on placental structure and prenatal ontogeny of the rat and further demonstrated the changes in the pattern of placental carbohydrates at 15 days of pregnancy.

30.

OXYGEN TENSIONS MAY ALTER NHE-3 EXPRESSION IN HUMAN PLACENTA

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It is known that in some pathological states such as preeclampsia (PE) the hypoxic environment during placentation modifies the expression and/or the function of some transport proteins. One of them could be the isoform 3 of the Na+/H+ exchanger (NHE-3) which plays a key role in the maintenance of intercellular pH.
Previously, we found that the molecular expression of NHE-3 was decreased in syncytiotrophoblast (hST) from preeclamptic placentas. Up to now, the mechanism that regulates NHE-3 expression remains unclear.
The aim of our work was to evaluate if different oxygen tensions alter NHE-3 expression in human placentas.
Explants from normal term placenta were incubated in normoxia, hypoxia, and in hypoxia/reoxygenation (H/R). Semiquantitative RT-PCR, Western blot analysis and immunohistochemistry were performed to study NHE-3 expression. We found that the expression of NHE-3 in hST is decreased when explants were cultivated under hypoxic treatment. However, H/R partially restored it but less than in normoxia ones. In all cases, NHE-3 was located in the apical membranes and in citoplasmatic regions.
We can conclude that hypoxia may alter the expression of the NHE-3 and reoxygenation may partially reverse this effect. Further studies are needed to elucidate if changes in oxygen tension may also modify placental pH homeostasis.

31.

HORMONE-REGULATED MITOCHONDRIAL REORGANIZATION IS REQUIRED FOR THE FORMATION OF A MITOCHONDRIAL PROTEIN COMPLEX NECESSARY FOR STEROID SYNTHESIS

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In the hormonal regulation of steroid synthesis we have previously demonstrated the participation of an acyl-CoA synthetase (ACSL4) to generate arachidonic acid into the mitochondria. In this mechanism ACSL4, as a part of the mitochondria associated membrane (MAM), is translocated to the mitochondria; however ACSL4 has no mitochondrial signal peptide. Thus we postulate that a reorganization of the mitochondria after hormone treatment will trap ACSL4 at this organelle. In order to detect the reorganization of the mitochondria we developed a stable transfected MA-10 Leydig cell line expressing the green fluorescence protein associated with the mitochondrial signal peptide. Hormone treatment produces the reorganization of the mitochondria. Decrease in SHP2 expression in hormone-induced cells inhibited mitochondrial reorganization, ACSL4 translocation and steroid synthesis. These results support the concept that tyrosine dephosphorylation and mitochondria reorganization are crucial events in the regulation of Leydig cell function.

32.

PARTICIPATION OF FEMALE REPRODUCTIVE TRACT CRISP1 IN THE FERTILIZATION PROCESS

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Recent results from our laboratory indicate that epididymal CRISP1 protein is present in the rat female reproductive tract. Considering the participation of sperm CRISP1 in different stages of fertilization, in the present work we investigated the involvement of female reproductive tract CRISP1 in the mouse fertilization process. The presence of CRISP1 mRNA was analyzed in the uterus, oviduct, ovary, and cumulus cells by rt-PCR. Protein expression studies by Western blot (Wb) using a specific anti-CRISP1 antibody, indicated the presence of CRISP1 mRNA in the three tissues. Indirect immunofluorescence of cumulus treated with hyaluronidase revealed the presence of CRISP1 in the follicular cells. In view of these observations, we evaluated the possible participation of cumulus CRISP1 in fertilization by *in vitro* fertilization assays using cumuls-oocyte complex from CRISP1 knock out mice (KO) generated in our laboratory. Results showed a significant decrease in the percentage of fertilized eggs compared to controls. Together, these results confirm the existence of CRISP1 in the female reproductive tract as well as the participation of cumulus CRISP1 in the fertilization process.

33.

PRESENCE AND CHARACTERIZATION OF GLIPR1 FAMILY MEMBERS IN THE MALE REPRODUCTIVE TRACT

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The analysis of plasmatic membrane lipid RAFTs of mouse sperm reveals the presence of GLIPR1l1 and GLIPR1l2 proteins which, as CRISP proteins, are members of the CAP superfamily. Considering the CRISP participation in different events of fertilization, in the present work we cloned and characterized Glipr1l1 and Glipr1l2 as a first step towards the study of their potential role in the fertilization process. rt-PCR studies revealed the testicular expression of Glipr1l1 and both the testicular and epididymal expression of Glipr1l2. Semi-quantitative rt-PCR showed a decrease in mRNA expression from birth to day 7, and a gradual increase thereafter reaching maximum levels at 60 days. The positive clones were subjected to IPTG induction and the detected recombinant proteins were then successfully purified. The use of specific antibodies against the human homologous of Glipr1l1 and Glipr1l2 indicated the presence of both proteins in fresh human sperm as well as their permanence after capacitation, supporting their possible participation in subsequent sperm-oocyte interaction. Altogether, the results obtained in mouse and human will allow the study of the potential role of GLIPR1 proteins in the maturation, capacitation and fertilization processes.

34.

HEPARAN SULFATE EXPRESSION IN MURINE OOCYTES AT DIFFERENT STAGES OF DEVELOPMENT

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Previous studies from our laboratory demonstrated the role of Heparan sulfate (HS) as decondensing agent of human spermatozoa *in vitro* and its presence in mature mouse oocytes. The aim of this study was to determine at what time in follicular development the oocyte begins to express HS. Eight weeks old female CF1 mice were stimulated with PMSG and hCG (7.5 UI for both) to recover mature oocytes (M_{II}) or with PMSG alone to retrieve immature oocytes. M_{II} were recovered from the oviduct and cumulus cells were removed. Immature oocytes were recovered puncturing the ovary. In both cases, zona pellucida was removed and oocytes were fixed and permeabilized with formaldehyde and Tween-20. Immunocytochemistry was performed using anti-HS (monoclonal) as first antibody, anti-mouse IgM as second and propidium iodide to label DNA. Fluorescent label was observed both in MII and immature oocytes. Results demonstrate that HS is already present at early stages of follicular development.

35.

EXPRESSION OF DIFFERENT DETOXIFICATION PATHWAYS IN *Fasciola hepatica* RECOVERED FROM TRICLABENDAZOLE TREATED SHEEP

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Fasciolasis is a parasitic disease caused by *Fasciola hepatica*. Its control is mainly based on the use of triclabendazole (TCBZ). All the helminth possess different biochemical mechanisms for detoxification. The knowledge of these mechanisms in *F. hepatica* is needed. This work was aimed to assess the enzymatic activity of Glutathione-S-transferase (GST), Carbonyl reductase (CR) and Nitrophenyl acetate esterase (NFAE) in adult *F. hepatica* specimens recovered from TCBZ treated sheep. Four untreated sheep were inoculated with 200 metacercaries of *F. hepatica*. Confirmed the infection, the sheep were treated with TCBZ (10 mg/kg) and sacrificed at 3, 24, 48 and 60 h post-treatment. Adult fluke were collected from bile ducts and the cytosolic and microsomal fractions were obtained. The highest activities were observed into 24 and 48 h post-TCBZ administration. The activity, 60 h after TCBZ treatment, returned to levels similar to those measured in non-exposed flukes. TCBZ action may induce secondary oxidative stress in *F. hepatica,* which may explain the observed increment in activities as a defensive mechanism. In fact, the highest activity was observed when the peak TCBZSO concentration was measured within the flukes recovered from treated sheep. These preliminary results may be useful to further understand the mechanisms underlying the drug metabolism/disposition and activity in target helminth parasites.

36.

EXPRESSION OF 11β-hsd2 DURING THE TEMPERATURE-DEPENDENT MASCULINIZATION PROCESS IN PEJERREY

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Recently, our group has associated cortisol (a stress hormone) with masculinization by warm temperature (MPT, 29ºC) in the pejerrey, *Odontesthes bonariensis*. In this species, all larvae exposed to MPT during 4 weeks after hatching develop as males. Moreover, a significant increase in 11-ketotestosterone (11-KT, the main bioactive androgen in fish) levels is observed during the same period. In order to clarify the role of stress on masculinization, the expression of the stereodogenic enzyme *11β-hsd2*, and the glucocorticoid receptors *gr1* and *gr2* was analyzed by Real Time PCR and *IS*H in larvae reared at MPT, FPT (female promoting temperature, 17°C), and MixPT [with our without cortisol (50 mg/kg food) supplemented food] during the sex determination period. In addition, an *in vitro* experiment with adult testicular explants was done. Both MPT and cortisol treatments showed significant increases in gene transcription levels, mainly in *11β-hsd2*, whose mRNAs in larval gonads were restricted to somatic cells, presumably Leydig cells The administration of increasing doses of cortisol *in vitro* induced an increase in both *hsd11b2* expression and 11-KT levels. Overall, these results suggest that cortisol may increase 11-KT production through the action of *11β-hsd2* during the masculinization process by warm temperature.

37.

EFFECT OF FASTING ON SOMATIC GROWTH IN THE CICHLID FISH *Cichlasoma dimerus*

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Fish can withstand long periods of food restriction, by modulating the expression of different factors associated with growth hormone (GH) / insulin-like growth factor-I (IGF-I) system. If food is limited, energy is used for essential physiological processes and a reduction in growth can be observed. However, little is known about the mechanisms involved in these processes.
The aim of this study was to determine in this specie if fasting affects somatic growth, and GH /IGF-I expression.
Fish were divided in two groups. The first was fasted for 21 days and the second continued to be fed (control group). During this period the weight and length were recorded twice a week. After this time they were sacrificed, previous blood extraction, and pituitary and liver sampled in order to analyze the GH and IGF I mRNA levels respectively by RT-PCR.
Results showed a significant reduction in somatic growth and in the hepatosomatic index in fasted animals. There were no differences in cortisol levels between the two groups. The fasted animals showed a significant reduction in IGF mRNA levels, but no difference in GH mRNA levels was observed. These results suggest that fasting affects somatic growth probably acting on GH / IGF-I at different levels.

38.

A CELLULAR MODEL TO STUDY PROLACTIN PARTICIPATION IN HUMAN ENDOMETRIUM DIFFERENTIATION

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The expression of prolactin (PRL) and its receptor (PRL-R) increase during endometrial decidualization, however, the role of PRL in the endometrium differentiation is still unknown. Previous results in endometrial epithelial cells primary cultures have shown that PRL induces the expression of MFG-E8 protein ("milk fat globule EGF-factor 8"), suggesting a possible role of PRL in endometrial differentiation. The aim was to characterize a model based on established cell lines to study the role of PRL on human endometrium differentiation. In cultures of Ishikawa and HEC-1A endometrial epithelial cell lines, the presence of the PRL-R was analyzed by Western blotting and immunofluorescence. Both cell lines showed the presence of two immunoreactive bands of 80 and 50 kDa corresponding to the long and intermediate receptor isoforms, respectively. The cells showed fluorescence label on the surface as well as at intracellular sites. We conclude that the presence and localization of the PRL-R in two established cell lines indicate its possible use to study the role of PRL in endometrial differentiation *in vitro*.

39.

THE MICROINJECTION OF MARCKS EFFECTOR DOMAIN INHIBITS CORTICAL GRANULE EXOCYTOSIS IN MOUSE EGGS

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Cortical granule exocytosis is a crucial secretory process that blocks polyspermy and enables successful embryonic development. Nevertheless, the molecular mechanism of this particular exocytosis remains unknown. We have reported that MARCKS, a prominent substrate of PKC involved in exocytosis in different cell types, is expressed in mouse eggs. We hypothesized that MARCKS is involved in cortical granule exocytosis in mouse eggs. To test this hypothesis, we analyze the effect of MARCKS effector domain in a functional assay of cortical granule exocytosis. We purified MARCKS effector domain as a GST-fusion protein and microinjected mouse eggs before activate them parthenogenetically with $SrCl_2$. As a control, we microinjected GST protein. Results showed that only the microinjection of MARCKS effector domain was able to inhibit cortical granule exocytosis stimulated by $SrCl_2$ when compared to control. In addition, the extent of this inhibition was dependent on the concentration of MARCKS effector domain. These data validate our hypothesis and suggest that MARCKS is involved in the signal transduction pathways that lead to cortical granule exocytosis in mouse eggs.

40.

REGULATION OF THE ENDOCANNABINOID SYSTEM BY OVARIAN HORMONES IN BOVINE SPERM-OVIDUCT INTERACTION

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We have previously demonstrated that anandamide (AEA), the major endocannabinoid, is involved in sperm-oviduct interaction by promoting the release of spermatozoa (SPZ) from the bovine oviductal epithelium (BOEC). In addition, it has been shown that estradiol (E) and progesterone (P) regulate the enzymes that synthesize (NAPE-PLD) and degradate (FAAH) AEA in the uterus and the oviduct of mammals. Therefore, the objective of this study was to assess whether E and P are capable of regulating these enzymes and thus induce SPZ release from BOEC. SPZ-BOEC co-cultures were treated with E and/or P for 1, 2 and 4h. The results indicate that both hormones induce the release of the SPZ from the BOEC at 2 and 4h of incubation ($p<0,05$). The effect of hormones was blocked when co-cultures were treated with a CB1 receptor antagonist ($p<0,05$). On the other hand, oviductal FAAH and NAPE-PLD mRNA expression was assessed by PCR in BOEC incubated with hormones for 2 and 4h. Both E and P increased the expression of NAPE-PLD but did not change the expression of FAAH. Overall, our results suggest that ovarian hormones could be modulating oviductal levels of AEA and thus regulating the release of SPZ from oviductal epithelium in bovines.

41.

NUTRITIVE VALUE OF PEANUT HULLS (*Arachis hipogaea*) FOR RUMINANTS

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Around 200.000 tn of peanut hulls are available each year in Argentina. The objective was to determine the proportion, composition and degradation parameters of the fractions that make up the hulls. Hulls samples of 4 different regional industries were obtained. By hand scraping, 400 g of each sample was separated in to endocarp (E) and the rest (R). Dry matter (DM), crude protein (CP), lignin (ADL), neutral and acid detergent fiber (NDF, ADF) and ashes in each fraction were determined. Degradation parameters were estimated by the gas production technique, fitting the Mitscherlich model with a lag phase, to the gas production values. Means were compared by Student t test. E was 8.88 ± 1.41% of the DM hulls. NDF (53.25 vs 75.67%), FDA (46.07 vs 59.15%) and ADL (6.77 vs 28.06%), for E and R respectively, were statistically different (P<0.001). Fractional degradation rate (0.064 vs 0.033 hr^{-1}), total gas production at 48 hr (526.31 vs 215.43 ml/g) and lag time (1.81 vs 3.56 hr), for E and R respectively, differed (P<0.01). Peanut hulls have 2 fractions spatially separated, very different in composition and degradation. The endocarp proportion, which varies with maturity, alters the nutritional value of peanut hulls.

42.

SPERMATOZOA WITH AN INTACT ACROSOME MAY RESPOND BY CHEMOTAXIS TOWARD PROGESTERONE

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Sperm chemotaxis is the main transport mechanism that orients spermatozoa toward the oocyte. Progesterone (P_4) is secreted by cumulus cells forming a concentration gradient around the oocyte. P_4 may induce the acrosome reaction at higher concentrations and chemotaxis at lower doses. Hence, it is expected that these two processes do not coexists at the same time. The aim of this work was to evaluate simultaneously the sperm acrosome state and the chemotactic response toward P_4 in real time. The assays where performed in a chemotaxis chamber using transgenic mouse spermatozoa with a green fluorescent label in the acrosome and a red label in the mid piece. The simultaneous evaluation of chemotaxis and acrosome status was performed by videomicroscopy and image analysis. The results show that only intact spermatozoa may be chemotactically oriented toward 100pM P_4 in a dose-response assay. This result was verified inhibiting chemotaxis by altering the gradient of P_4 and the chemotactic signaling cascade. In conclusion, in order to respond chemotactically toward P_4, spermatozoa may need an intact acrosome and also the plasma membrane that covers it.

43.

ROLE OF NOTCH 1 AND NOTCH 4 RECEPTORS IN A HUMAN TUMORAL OVARIAN CELL LINE (KGN)

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Introduction: Ovarian cancer involves a type of solid tumours and in spite of having a good response to treatments, it is frequently recurrent. Notch system is an intracellular pathway that regulates cell growth and homeostasis of embryonic and adult cells. Objectives: a. To evaluate the expression of NOTCH 1 and 4 receptors in a tumoral granulosa cell line KGN (human ovarian granulosa) and in human granulosa cells obtained from women subjected to assisted reproductive techniques (ARTs). b. To study the effect of NOTCH 1 and 4 receptors signaling blockade on the proliferation of KGN cell line. Methodology: we performed western blot to analyze the protein content of the NOTCH 1 and 4 receptors in KGN cell line and human granulosa cells obtained from women subjected to assisted reproductive techniques (ARTs). We studied KGN cellular proliferation by ^{3}H-thymidine incorporation in the presence of different concentrations (10-30μM) of a Notch pathway inhibitor (DAPT). Results: we observed higher expression for both receptors in KGN cell line compared to granulosa cells obtained from women subjected to ARTs. When KGN cells were cultured in the presence of the inhibitor DAPT, we detected a dose-dependant significant decrease (P<0.05) in cell proliferation. Conclusion: these results show that NOTCH 1 and 4 receptors are differentially expressed in tumoral granulosa cells and in their normal counterparts. They also suggest that Notch signaling is involved in KGN cell proliferation.

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44.

THE FEEDING OF *DIPTURUS TRACHYDERMA* AND *DIPTURUS CHILENSIS* IN SAN MATÍAS GULF

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The food consumption information of the rays *Dipturus trachyderma* and *D. chilensis* in San Jorge gulf indicates that these species prey of *Pleoticus muelleri*, *Merluccius hubbsi* and amphipods. Our goal was to determinate the food consumption of these species in San Matias gulf. 25 specimens of *D. trachyderma* and 58 of *D. chilensis* from the commercial catch fishery of the San Matias gulf were sampled. The stomachs were extracted and prey items were identified, counted and weighed. Diet composition was evaluated using the index of relative importance (IRI%). For *D. chilensis* were identified 25 prey items: fishes, mollusks, crustaceans and echinoderms. The most important item according with the IRI% were crustaceans (62,7), then fishes (37), mollusks (0,3). *Peltarion spinosulum* was the crustacean which contributed more to the diet (20,9), *Engraulis anchoita* between the fiches (22,8) and *Loligo* sp. from the mollusks (0,3). *D. trachyderma* feeds on 16 prey items. The IRI% were for fishes (89,3), crustacean (9,5) and mollusks (1,3). The species which more contribute per group were *M. hubbsi* (36,5), *Platyxanthus patagonicus* (9) and *Loligo* sp. (1,3) respectively. We observe a difference of the food habit between these species, showing bentonic habit for *D. chilensis* and demarsal for *D.trachyderma*. The difference could be explained by the size range of each species sampled.

45.

THIOL REDUCTION AND PROTAMINE EXCHANGE: SIMULTANEOUS EVENTS DURING HUMAN SPERM DECONDENSATION?

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Previous results indicate that heparin (Hep) cooperates with glutathione (GSH) in protamine thiol reduction during human sperm decondensation. The aim of this study was to evaluate the effect of Hep on chromantin thioreduced status using a direct methodology. Isolated nuclei from normospermic donors (OMS) were incubated (37°C) with GSH (10mM) or DTT (0.1mM) (30') and Hep (46uM) (0, 5, 15, 30'). Chromatin thioreduced status was evaluated by fluorescence microscopy with thiol reagent monobromobimane and quantified (arbitrary units) using ImageJ. DTT was a more potent thiol reducing agent than GSH (98±2 vs. 62±2, ANOVA-Dunn, p<0,01). Thiol reduced status increased significantly after 5' exposure to Hep with GSH as thiol reducing agent (62±2 vs. 102±3, ANOVA-Dunn, p<0.001) but not with DTT. These results confirm that Hep not only behaves as protamine acceptor but also cooperates in chromatin thiol reduction by GSH, suggesting that both processes take place simultaneously.

46.

GESTATIONAL DIABETES (GD) ALTERS APOPTOSIS IN RAT FETAL BRAIN

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The GD is an obstetric disease with severe effects on offspring. Despite insulin treatment the rate of malformations is high in mothers having this disease. We have previously shown that hyperglycemia caused by GD alters intracellular signaling pathways in the fetal brain with a decreased expression of Bax and phospho-Akt proteins in the present work we studied the effect of GD on the anti-apoptotic protein Bcl -2 as well as the state of axonal neurofilaments in perinatal fetal brain. The tissues were obtained from fetuses of embryonic day 19 from sham injected mothers and mothers injected on day 1 of pregnancy with streptozotocin (40mg/kg body weight i.v.). Studies were conducted by Western blot using specific antibodies. The immunostaining for Bcl-2 increased in hyperglycemic fetal tissue, but this increase was not significant (p = 0.06) however it was a significantly decreased on Bax expression (P <0.01). There were no changes in the expression on heavy neurofilaments NF-200. Unlike what happens during the period of organogenesis, the Bax/Bcl-2 balance is diminished in the perinatal fetal brain exposed to GD. The no differences found on NF200, an essential component of axons, suggest that the natural death of neurons characteristic of this stage of neurodevelopment, is decreased, which can compromise the brain citoarquitecture in adult brain.

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47.

NEUROPATHOLOGICAL MECHANISMS TRIGGERED BY HYPOXIA IN THE POST-NATAL RAT HIPPOCAMPUS

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Perinatal asphyxia is a frequent obstetric complication that may produce neurological damage in the newborn. To evaluate the therapeutic use of different natural and synthetic steroids we started studding molecular changes produced by hypoxia *in vitro*. Previously we described the hypoxic effects on organotypic cultures from striatum, neocortex and hippocampus. In this work we used another model where postnatal fresh tissue is kept alive during 2-6 hours. Hippocampus from 21 days old male rats were kept in a 37ºC cerebrospinal fluid-like solution (aCSF) for 90 min then subjected to hypoxia for 30 min and finally transferred to a fresh oxygenated aCSF for 2, 4 and 6 h. By Western blot we evaluated the expression of MAP-2, that showed a significant decrease at 4 and 6 h post hypoxia (20% p <0.05), meanwhile GFAP showed a non significant 10% increase (p = 0.058) and the expression of caspase-3 and iNOS resulted unchanged at any time. This experimental model reproduces some changes previously observed *in vivo* by hypoxia as well as in organotypic cultures. Using this experimental model will allow us to rapidly assess the effect of compounds with probable protective action on hypoxic phenomenon.

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48.

BMP4 AND NOGGIN DURING OOCYTE *IN VITRO* MATURATION (IVM) IN BOVINE: EFFECT ON EXPRESSION PATTERN OF BLASTOCYSTS

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BMP4 is implicated in maintaining the pluripotent state of embryonic stem cells (ESCs) in mice. In bovine, ESCs has not been established yet therefore, the effects of BMP4 on these cells are still unknown. The aim of this work is to study the effects of BMP4 and its inhibitor, Noggin during IVM on the expression pattern of Oct-4 (a pluripotency marker) in bovine blastocysts. Oocytes were *in vitro* matured in TCM (Control) or supplemented with 100 ng/ml of BMP4 or Noggin. After 24 h oocytes were activated with 5uM of Ionomicyne and 1.9 mM of 6-diaminopurine and culture in CR2 medium. On day 9, blastocysts were fixed and Oct-4 expression pattern was studied by immunocytochemistry and confocal analysis. Proportion of Oct-4 expressing cells over total cell number was analyzed with a test of proportions (INFOSTAT software). BMP4 blastocysts had a higher proportion of Oct-4 expressing cells (79.0% n=2) vs Control (63.0 % n=3; p< 0,01) and vs Noggin (65,4% n=3 p< 0,01). Oct-4 was found in trophoblastic and inner cells, localized nuclear as well as cytoplasmatic. Our results suggest that exogenous BMP4 during maturation is affecting bovine pluripotent state of parthenogenic bovine blastocysts.

49.

IDENTIFICATION OF MOLECULAR MARKERS IN BLADDER CANCER USING MURINE AND HUMAN MODELS

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Introduction: Bladder cancer affects over 1 million people worldwide, but the molecular basis remains unknown. Downregulation or loss of epithelial cadherin (cadE) is a prognostic marker for this cancer. Presence of negative modulators of its expression (Snail) and of mesenchymal cadherins (Neural cadherin, cadN) has been associated to tumor progression. Dysadherin (Dys) is a transmembrane glycoprotein that regulates cadE adhesive functions, but its expression in bladder cancer has yet not been reported. Objective: To evaluate the expression of cadE and its modulators in 3 bladder cancer models: a) an orthotopic animal model (MB49/MB49-I) b) a human invasive cell line (T24) c) human tumors. Methods: RT-PCR, Western Immunoblotting and immunocytochemistry. Results: In the 3 models, low levels of cadE expression were found, and cadN and Snail trancripts were detected. Expression of Dys was determined in bladder tumors of human and murine origin. Discussion: The study describes changes in expression of cadE, cadN and Snail and reports, for the first time, the detection of Dysadherin, a new molecular marker in the evaluation of bladder cancer.

50.

PORCINE TRANSGENESIS BY INTRACYTOPLASMIC DNA INJECTION

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Porcine transgenesis is an essential tool in agriculture, pharmacology, medicine and biotechnology. There is a wide variety of methods in pig transgenesis but they show some limitations like low efficiency and high cost. The aim of this work was to develop a simpler and economic method in pig transgenesis than those frequently used. Parthenogenic pig embryos obtained from *in vitro* matured oocytes activated by an electric pulse and posterior incubation with 6-DMAP were cultured in SOF medium at 39ºC, 5% CO_2 (Control, N=289) or subjected to intracytoplasmatic injection with the circular plasmid pCX-EGFP at concentrations of 3 or 300 ng/ul and cultured like Control (N=146 and 41 respectively). The group injected with 300 ng/ul presented a higher % of embryos expressing the transgene and a lower % of blastocysts compared to the group injected with 3 ng/ul (22.0% vs 8.9% GFP+ and 0.0 vs 4.8% blastocysts). Evaluation of genomic integration remains to be tested but these results suggest that the plasmid intracytoplasmic injection is a useful tool for obtaining porcine GFP+ embryos. It is necessary to adjust the plasmid concentration for obtaining embryos expressing the transgene without a reduction in embryo viability.

51.

MOLECULAR MECHANISMS INVOLVED IN PROTEIN TYROSINE PHOSPHORYLATION INHIBITION DURING CRISP1 KNOCKOUT SPERM CAPACITATION

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CRISP1 protein associates with sperm during epididymal maturation and participates in fertilization. Interestingly, sperm from *Crisp1*$^{-/-}$ mice generated in our laboratory exhibit lower levels of capacitation-associated protein tyrosine phosphorylation. In the present work, we further investigated the mechanisms underlying this inhibition in CRISP1-deficient sperm. Capacitation of *Crisp1*$^{-/-}$ sperm in the presence of purified native CRISP1 did not restore tyrosine phosphorylation levels. Considering that tyrosine phosphorylation is regulated by a cAMP-dependent pathway, we evaluated the occurrence of this event in *Crisp1*$^{-/-}$ sperm exposed to both a cAMP analog and a phosphodiesterase inhibitor. Results showed that, under these conditions, there was a reversion in *Crisp1*$^{-/-}$ sperm phosphorylation levels supporting the existence of a lower content of cAMP in mutant sperm. This possibility was confirmed by the significantly lower levels of cAMP determined by RIA. Considering that sperm acquire both CRISP1 and the ability to undergo capacitation during their transit through the epididymis, our results suggest that the inhibition in tyrosine phosphorylation in *Crisp1*$^{-/-}$ sperm might be due to the lack of CRISP1 association during epididymal maturation.

52.

REGULATORY ASPECTS OF CAT ACTIVITY DURING SHADE-INDUCED SENESCENCE IN WHEAT LEAVES

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In previous works it was shown that the suppression of blue light (400-450 nm) by means of selective light filters accelerates the senescence rate of wheat leaves exposed to shading. This phenomenon was correlated to an increase in the sensitivity to oxidative damage, in part due to a marked decrease in the activity of catalase (CAT). In order to better understand how CAT activity is regulated during shade-induced senescence, zymograms were performed, and the study of the expression of the two main CAT genes found in wheat (CAT 1 and CAT 2) was initiated, on extracts of detached wheat leaves exposed during different time periods to light selective Lee ® filters. The effect EGTA (2.0 mM) was also assayed, considering that calcium would be involved in the regulation of CAT activity. While CAT 2 activity was found to be very high and almost constant during most of the experimental period, the activity of CAT 1 changed with the development of leaf senescence. No clear relationship was observed between the enzyme activities recorded and the expression patterns of their respective genes, suggesting that post-transcriptional effects might be involved. CAT activity decreased due to the presence of EGTA only when blue light was suppressed.

53.

GROWTH HORMONE (GH) SIGNALING DURING GROWTH PERIOD IN MICE

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Growth hormone (GH) is the major endocrine postnatal body growth regulator; it acts mainly through the JAK2/STAT5 pathway. Suppressors of cytokine signaling (CIS/SOCS) and phosphatases (PTP-1B, SHP-1 and SHP-2) regulate the pathway negatively, while transcription factors GR and HNF-1 modulate it positively.
Activation by GH of the JAK2/STAT5 pathway during the growth period was evaluated in Swiss mice of 1 week (GH independent growth), 2.5 weeks (GH dependent growth) and 9 weeks (adults, reference) of age. These mice were stimulated with GH and after 7.5 minutes liver was obtained (key GH target tissue) in order to determine the content and/or phosphorylation of proteins of interest by immunoblotting.
Results showed that STAT5 presents greater activation in GH-dependent rapid growth period ($p < 0.05$; $n = 6$). This elevated phosphorylation is related with: 1) low level of proteins involved in signaling termination such as CIS, SOCS-3 and PTP-1B, 2) high level of GR and HNF-1 and 3) higher STAT5 content at the beginning of GH dependent rapid growth period.
GH sensitivity during growth is age dependent in liver, and it could be associated with changes in the expression of modulators of the pathway in study.

54.

MIX-cAMP SIGNALING IN PRE-ADIPOCYTE DIFFERENTIATION

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Adipogenesis is stimulated in 3T3-L1 fibroblast by a combination of insulin, dexamethasone, and methylisobutylxanthine (MIX). Mitotic clonal expansion (MCE) precedes differentiation of 3T3-L1 fibroblast to adipocytes. MIX increases cAMP content, which activates protein kinase A (PKA). However, PKA-independent cAMP signaling through EPAC (exchange protein activated by cAMP) has been described. We found that H89, an inhibitor of PKA, was able to block MCE but not differentiation of 3T3-L1 fibroblast. Differentiation, evaluated by Oil-Red-O staining or quantification of triglycerides, did not occur when MIX was not present in the differentiation mixture but it was restored by addition of either dibutyryl-cAMP (db-cAMP) or 8 CPT-2 Me-cAMP. The latter activates cAMP-EPAC but not PKA signaling. In addition, we found that MIX signaling contributes to the activation of PPAR gamma, a transcription factor required for adipocyte differentiation and also the kinase JNK but not PI3K-PKB. On the other hand, we found that transfection of pre-adipocytes with a dominat-negative Rap, a usual substrate of EPAC, was able to inhibit pre-adipocyte differentiation. These results indicate that MIX signaling activates JNK and EPAC-Rap during 3T3-L1 fibroblasts differentiation to adipocytes.

55.

IDENTIFICATION OF PROGESTERONE RECEPTOR ON HUMAN SPERMATOZOA

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Progesterone (P) induces chemotaxis, hyperactivation and acrosome reaction concentration-dependent in mammalian spermatozoa (S). However, there is discrepancy on the identity and localization of the P receptor (PR). The aim was to identify and localize PR on human S. Cell-surface and intracellular proteins were isolated by biotinylation and membrane and cytosol proteins were purified by ultracentrifugation. PR detection was performed by inmunoblotting and immunofluorescence with an antibody against the C-terminal tail of the genomic PR. A protein band corresponding of ~85 kDa was detected in the biotinylated cell-surface proteins, as well as in the membrane and cytoplasmic fractions. The percentage of positive S for the PR observed in the equatorial segment was significantly higher in permeabilizated S. In conclusion, the results suggest that PR in human S would be localized in both membrane and cytoplasm of the equatorial region. This finding could be in agreement with the different sperm processes stimulated by distinct P concentrations.

56.

β-HEXOSAMINIDASES FROM *X. laevis EGG*: BIOCHEMICAL CHARACTERIZATION

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The enzyme *N*-acetyl-β-D-glucosaminidase (β-hexosaminidase or Hex) has been reported to participate in phylogenetically distant animals both in gamete binding and polyspermy prevention. We previously demonstrated that the Hex was the main glycosidic activity in *X. laevis* oocytes. In addition, we presented by first time the putative *X. laevis* Hex sequence. Our objective now was to characterize the Hex of *X. laevis* oocytes. Based on native gel assays using oocytes extract two different Hex activity bands were detected. Using specific substrates we determined that both bands correspond to Hex A or S isoforms. The molecular composition of both Hex proteins was determined by Western blot. The isoform of higher electrophoretic mobility in native gels was composed by two polypeptidic chains of 59 and 49 kDa. Only a 59 kDa chain was observed in the isoform of lower electrophoretic mobility. While the 59 kDa polypeptides were in agreement with the translated *X. laevis* ORF, the 49 kDa chain seems to be a proteolytic derivate from the 59 kDa polypeptide. Finally, by immunohistochemistry, the Hex was localized in the cortex of animal hemisphere. Since this hemisphere has been reports as the sperm entry site in amphibians and that the Hex in *X. laevis* eggs has been linked to the polyspermy prevention, our experiments suggest the existence of a strategic location of Hex that might directly impact in the polyspermy prevention efficiency.

57.

INVOLVEMENT OF BUFO ARENARUM INTEGRINS IN THE SIGNALING CASCADE ACTIVATED BY FERTILIZATION

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The incubation of *B. arenarum* oocytes with the integrin interacting motif RGDS provoked a significant decrease in the fertilization scores suggesting a role for this protein in fertilization. Our aims were: i) to study the effect of a polyclonal antibody against the β1 integrin subunit (M-106) in the fertilization process ii) to analyze the effect of RGDS treatment over the oocyte. The effect of M-106 antibody was evaluated by *in vitro* fertilization assays done in the presence 50 µg/ml of M-106 or control antibody. Oocyte activation was evaluated by macroscopic analysis (vitelline envelope elevation) and studying the phosphorylation state of p42-MAPK. We isolate cytosolic and plasma membrane fractions from oocytes, RGDS-treated oocytes and eggs after 1 or 10 minutes post-fertilization and analyze the tyrosine phosphorylation patterns of these fractions by western blot. The M-106 antibody provoked a significant inhibition of fertilization (100,5 ± 4,3 vs. 58,5 ± 2,1%, $p<0.05$). RGDS peptide treatment did not induce oocyte activation but induced protein tyrosine phosphorylation, partially mimicking the fertilization-induced changes in the tyrosine phosphorylation pattern. These results suggest that integrins (in particular the β1 subunit) are involved in fertilization and due to the fact that RGDS peptide is an integrin-interacting peptide, our results demostrate that these proteins are members of the signaling cascade that follow fertilization.

58.

THE LIPOPOLYSACCHARIDE (LPS) STIMULATES THE PROTEIN EXPRESSION OF THYROID PEROXIDASE (TPO) IN A NUCLEAR TRANSCRIPTION FACTOR (NFκB) DEPENDENT MANNER

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LPS is a powerful activator of gene expression in immune and endocrine cells, with the transcription factor NFκB as one of its main intermediaries. We previously demonstrated the presence and function of TLR4 in thyroid cells and that LPS stimulates the expression of the cotransporter Na^+/I^- (NIS) in a NFκB dependent manner, and thyroglobulin (TG). TPO, an enzyme involved in the biosynthesis of thyroid hormones, is the main microsomal component responsible of thyroid autoimmunity. Objective: To study the effect of LPS on TPO expression and the role played by NFκB. Methods: FRTL-5 cells deprived of TSH for 5 days were treated with LPS in the presence or absence of TSH, carrying out assays of RT-PCR, Western blot, ChIP and promoter activity. Results: LPS increased transcriptional activity, mRNA and protein expression of TPO induced by TSH. These increases obtained were suppressed in the presence of NFκB inhibitor, BAY 117082. Discussion: LPS can stimulate the expression of TPO, mediated by NFκB. Growing evidence suggests that NFκB participates in the pathogenesis of autoimmune diseases relating to chronic inflammatory processes.

59.

GENO-CYTOTOXICITY OF THE HERBICIDE IMAZETHAPYR AND ITS COMMERCIAL FORMULATION PIVOT® ON MAMMALIAN CELLS

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Imazethapyr (IMZ) is a systemic residual herbicide used for the pre-postemergent weed control. Despite its increasing use worldwide, and particularly in our country, data about its toxic potential are limited, being classified by the EPA as class III member. The aim of this study was to evaluate the genotoxic and cytotoxic potential of IMZ and its formulation Pivot® (10% IMZ) on CHO-K1 cells (0.25-15 µg/ml, 24 h) using genotoxicity [sister chromatid exchange (SCE)], and cytotoxicity assays [cell-cycle proliferation (CCP), mitotic index (MI), neutral red and MTT]. The results showed: 1) For both compounds, an increased SCEs frequencies throughout all concentrations ($p<0.05$); 2) Both compounds induced CCP delay with concentrations higher than 0.25 µg/ml ($p<0.05$); 3) MI decrease with concentrations higher than 1 µg Pivot®/ml ($P<0.05$); 4) Decrease in lysosomal activity with concentrations higher than 0.25 µg Pivot®/ml; 5) A decrease in mitochondrial activity with concentrations higher than 5 µg Pivot®/ml ($P<0.05$). These results demonstrate that Pivot® possess a higher *in vitro* cytotoxic effect than IMZ. Furthermore, they can suggest the presence of xenobiotics within the excipients that would enhance the deleterious effect of the active ingredient.

60.

ENHANCEMENT OF TRANSFECTION EFFICIENCY OF FETAL BOVINE FIBROBLAST BY MANIPULATION OF THE CELL CYCLE

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Successful transient and stable transfection of cells represents the first stage in many transgenic strategies based on somatic cell nuclear transfer (SCNT). Therefore, development of more efficient and reliable methods to transfect bovine cells in culture is needed. We studied the effect of the mitotic-blocking agent colchicine on the transient transfection of bovine fetal fibroblasts (BFFs) with a plasmid (pZsGreen1-N1) expressing a green fluorescent reporter protein. Transfection was carried out in presence of different transfection agents: GeneJammer (Stratagene) or FuGene (Roche) in BFF cultures previously treated with increasing colchicine concentrations (0-40 ng/mL) for different incubation periods (3-12 hs). We observed a positive effect of colchicine pretreatment on transfection efficiency at 10 ng/mL for 6 hs as evidenced by a significative increase in the percentage of fluorescent cells. High colchicine concentrations and/or long incubation periods lead to cytotoxicity. Further studies are warranted to define conditions leading to high transfection efficiencies and the karyotypic normalcy of treated cells. Results from this study set the bases to develop improved strategies that will facilitate transfection of BFFs for SCNT transgenesis.

61.

ANANDAMIDE (AEA) INCREASES NITRIC OXIDE (NO) LEVELS IN BOVINE SPERM CAPACITATION

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We have previously demonstrated that AEA, the major endocannabinoid, regulates sperm capacitation promoting the release of spermatozoa (SPZ) from bovine oviductal epithelium (BOEC). Our results also suggest that AEA exerts its effect by activating the NO pathway.
In the present study we investigated whether AEA modulates NO levels both in SPZ and BOEC. We performed *in vitro* sperm capacitation using bovine cryopreserved SPZ incubated with MetAEA (AEA analogue) and DAF-FM diacetate probe (fluoresce in the presence of NO). The fluorescent complex was measured by flow cytometry. Incubation of SPZ with MetAEA increased NO levels ($p<0.001$). Furthermore, immunocytochemistry assays revealed an increase of the neuronal NO synthase immunostaining in SPZ capacitated with Met-AEA.
On the other hand, BOEC cultures were incubated with MetAEA and nitrite concentration was determined by Griess technique. In addition, we assessed NOS activity in BOEC through the conversion of L-[^{14}C]-arginine to L-[^{14}C]-citrulline. MetAEA did not modify either nitrite concentration nor BOEC NOS activity. The results suggest that AEA may activate the NO pathway in SPZ during sperm capacitation and thereby promote SPZ release from oviductal epithelium in bovines.

62.

MORPHOLOGICAL CHARACTERIZATION OF *Crotalaria juncea* L. FIBERS

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Crotalaria juncea (Fabaceae) is an herbaceous plant of rapid growth that provides alternatives to wood fiber for papermaking. The aim of this study was to characterize anatomically the fibers of this species cultivated in Villa del Totoral, Córdoba, Argentina. The seeds from multiplication trials carried out in the chair of Industrial Crops (Universidad de Buenos Aires), were sown in experimental plots in Villa del Totoral. Plants were harvested at the beginning of flowering, when the fiber quality is optimal. Free-hand stem sections and phloem fibers macerations were made in order to determine fibers density / mm ^{2}, wall thickness and fibers diameter and length. The measurements were taken using ocular micrometer. The species were observed to have 673, 18 fiber/mm2 of bark, and that fibers were 32,83 µm average diameter, 5, 42 µm thick cell walls and 3 mm in length. The results are similar to those reported in the literature. This type of study provides basic information on fiber dimensions that determine quality for paper production.

63.

EFFECT OF ANTIPARASITIC TREATMENT ON THE DEVELOPMENT OF MAMMARY GLAND PARENCHYMA IN HOLSTEIN HEIFERS

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Nematode gastrointestinal parasites are known to delay growth and puberty onset in grazing cattle and to decrease serum IGF-I levels. We wished to investigate if nematode burden may impair normal mammary development. To this end 40 female Holstein calves were randomly assigned, at birth, to an untreated control group (C) or to the treated group (T) which received monthly, from birth onward, atiparasitic drugs (ivermectin, fenbendazol and/or levamisol). At 20, 30, 40 and 70 weeks of age blood and fecal samples were taken for serum IGF-I determination and nematode egg counting (EPG) in feces, and mammary biopsies were taken to 6 heifers in each group for histological and immunohistochemical studies. IGF-I increased with age ($P=0.003$) and was higher in T calves ($P=0.042$), whereas C calves had always higher EPG ($P=0.020$). Mammary parenchyma was embedded in fat pad, conforming ductal developing structures of epithelial cells. Mammary samples from T heifers had higher ratio of parenchyma/total area ($P= 0.036$), PCNA index ($P= 0.037$) and expression of estradiol-receptor alpha ($P=0.006$), compared with C heifers. These results indicate that lowering parasite burden induces a higher mammary parenchymal development and cell proliferation, as well as a higher density of parenchymal E2 receptors in heifers.

64.

GROWTH PARAMETERS CHANGES ON A CELL LINE DERIVED FROM *Oryctolagus cuniculus*

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One of the landmarks occurring during the establishment of a cell line is the presence of alterations affecting cell proliferation mechanisms. The aim of this study was to assess those changes affecting the cell growth parameters that occur during the maintenance of a cell line established from New Zeeland skin rabbit between subcultures (SCs) 5-50. The analysis included the comparison of cell growth curves with 10% fetal bovine serum (SBF) and those obtained from SBF growth concentration-dependent (0-10%). The results showed: 1) An increased growth rate among SCs ($P\leq0.05$); 2) An enhancement of cell density at growth confluence of the different SCs, achieving at SCs 40 and 50 the highest parameters ($P\leq0.05$); 3) An increased independence of SBF supplement in latest SCs, regardless of its concentrations ($P\leq0.05$). These data could indicate that among the maintenance of the cell line under study, the inhibition of cell proliferation by contact is a time-dependent gradual process as well as the synthesis of endogenous growth factors is inherent to the cellular system employed.

65.

MALE MEIOSIS AND KARYOTYPE EVOLUTION IN HARPACTORINAE AND ECTRICHODIINAE (HEMIPTERA: REDUVIIDAE)

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Up to now, there have been cytogenetically analyzed 152 species of the family Reduviidae, which are characterized by holokinetic chromosomes, a wide range in the number of autosomes, and simple and multiple sex chromosome systems. It is proposed that the reduviids have originated from a cimicoid ancestral stock characterized by 2n=28A+XY/XX, and two evolutionary trends have occurred in the karyotype evolution: a decrease in the number of autosomes through fusion mechanisms, and an increase in the number of X chromosomes by fragmentations. In this work, we analyze for the first time the karyotype and male meiosis of *Graptocleptes bicolor* (2n=22A+XY) and *Zelus obscuridorsis* (2n=16A+XY) (Harpactorinae), and *Brontostoma colossus* (2n=28A+XY) and *B. discus* (2n=34A+X_1X_2Y) (Ectrichodiinae). All species studied have the characteristic meiotic behaviour of Reduviidae. Our results and published data allow us to suggest the following trends in the evolution of karyotype: in Harpactorinae the two evolutionary trends characetristic of Reduviidae were observed, i.e. a decrease in the number of autosomes through fusion mechanisms and an increase in the number of X chromosomes through fragmentations, whereas in Ectrichodiinae the increase in the number of autosomes and, also, in the number of X sex chromosomes were occurred, and even loss of the Y chromosome giving rise an X0 sex chromosome system.

66.

ENVIRONMENTAL EXPOSURE TO PESTICIDES: A BIOMARKER'S PRELIMINARY STUDY IN UMBILICAL CORD BLOOD

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Considering fetal vulnerability to enviromental toxicants and the exposure through placental transfer, the aim was to evaluate the impact of intensive pesticide use, mainly organophosphate, on umbilical cord blood biomarkers. In a rural population, samples were collected using inclusion/exclusion criteria during pesticides pulverization period (PP) (n=14) and recess period (RP) (n=11). The activity of cholinesterases (plasmatic and erytrocytic) and red cell's catalase (CAT) were determined spectrophotometrically. Hematologic and genotoxic damage indeces (comet assay of lymphocytes) were also evaluated. Samples obtained in PP demonstrated, in comparison to RP, a significant 51 % enhancement in CAT activity (p=0.02), a 40% enhancement in leukocytes number (p=0.02) and in lymphocytic subpopulation number (43%; p=0.04). There were not significant variations in blood cholinesterases. Preliminary study on genotoxic damage indicated that the damage index was 258±34 (mean±SEM:) while the index in a control group was 194.5±1.44. Our data show an alteration of white cell count. CAT resulted a more sensitive biomarker than cholinesterases. Its increased activity could be associated to an adaptive response to pesticide's *in utero* exposure.

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67.

VASOTOCIN: A NEUROHYPOHYSIAL HORMONE INVOLVED IN SOCIAL BEHAVIOR AND REPRODUCTION OF CICHLID FISH

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In non-mammalian vertebrates, the nonapeptide arginine-vasotocin (AVT) is involved in the regulation of social behavior related to reproduction and aggression. The cichlid fish *Cichlasoma dimerus* is a monogamous species with complex social hierarchies. In this work we studied the peripheral AVT system. The effect of AVT on pituitary gonadotropin secretion was analyzed by single pituitary culture while expression of AVT and in peripheral organs was studied by RT-PCR using specific primers. Finally, the role of AVT on testicular steroidogenesis was assessed by *in vitro* incubation of testis. Results showed a positive effect of AVT on gonadotropin secretion, androgens synthesis and aggression and establishment of social hierarchies. Vasotocin expression was observed in testicular somatic tissue located in the interstitial compartment.

68.

PROSTAGLANDIN E_2 INDUCES THE EXPANSION OF CUMULUS-OOCYTE COMPLEX (COC) CELLS IN TRANSGENIC MICE HYPERSECRETING HUMAN CHORIONIC GONADOTROPHIN (HCG)

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It has been previously described that transgenic female mice hypersecreting hCG (hCGαβ+) present alterations of the cumulus cell expansion. The cyclooxygenase-2 synthesizes prostaglandin E_2 (PGE_2), which is a key mediator of the ovulation process. The objective of this work was to study the influence of the COX-2-PGE_2 system on the ovulatory capacity of hCGαβ+ females. The gene expression of COX-2 from ovaries of hCGαβ+ and wt females induced to ovulate was analyzed by Real Time RT-PCR. The COX-2 expression in hCGαβ+ ovaries was decreased compared to wt. COC were incubated with 1μM of PGE_2 for 20 hs *in vitro*; after the period of incubation, COC expansion occurred in both hCGαβ+ and wt. In addition, histology of the ovaries from hCGαβ+ females injected with 40 μg/mouse of PGE_2 was analyzed, showing the absence of COC expansion 6 hs post-injection. In conclusion: alterations of the COX-2-PGE_2 system in the ovary would be responsible for the COC expansion failure of hCGαβ+ female mice.

69.

HYPOXIA MODULATION OF CAVEOLIN-1 IN HUMAN PLACENTA

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Increasing evidence indicates that O_2 is a key regulator of trophoblast differentiation, and failure of the oxygen-associated developmental events contributes to placental disease such as preeclampsia. It has been suggested that alterations of cellular caveolin-1 levels may lead to different angiogenic responses. Caveolin-1 protein is expressed at very high levels in placental artery and villous microvessels. Previously, we have reported a reduced expression of caveolin-1 in preeclamptic placentas.
The aim of our work was to evaluate if different oxygen tensions may alter caveolin-1 expression in human placentas.
Explants from normal term placenta were incubated in normoxia, hypoxia, and in hypoxia/reoxygenation (H/R). Semiquantitative RT-PCR, Western blot analysis and immunofluorescence were performed to study Caveolin-1 expression. We found that the expression of Caveolin-1 decreased significantly when explants were cultivated under H/R and it was almost undetectable in endothelial vessels. Our results may indicate that Caveolin-1 expression may be modulated by O_2 levels and this could be associated to deficient trophoblast invasion of the endometrial arteries observed in preeclampsia.

70.

REGULATION OF GLUCOCORTICOID PRODUCTION IN THE TOAD *Rhinella arenarum*

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Amphibians present seasonal changes in plasmatic concentrations of glucocorticoids (GC), showing the highest levels during the reproductive period. *Rhinella arenarum* is characterized by having an androgen-dissociated breeding pattern with the lowest concentration of testosterone during this period and the highest during the pre-reproductive period. Also, GC regulate the production of testicular androgens by inhibiting the cytochrome $P450_{c17}$. The aim of this work was to analyze if androgens, which in this species are only from testicular origin, regulate GC production in the adrenal gland of *R. arenarum*. Adrenals from adult male toads were incubated *in vitro* with physiological concentrations of testosterone corresponding to the reproductive (10 nM) and pre-reproductive (100 nM) period. After 24 h, adrenals were incubated for 1 h in fresh medium without hormones and corticosterone production in the media was evaluated by radioimmunoassay. The treatment with 100 nM testosterone produced a decrease in the corticosterone synthesis respect the incubation with 10 nM. In *R. arenarum* an interaction between the gonadal and adrenal system seem to be present, while GC down-regulate the testosterone production during the reproductive period, androgens might regulate the GC production during the pre-reproductive period.

71.

SYNTHETIC STEROIDS ACTION ON 3βHSD ENZYME ACTIVITY IN ADRENAL GLAND

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The 3 beta-hydroxi-steroid-deshydrogenase (3βHSD) an enzyme present in the steroidogenic tissues produces progesterone from pregnenolone (P5). Allopregnanolone (Allo) a reduced derivate from progesterone acts as an inhibitor of this enzyme. In previous studies, we showed the binding behavior to GABA**A** receptor complex, of synthetic steroids with spatial conformation similar to the progesterone-reduced metabolites. According with that these steroids may be useful as therapeutic drugs for neuroprotection. In this presentation, we evaluate the action of one of these steroids, the analog oxo 6-19 (Ns1), on the 3βHSD activity. Adrenal rat homogenate was incubated in a glicine-BSA-NAD+ solution, at 37ºC for 5 min, then different concentrations of the steroids Allo or Ns1 was added (10-100μM) followed by P5 as substrate. Absorbance was measured at 340 nm. Twice the amount of Ns1 was required to produce the same enzyme inhibition that Allo. Considering that the effects on the GABA**A** receptor binding described was similar for both steroids, its minor effect of Ns1 on the enzyme activity at the same concentration, might represent an advantage for a possible systemic treatment. More studies must to be done *in vivo* to better characterize the Ns1 action.
PICT00727/06 – UBACYT M012.

72.

BINDING ACTIVITY OF A-HOMOPREGNANES ON $GABA_A$ RECEPTOR

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Previously we have characterized the binding of synthetic steroid with allopregnanolone (Allo) or pregnanolone like conformation to compare their ability as GABAA receptor modulators. Here we evaluated other compounds with more flexibility between A-B rings that the natural steroids.These compounds call A-Homopregnanes have a 7 carbons A ring and where used to study their effects on ^{3}H-muscimol (MUS 10nM) and ^{3}H-flunitrazepam (FLU 1nM) binding. Four A-Homopregnanes(MVD36:3βΔ^5;MVD96: 3αΔ^5;MVD100: 4αΔ^5;PD575:3β5α) were used. Incubations were carried on with rat brain sinaptosomes at 4ºC by 60-90 min with a range of 5 to 1000 nM of Allo, Preg, MVD36/96/100 and PD575. GABA (10μM) or Diazepam (1μM) were used for non specific binding respectively. Allo, MVD96/100, PD575 and Preg stimulate the binding of MUS (EC_{50}= 22; 23,9; 21,8; 8,3 y 17,7 nM) but MVD36 inhibits it (IC_{50}=1,36$_x$10^{-14}M).Allo, MVD36/96 and Preg stimulates the binding of FLU(EC_{50}= 180;6,1;25;14,4 nM) meanwhile MVD100 inhibits it (IC_{50}=0,3nM) and PD575 has a biphasic behaviour. MVD100 has a reverse action on the binding of both ligands studied, making it an interesting compound to study its pharmacological action by other methodologies.
PICT00727-UBA M012.

73.

INVOLVEMENT OF MEMBRANE ADENYLYL CYCLASE IN CAPACITATION OF CRYOPRESERVED BOVINE SPERMATOZOA

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It has been demonstrated that sperm capacitation involves cAMP production by membrane adenylyl cyclase (mAC) and protein tyrosine phosphorylation in different mammalian species. Our aim was to evaluate mAC involvement in capacitation of cryopreserved bovine spermatozoa. Sperm samples were incubated with a) heparin (H), b) different concentrations of forskolin (FSK, activator of mAC), c) H + 2′,5′- dideoxiadenosine (mAC inhibitor) and d) FSK + 2′,5′- dideoxiadenosine. Capacitation was determined by CTC. Acrosome reaction (AR) and true AR were determined by CTC and differential-interferential optical contrast microscopy, respectively. Progressive motility and sperm viability were evaluated by optic microscopy and eosin-nigrosin technique, respectively. FSK (25µM) achieved a percentage of 27,80 ± 2,59% of capacitation, without differing significantly with the one obtained with H (33 ± 4,47%). Follicular fluid-induced AR was not significantly different in spermatozoa previously capacitated with FSK or H. Inhibitor of mAC (12 µM, 2′,5′- dideoxiadenosine) prevented capacitation induced by FSK or H. Our results indicate that mAC plays a pivotal role in capacitation of cryopreserved bovine spermatozoa.

74.

METABOLIC PROFILE OF TRICEPS SURAE MUSCLE OF SELECTED MICE (*Mus musculus*)

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The biochemical properties of the fiber types of mammal skeletal muscles are determinants in functional adaptations before motor and posture requests and they have been studied in animal production species and in animal models. The aim of this research was to analyze variations in the metabolic profile of M. triceps surae in mice lines selected by body structure: CBi + (high corporal weight and long skeleton), CBi- (low corporal weight and short skeleton) and CBi (witness without selecting). Six male adults of each line were killed by overexposure to CO2. Mm. triceps surae were identified, extracted and frozen in liquid nitrogen to -80ºC. The muscles samples were cut in cryostat. By means of NADH reaction was considered the oxidative capacity of the muscle from the optical density obtained by an image analizer. A significant increase in the oxidative metabolic capacity was observed in soleus and medial gastrocnemius head of the lines CBi + and CBi- with regard to CBi. No significant differences were observed among lines in the gastrocnemius lateral head. The direction of the selection did not affect the sense of the oxidative capacity modification possibly by supporting the practically equal biomass by skeleton unit in selected lines.

75.

LANGERHANS CELLS: ASSESSING THEIR ROLE IN THE PHAGOCYTOSIS OF APO/NEC MURINE B16 MELANOMA CELLS

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Our research focuses on DC loaded with apoptotic/necrotic tumour cells-based vaccines (DC/Apo-Nec) against B16 melanoma in syngeneic mice. Previous work by our group has shown that most DC does not leave the vaccine administration site. In order to determine whether recipient's Langerhans cells (LC) have a role in this protective mechanism, we first assessed LC's phagocytic activity *in vitro*. Epidermal cell suspensions obtained by trypsinization of skin from C57/BL6 mice were stained with PKH26 and cocultured with irradiated B16 cells (Apo-Nec), which had been previously stained with CFSE. The % of $PKH26^+CFSE^+$ cells was assessed by flow cytometry. Secondly, in order to evaluate LC's migratory activity *in vivo*, we performed skin painting experiments. FITC/EtOH was applied to the skin of healthy mice, which then received an inflammatory stimulus (LPS). The % of $FITC^+CD11c^+$ cells in draining lymph nodes was assessed at different times after LPS injection. Results: epidermal cell suspensions contained (2±1)% LC, and the % of phagocytosis observed was (6±2)%. Skin painting experiments allowed us to keep track of LCs, given that we could identify a $FITC^+CD11c^+$ population (15%±5%) 48hs after the stimulus. Our next goal is to evaluate LC's phagocytic, migratory and antigen-presenting activity *in vitro* and *in vivo*, in response to CD/Apo-Nec vaccination.

76.

DIFFERENCES IN HUMMAN AND MURINE SPERM CHROMATIN DECONDENSATION

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Heparan sulfate (HS) and glutathione (GSH) decondense human spermatozoa *in vitro*. The presence of HS has been demonstrated in mouse oocytes, but the details of murine sperm decondensation are still unknown. The aim of this study was to compare human and murine sperm decondensation. Cauda epidydimal sperm from 60-90 day old CF1 mice were capacitated for 2 h at 37°C and decondensed with 10 mM GSH + heparin (Hep), dermatan sulfate (DS), chondroitin sulfate (CS) (46 µM) or 2 µM hyaluronic acid (HA) for 15, 30, 45 and 60 min.. The % sperm decondensation was determined by phase contrast microscopy. Total decondensation was higher in murine than human sperm, for both DS and Hep: 68 + 3% vs 10 + 1% ($p<0.001$) for DS and 90 vs 22% for Hep. CS and HA had no decondensing activity in either species. Murine decondensation kinetics was similar for Hep and DS. Hep decondensation kinetics was faster for human than mouse sperm ($t1/2 = 15$ vs 21 min). Results show that Hep decondensation kinetics and DS decondensing activity differ in murine and human spermatozoa.

77.

FLUORESCENT BANDING (DAPI/CMA_3) AND FLUORESCENT *IN SITU* HYBRIDIZATION IN *CALLIGRAPHA POLYSPILA* GERMAR (COLEOPTERA: CHRYSOMELIDAE)

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In Calligrapha (Chrysomelidae), cytogenetic data refer to 13 bisexual species, and show that this genus is relatively homogeneous, with 11 pairs of autosomes and an X0 sex male chromosome system, except for a population of *C. polyspila* from Uruguay which has a Xy_p sexual system, considered as ancestral in Coleoptera. In this work we analyzed the fluorescent DAPI/CMA_3 banding pattern and the localization of nucleolar organizer region (NOR) by fluorescence *in situ* hybridization in spermatogonial chromosome preparations of *C. polyspila* (Argentina). The results revealed that this species has 2n = 22 A + X0, n = 11A + X0 and metacentric chromosomes; possesses an interstitial DAPI-/CMA_3+band in one arm of an autosomal pair; the X sex chromosome is DAPI+/CMA_3+, and the NOR is located at interstitial position in one arm of an autosomal pair. From our observations and from previous data of Coleoptera, we can conclude that: a) *C. polyspila* possesses a symmetrical karyotype with scarce heterochromatin, b) the CMA_3+ band would correspond to the NOR, being this region rich in GC base pairs, and c) its derived X0 sex system would have been originated from an ancestral Xy_p sex chromosome system by the loss of y_p chromosome.

78.

ALPHA-TOCOPHEROL CRYOPRESERVATION AND SEPHADEX FILTRATION IMPROVED BOAR SPERM QUALITY

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Alpha-tocopherol improves boar sperm functionality. Sephadex filtration removes dead and abnormal spermatozoa. The aim of this study was to compare the effects of spermatozoa separation techniques on sperm quality and the response to *in vitro* capacitation and acrosome reaction inducers, for cryopreserved boar sperm with (VE) or without (C) α-tocopherol. All separation techniques enhanced sperm motility, plasma membrane integrity and functionality and acrosome integrity, for C and VE samples (P<0.05). Better results were obtained with neuter and ionic Sephadex column. It was a significative decrease of cryocapacitate state in C samples separated with Sephadex. Cryopreservation with α-tocopherol decreased (P<0.05) the percentage of cryocapacitated sperm respect to C samples, without differences between selection techniques. Samples cryopreserved with α-tocopherol and subsequent separated decreased lipoperoxidation with respect to unselected and separated C samples. Percentages of bicarbonate-induced capacitation were significantly higher for neuter Sephadex-separated VE samples, respect to Percoll-separated and C samples. These results were confirmed by a higher level of tyrosine phosphorylation proteins (p32) and follicular fluid-induced acrosome reaction. Semen cryopreservation with α-tocopherol, and subsequent Sephadex columns-selection, optimize sperm quality and functionality for its use in different reproduction techniques of breeding swine.

79.

REGULATION OF AROMATASE IN THE BIDDER´S ORGAN OF *Rhinella arenarum*

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The Bidder's organ (BO) of male true toads of Bufonidae family is located in the anterior pole of the testis and it has been compared to a rudimentary ovary. In males, oogenesis in this organ is probably inhibited by the testis. The aim of this work is to determine the presence of aromatase in the BO of adult males *Rhinella arenarum*, and also analyze if several steroids are involved in the regulation of this enzyme. BOs were incubated *in vitro* in order to study steroidogenesis and determine aromatase activity in organs treated with several steroids. When testosterone is used as substrate, BO produce androstenedione, estradiol (E_2) and dihydrotestosterone (DHT), suggesting the presence of steroidogenic enzymes. Total aromatase activity is lower in pre reproductive period when compared to reproductive and post reproductive periods. The treatment with glucocorticoids (GC) or E_2 did not affect aromatase activity, and incubation with DHT produced a decrease in this activity. In conclusion, low aromatase activity during the pre reproductive period is associated to high levels of plasmatic androgens and low levels of GC, suggesting an inhibitory role of androgens and no effect of GC. *In vitro* treatment with DHT and GC confirmed the effect of these hormones on aromatase activity.

80.

EFFICIENT TRANSDUCTION OF BOVINE FETAL FIBROBLASTS BY AN ADENOVIRAL VECTOR

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Forced expression of signaling molecules and transcription factors has proven useful to manipulate cultured cells, e.g., to generate induced pluripotent stem cells. The study was aimed at examining the effect of FuGene (Roche), Lipofectamine 2000 (Invitrogen) and electroporation on transduction efficiency of bovine fetal fibroblasts (BFFs) by an adenoviral vector carrying the GFP gene (Ad-GFP; provided by C. Hereñú and R. Goya from INIBIOLP). BFF cultures were trypsinized 72 hs after transduction and the percentage of GFP positive cells was determined by cell counting. Addition of FuGene at a final concentration of 1.3% increased the percentage of GFP-expressing cells (control= 13.9% vs FuGene at 1.3%= 24.5%; p<0.05). Higher doses of FuGene (2% and 2.6%) were associated to a decrease in the proportion of fluorescent BFFs. Supplementation of the transduction media with Lipofectamine 2000 (0.66%) induced a 38% increase in the proportion of fluorescent BFFs compared with that in the control. A similar positive effect on the percentage of transduced cells was detected when Lipofectamine 2000 was included at concentrations of 1% and 1.33%. Electroporation of BFFs in presence of Ad-GFP did not affect the transduction efficiency. Thus, addition of FuGene or Lipofectamine 2000 during adenoviral infection of BFFs, but not electroporation, can revert the poor transduction efficiency of these cells.

81.

INFLUENCE OF THE COTYLEDONS PHOTOSYNTHETIC ACTIVITY DURING THE FIRST STAGES OF SEEDLINGS DEVELOPMENT OF *CUCURBITA MAXIMA* VAR ZAPALLITO

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The cotyledons play an important nutritional roll during the germination of the seeds and the first development stages of seedling. The epigeous cotyledon, besides being reservation organs, they photosynthesize. That photosynthetic ability, for some species, is key for the seedling vigor determination and other aspects related to the establishment, like the competitive ability. The aim of the present work is to assess the importance of the carbonated nutrition supply by the cotyledons on the absolute growth index (ACI), and the relationship aerial organs/root (A/R) in seedling of these specie. The work was performed with seedling of Cucurbita maxima var zapallito germinated and grown in pots, under watter controlled conditions and with uncovered (T1) and covered cotyledons (T2). An entirely randomized design was used. Based on dry weight, in 10 repetitions of 5 seedling each, was determining the relationship A/R, was 3,32 and 2,65, and the ACI 31,41 and 6,32 grams/day for T1 and T2 respectively,. this difference was significant for ANOVA. The results show the importance of the cotiledonal photosynthesis on the growth rate and consequently their larger competitive ability of seedling on the establishment. However, the no significant difference, in the relationship A/R shows a remarkable stability on the assimilates partition and on the seedling architecture.

82.

OVARIAN ANGIOGENESIS INHIBITION: ¿POSSIBLE THERAPEUTIC STRATEGY FOR OVARIAN HYPERSTIMULATION SYNDROME (OHSS)?

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OHSS is an iatrogenic complication caused by the induction of ovulation in fertility treatments, which involves an ovarian overproduction vasoactive substances such as VEGF. The objective was to analyze the *in vivo* effect of inhibition of VEGF in a model of OHSS developed in rats on follicular development and luteal cyst formation, proliferation and apoptosis in the corpus luteum (CL). We used prepuber rats. The OHSS group was injected with high doses of eCG (50 IU / day) for 4 days and 24 hours after hCG was injected (25 IU). TRAP+OHSS group was intraovarian injected with VEGF inhibitor (TRAP chimera, 0.2 mg / ul) on the day of hCG administration. The rats were sacrificed 48 h post-hCG injection. Ovaries were extracted, one for histology and the other for protein extraction. By H&E, in the OHSS group, the TRAP increased the number of preantral follicles, early antral and atretic, and decreased the number of CLs as ovarian cysts. In this group, IHC showed a decrease in immunostaining for PCNA and an increase in active caspase-3 in the CLs compared to untreated OHSS group. These results were corroborated by western blot studies. In conclusion, these results suggest that *in vivo* inhibition of VEGF in the OHSS model affects the follicular and luteal development, reduces the number of cysts and cell proliferation and increases luteal apoptosis. Therefore, this treatment would decrease the severity of OHSS.
This work was funded by grants from CONICET and Roemmers Foundation.

83.

PHOSPORYLATION OF ACYL-COA SYNTHETASE 4 (ACSL4) IN STEROIDOGENIC CELLS

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In steroidogenic cells, arachidonic acid (AA) levels and steroidogenesis are regulated by Acsl4 activity. Acsl4 is an AA- preferring enzyme acting in a dimer form, with a high turnover rate, located in mitochondria-associated membranes (MAM).
Steroid synthesis is modulated by different hormones or factors through PKA or PKC-mediated protein phosphorylation. Since the Acsl4 aminoacidic sequence shows phosphorylation consensus sites for PKA and PKC, the aim of this study was to determine whether Acsl4 is a substrate of these kinases and if it is indeed a phosphoprotein. By $^{32}P[H_3PO_4]$ incorporation, two-dimensional gel electrophoresis, and immunoprecipitation we demonstrated that Acsl4 is phosphorylated by the action of the adrenocorticotrophin hormone in Y1 adrenal cells.
We also demonstrated that recombinant Acsl4 is an *in vitro* substrate for PKA and PKC, these phosphorylation events being independent from one another. In addition, the activity of the enzyme is increased when Acsl4 is PKA-phosphorylated but the dimer formation is not affected.
Since Acsl4 participate in MAM/mitochondria association and given that protein phosphorylation is essential for this event, we consider that Acsl4 phosphorylation may play a role in this mechanism.

84.

ROLE OF LYSOPHOSPHATIDIC ACID IN THE REGULATION OF PROSTAGLANDIN PRODUCTION DURING IMPLANTATION

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Introduction: prostaglandins (PGs) and lysophosphatidic acid (LPA) are biologically active mediators in the implantation process. LPA3 receptor knockout mice, one of LPA receptors, have serious deficiencies in this process. In our laboratory we detected LPA3 receptor expression in the rat uterus. Also, LPA increased the expression of a marker of decidualization.
Objetive: to study LPA effect on PGs production during implantation.
Results: incubation of rat uterus from day 5 of gestation, before implantation, with 50 uM of LPA for 6 hours increased the expression of COX-2 mRNA and protein without affecting COX-1. Also, LPA treatment increased PGE2 production. This effect was mediated by LPA3 receptor and COX-2 isoform. LPA did not change PGF2alpha production.
Discussion: these results suggest that LPA could be a potent lipid mediator that promotes embryo implantation through the regulation of several mediators, including PGE2.

85.

STRUCTURAL AND FUNCTIONAL NETWORK OF THE KALLIKREIN-KININ AND THE RENIN-ANGIOTENSIN SYSTEMS

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The kallikrein-kinin and renin-angiotensin (KKS-RAS) represent two highly regulated proteolytic systems that participate in several physiological and pathological processes [1].
The multilayered interaction of the KKS-RAS was assessed at the structural and functional levels. A structural protein network was built-up on the 3D domain-domain interactions [2]. The essential domains that link these systems are: Cystatin, Peptidase_C1, Thyroglobulin_1, Insulin, CIMR, fn2, fn1, EGF, Trypsin, and Serpin. Unexpectedly, the CIMR domain was found at the core of the network, thus linking both systems. From the later, all domain interactors up to level 4 were retrieved.
In addition, a functional network based on various 'omics' tools was built-up. A level-1 signaling network of 104 proteins led to 1574 non-redundant protein-protein interactions where 72 significant complexes were identified.
In conclusion, we present an integrative and multilayered approach; thus providing a new framework to analyze complex biological systems.

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86.

HYPOXIA MAY REGULATE CFTR EXPRESSION IN HUMAN PLACENTA

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It has been proposed that intermittent placental perfusion, secondary to deficient trophoblast invasion of the endometrial arteries, leads to an ischemia-reperfusion [hypoxia-reoxygenation (H/R)] type insult in preeclamptic placentas (PE). Such variations in oxygenation can further alter the syncyciotrophoblast transport functions. We have previously reported that CFTR (cystic fibrosis transmembrane conductance regulator) is significantly reduced in PE.
Our aim is to identify the mechanisms implicated in the regulation of CFTR expression. We hypothesized that generated hypoxia-ischemia observed in preeclampsia may be responsible for the down-regulation in CFTR protein.
Explants from normal placenta were cultured in normoxia, hypoxia, and H/R. CFTR expression was analyzed by semiquantitative RT-PCR, Western blot, and immunofluorescence. We observed that CFTR expression decreased significantly in explants cultured under hypoxia conditions. However, H/R treatment did not restore CFTR expression. Immunofluorescence experiments also showed the same results.
Our findings provide new evidence suggesting that H/R may be the responsible for CFTR reduced expression in preeclampsia. Further studies are required to clarify the regulation of CFTR in human placenta.

87.

CHROMOGENIC AGAR ASSESSMENT FOR DETECTION OF TOTAL COLIFORM AND *Escherichia coli* IN FOODS AND WATER SAMPLES

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Detection and enumeration of coliforms and *Escherichia coli* is important in controlling water and food quality; Chromobrit® c.c. agar is used for determining these groups of bacteria. This agar contains chromogenic substrates to detect the enzimatic activity of β-galactosidase for coliforms and β-glucuronidase for *E. coli* within 24 h. Conventional methods are slow and laborious. In this study we evaluated the ability of Chromobrit® agar to differentiate coliform organisms from *E. coli*. We used a collection of 137 strains (21 *E. coli*, 53 total coliform and 63 non- coliform). Starting from cultures of strains in Tryptona Soya agar (24 h-37ºC), the growth of the colonies and their characteristics were observed. Each strain was inoculated by puncture in Chromobrit® agar and incubated at 35- 37ºC during 24 h. The 98, 6% of coliform strains were typical of the coliform group. Nine strains of *Aeromonas*, two of *Salmonella* and one of *Vibrio* were typical coliform colonies while one of *Klebsiella* had non-coliform development. Of 21 strains of *E. coli*, only one showed no β-glucuronidase activity (4, 8 %) while two strains of *Enterobacter agglomerans* showed enzymatic activity. Chromobrit® agar was efficient in detecting the difference in enzyme activity for coliform and *E. coli* in the collection of strains tested.

88.

EFFECT OF MEDIA AND STORAGE TIME ON ULTRASTRUCTURAL CHANGES IN FELINE EPIDIDYMIDES

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The aim of this study was to assess the ultrastructural changes of cat epididymides (n=28) stored at 4°C in two different media (saline solution [SAL] or tris-egg yolk [TEY]). Our hypothesis was that epididymides stored in TEY would have delayed epithelial cell autolysis. Four epididymides were fixed and process immediately, and the remaining 24 epididymides were stored at 4°C in SAL or TEY during 12, 24, 48 or 72 h. Chromatin distribution (CD), stereocilia morphology (EM), and mitochondrial number (MN) and area (MA) were examined in epididymides ultrathin sections with a transmission electron microscope at 30000 X. CD and EM changed differently with time and media ($P<0.01$). Conversely, MN an MA did not change with media or time ($P>0.05$). The morphological differences observed in epididymal cells could be related to differences in cellular preservation due to storage media. These results could support our previous findings where higher number of intact sperm cells were recovered from feline epididymides stored in TEY compared to SAL.

89.

INTRACELLULAR CALCIUM FLUCTUATIONS IN SPERMATOZOA EXPOSED TO A GRADIENT OF PICOMOLAR CONCENTRATION OF PROGESTERONE

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Capacitated spermatozoa respond to a gradient of pM Progesterone (P) by chemotactically orienting their movement or priming for the acrosome reaction (AR), sperm process that need intracellular calcium (iCa). The aim was to characterize iCa variations in spermatozoa exposed to a gradient of pM P. Spermtazoa were loaded with Fluo 4 and then stuck to a coverslip of a chemotaxis chamber where a pM gradient of P was generated. Variations in the iCa were determined in each spermatozoon by fluorescence videomicroscopy and image analysis. Results showed that the sperm population exposed to P slowly increased iCa. However, a subpopulation of cells (~10%) increased iCa at a higher extend than the mean value of the whole sperm population, augment that was simultaneously observed in different regions of the spermatozoon (acrosome, post-acrosome and midpiece). These preliminary results suggest that the gradient of pM P that stimulates chemotaxis and the priming of AR also stimulates variations in iCa in a subpopulation of capacitated spermatozoa.

90.

PARTICIPATION OF ZINC IN THE ASSOCIATION OF CRISP1 TO RAT SPERM DURING EPIDIDYMAL MATURATION

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Previous results from our laboratory indicated the participation of Zinc (Zn^{2+}) in the association of epididymal CRISP1 with the sperm surface during maturation. Based on this observation, the aim of the present work has been to investigate the molecular mechanism by which Zn^{2+} is involved in this process. Flow cytometry assays using biotinylated CRISP1 confirmed the requirement of the cation for the binding of the protein to epididymal caput sperm. Posterior localization studies by fluorescent microscopy revealed the association of CRISP1 with the flagellum of caput sperm and to both the head and tail of caudal cells. While the analysis of the tryptophan fluorescence spectrum did not show conformational changes in CRISP1 after its exposure to Zn^{2+}, native gel electrophoresis revealed the formation of high molecular complexes induced by the incubation of the protein with the cation. These complexes were also detected in epididymal luminal fluid and were absent when the samples were pretreated with EDTA. Altogether, these results support the participation of a molecular complex formed by CRISP1 and Zn^{2+} in the association of the protein with the sperm surface during epididymal maturation.

91.

INHIBITON OF ISOCITRATE DEHYDROGENASE ENZYME AFFECTS *IN VITRO* MATURATION OF PORCINE OOCYTES

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Maturation depends on the metabolic activity of the cumulus-oocyte complex (COC) that performs nutritive and regulatory functions during this process. However, there is little information about the metabolic profile of porcine COCs during *in vitro* maturation. Our aim was to determine the participation of isocitrate dehydrogenase enzyme in the *in vitro* maturation of porcine oocytes. COCs were recovered by aspiration of antral follicles (3-8mm) from ovaries of slaughtered gilts. *In vitro* maturation was performed during 48 h, 39ºC, 5% CO_2 in medium 199 supplemented with gonadotropins and different concentrations (0-30 mM) of oxalomalate (inhibitor of isocitrate dehydrogenase). *In vitro* fertilization was performed in mTBM with fresh semen during 18 h. Nuclear and cytoplasmic maturation were evaluated by the presence of metaphase II chromosome configuration or decondensed sperm heads and pronuclei, respectively. The percentages were analized by the Chi-square test. Nuclear maturation diminished with 20 and 30 mM of oxalomalate ($p<0.05$) and cytoplasmic maturation decreased from 10 mM ($p<0.05$). The inhibition of the key enzyme of the Krebs cycle affects negatively both nuclear and cytoplasmic *in vitro* maturation of porcine oocytes.

92.

ALTERATION IN INTRAHEMISFERIC THETA OSCILLATIONS ASSOCIATED WITH IDIOPATHIC GENERALISED EPILEPSY

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Quantitative electroencephalogram (QEEG) analysis techniques can provide additional measurements of electroencephalogram technique. The cross spectral power analysis (CSP) allow evaluate the common frequency of high power between two channels. Epilepsy presents as physiopathological substrate abnormal electrical activity of a group of neurons how has abnormal excitability membrane proprieties. It could be detected by CSP.

The purpose of this study is to analyze and compare the CSP in adults' healthy dogs and idiopathic generalized epilepsy dogs.

We worked with twenty four healthy adult dogs and twenty four idiopathic generalized epilepsy dogs, with electroencephalogram without transitory paroxystic events. The electroencephalographic record was obtained with a computer electroencephalographic and brain mapping software, with 12 simultaneous channels of registry. For restriction xylazina was used (1 mg/kg)

Spectral estimation was doing over selected segments of the base trace. The cross spectral power analysis was doing for intrahemispheric pair of electrodes (O-P), (O-F), (O-Fp), (O-T), (T-Fp) in both hemispheres. Statistical analyses design of parcels in space divided for **state** (healthy/epileptic) and channel variable was applied.

The analysis show difference between states (F: 15,429; p: < 0.001). The epileptic animals present a value of smaller frequency (χ 5.22 ± 0.35 Hz) than the healthy dogs (χ 7.02 ± 0.35 Hz) for CSP.

Results suggest the presence of a permanent alteration in the brain rhythms activity, respect to healthy dogs. The changes consist basically in a decrease in the frequency of CSP respect from a population witness.

93.

METABOLIC STUDIES IN RELATION TO CHRONOLOGICAL AGE OF A *DIPLODON CHILENSIS* POPULATION FROM NORTH-PATAGONIA

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Diplodon chilensis (Bivalvia, Hyriidae) is one of the most representative mollusks from North Patagonia. It s an excellent model for growth and cellular ageing studies due to its longevity and abundance. The aim of this project is to study the relationship between oxidative stress-antioxidant defense markers and chronological age in *D. chilensis*. Bivalve population from Nonthué Lake (Neuquén province) was characterized by the hepatosomatic index (HSI), morphometric and metabolic parameters (CAT, GST, SOD, GSH, MDA, glycogen and proteins), in relation to size and chronological age. The IHS increased with size, due to an increase in glycogen content. Antioxidant enzymes showed the highest activities for the sizes 5-5.5 cm and decreased at the largest sizes (6-8 cm). CAT and GSH antioxidants presented the most important decreases. Total protein amount decreased with size meanwhile MDA content increased until 5.5 cm and then remained constant until 6-8 cm. We can conclude that the decrease in CAT activity and GSH content in old individuals are closely related to cellular ageing process in this species.