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SOCIEDAD URUGUAYA DE BIOCIENCIAS

Abstracts

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The abstracts were evaluated by a scientific committee prior to publication

C1.

ALTERED O-GLYCOSYLATION IN CANCER. BIOLOGICAL IMPORTANCE AND CLINICAL APPLICATIONS

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Abnormal O-glycosylation is one of the most common changes in carcinomas, leading to the expression of short truncated O-glycan antigens (such as Tn, sialyl-Tn, and TF). These structures which are related with the malignant behavior are actively investigated as immunotherapeutic targets. The GalNAc-T family enzymes catalyze the initial step of mucin O-glycosylation and could be responsible for the altered glycosylation observed in cancer. We characterized the abnormal expression of GalNAc-Ts in different tumors. We demonstrated that GalNAc-T6 is expressed in breast cancer but not in normal breast epithelium. We found an association between GalNAc-T6 expression in bone marrow samples and poor clinical outcome in lymph node–negative breast cancer patients. We found the brain-specific isoform *GALNT9* (the gene coding the GalNAc-T9) to be mainly expressed in neuroblasts displaying low-aggressive behavior. In neuroblastoma patients *GALNT9* expression correlated with improved clinical outcome providing a valuable prognostic marker for personalized therapy. We also found that *GALNT13* was the most strongly up-regulated gene in metastatic malignant neuroblasts compared with primary tumor, and found that *GALNT13* expression in bone marrow at diagnosis was a strong predictor of poor clinical outcome. GalNAc-T13 is frequently expressed in non-small-cell lung cancer (NSCLC) and associates with worse prognosis in patients with adenocarcinoma who received neoadjuvant chemotherapy. Our data suggest that GalNAc-T13 is a novel marker associated to chemoresistance in NSCLC.

C2.

DR. BERNARDO HOUSSAY PRIZE CONFERENCE: CHARACTERIZATION OF PITUITARY TGF-β1 SYSTEM: IN THE SEARCH OF THERAPEUTIC TARGETS FOR RESISTANT PROLACTINOMAS

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Prolactinomas are benign adenomas usually treated with dopaminergic agonists. 15% are resistant and there are no alternative therapies. As TGF-β1 inhibits lactotrope proliferation, mediating dopamine (DA) inhibitory action, we characterized pituitary TGF-β1 system and its regulation by DA and estradiol (E2). The TGF-β1 system was down-regulated in our two experimental models of prolactinoma: female knock-out mice for dopamine D2 receptor and female rats chronically treated with E2. We found that DA and E2 regulated, not only the synthesis, but also pituitary TGF-β1 activation *in vivo*. We identified TSP1 and KLK1 as candidates involved in TGF-β1 activation induced by E2 and DA. Furthermore, we described, the latent TGF-β1 binding proteins expression in the pituitary, which are regulated by DA and E2, and differentially expressed in normal vs. tumoral pituitaries. Finally, we assayed the efficacy of an *in vivo* treatment with TSP1 mimetic peptides (ABT-510 and ABT-898) in prolactinomas. ABT treatment reduced prolactin serum levels and pituitary weight, effects mediated by their anti-angiogenic properties and the recovery of active TGF-β1 levels. We conclude that recovering active TGF-β1 would be an effective approach to inhibit tumor growth of dopamine agonist resistant prolactinomas.

S1- SIMPOSIUM OF YOUNG SCIENTISTS

S1-1.
GENERATION OF CORTICAL INTERNEURON DIVERSITY IN THE MOUSE

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Gamma-aminobutyric acid-containing (GABAergic) interneurons play major roles in the function of the cerebral cortex. Through mostly inhibitory mechanisms, interneurons regulate the activity of pyramidal cells, prevent hyperexcitability, and synchronize the rhythmic output of cortical activity. Our knowledge on interneuron specification has grown incredibly during the last ten years thanks to the invaluable use of transgenic mice lines. In addition, growing evidence suggest that disruption of interneuron function is common to several psychiatric conditions, such as schizophrenia. They show an extraordinary diversity and, thus, understanding cortical interneuron development seems crucial to shed light into their role in cortical processing, both in health and disease. They arise from the subpallial region of the telencephalon, the most rostral part of the brain, mainly from three defined structures: the medial ganglionic eminence, the caudal ganglionic eminence and the preoptic area. Interneuron diversity is accomplished by the combined expression of different transcription factors during development. So, time and space are key features of interneuron fate. The talk will be focused on the molecular mechanisms controlling the development of cortical interneurons in the mouse.

S1-2.
EXTRACELLULAR KINASE TRAFICKING INVOLVED IN SPERM CAPACITATION

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Mammalian sperm are not able to fertilize eggs immediately after ejaculation. They acquire fertilization capacity in the female tract in a process known as capacitation. Capacitation involves changes in the motility pattern and the preparation to undergo an agonist-stimulated acrosome reaction (**AR**). The capacitation process can be mimicked *in-vitro* by incubation of sperm in medium containing HCO_3^-, Ca^{2+} y BSA. Albumin is involved in the removal of cholesterol from the sperm plasma membrane, which in turn, facilitates Ca^{2+} influx. The increase of intracellular HCO_3^- and Ca^{2+} activate an atypical adenylyl ciclase, promoting intracellular cAMP rise with the consequent activation of PKA, a key player during capacitation. Downstream PKA activation, tyr-phosphorylation of sperm proteins, by still unknown kinases, is also observed and known as a hallmark of sperm capacitation. Our results indicate that the tyr kinase Src is present in mouse sperm. Moreover, this kinase is activated during capacitation. We have also shown that Src-KO mice are infertile, even though they produce morphologically normal sperm, pointing towards an important role of Src in sperm capacitation. In order to be capacitated, sperm must first accomplish epididymal maturation. During this poorly understood process, we show that sperm incorporate Src delivered through small vesicles named epididymosomes. Most importantly, once activated in sperm, Src appears to be involved in the acrosome reaction. This is the first report of extracellular kinase trafficking with activity in the target cell.

S1- 3.
COMPARED PHYISIOLOGY AND ANATOMY IN THE ELECTROGENESIS IN PULSE GYMNOTIDS

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The Electric Organ Discharge (EOD) of Gymnotiform fish is a fixed action pattern. The effector organ (Electric Organ, EO) is easily identifiable by classical methods of anatomy. The function of this organ is to generate the energy (carrier) of the signals that stimulates the self sensory system and the congeners. Further, the system output is a fixed electrogenic pattern, all or nothing, in which the variability of behavior intensity is suppressed to avoid ambiguity. This electrogenic pattern is unique for each species, the differences are the result of changes in OE structure and in mechanisms of its activation. We studied the similarities and differences in the OE and the DOE in 11 species of genus Gymnotus to determine how and where these differences are. The results indicate that the structure of the OE, studied with light microscopy, is relatively preserved, while small changes in the electrophysiological properties of EO and the central pattern generator controlling the DOE, are responsible for the main differences between species.

S2 – SYMPOSIUM OF THE SOCIETIES OF BIOLOGY OF ARGENTINA

S2-1.
PHENOTYPIC AND MOLECULAR DIVERSITY FOR TOMATO FRUIT MORPHOLOGY

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The tomato wild species produce tiny and round fruits in contrast to the enormous morphological diversity displayed by the cultivated tomatoes (*Solanum lycopersicum*). Two genes that control fruit elongation (*SUN* and *OVATE*) as well as others two genes controlling high locule number and flat shape fruits (*FASICIATED* or *FAS* and *LOCULE NUMBER* or *LC*) have been identified in tomato by positional cloning. The distribution of fruit shape alleles for these four loci was evaluated in a collection of 368 tomato accessions which it was diverse in origin, germplasm class, and fruit morphology. Fruits were visually classified into eight shape categories that were supported by objective measurements obtained from image analysis using the Tomato Analyzer software. These fruit shape categories were: round, long, flat, obovoid, rectangular, ellipsoid, heart and oxheart. The allele distribution in all accessions was strongly associated with fruit shape classification. For example, obovoid fruits carry the OVATE gene and long fruits have SUN and LC. The flat fruits carry LC and/ or FAS whereas the oxheart fruits are mainly explained by *LC*, *SUN* and/or *FAS.* Through a model-based clustering done with the four fruit shape genes and additional 25 markers distributed evenly across the genome we demonstrated that five groups are defined by shape categories, germplasm classes, and these four genes. Based on the results, hypotheses about where and when the fruit shape mutations arose during the domestication and improvement of the crop are discussed.

S2-2.
PROTEINS ASSOCIATED TO CASEIN MICELLES IN MILK OF SEVERAL SPECIES OF MAMMALS

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The structure of casein micelles is determined by the need to maintain these proteins in suspension in the milk medium. Caseins are phosphoproteins that in most of species studied represent the protein contribution from mother to offspring during lactation. These molecules, mainly β, α_{s1}, and α_{s2}-caseins are sufficiently hydrophobic, so they would precipitate in milk environment if there were no other molecules that keep them in suspension due to its anphipatic properties, such as κ-casein. Casein oligomers, formed by junctions between α_s-casein and κ-casein through disulfide bridges, are also important constituents of micelles. In addition, a number of proteins that we have called proteins associated to casein micelles (PAM) are part of these micelles. We could observe in several species of wild mammals that PAM represents a high percentage of micellar proteins. Some of these PAM have been identified in our laboratory and we found variations in their concentration along different stages of lactation and between different species studied. A group of these proteins would play a role in maintaining the solubility of casein micelles, and on the other hand, another group would fulfill specific functions in the neonate, especially as related to defense mechanisms against pathogens.

S2-3.
TEMPERATURE DEPENDENT SEX DETERMINATION AND GONADAL DIFFERENTIATION IN PEJERREY *Odontesthes bonariensis*

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The development of a functional gonad implies consequences not only for the phenotypic sex but also for the reproductive capacity during adulthood. In recent years, fish have been extensively studied because they present a variety of and sometimes contrasting mechanisms of sex determination/differentiation.
In the present work we review the sex determining mechanisms and the gonadal differentiation process in *Odontesthes bonariensis*, commonly known as pejerrey, in order to discuss morphological aspects of the gonadal differentiation process in males and females and its relation to water temperature, the expression profile of some selected genes involved in this process, and the involvement of cortisol and 11-ketotestosterone in the masculinizing process induced by warm temperature.
The process of sex determination/differentiation in *O. bonariensis* makes this species an emerging model to study the environmental factors that ultimately lead to determination of the gonadal fate.

S3- SYMPOSIUM OF THE SOCIETY OF BIOCIENCES OF URUGUAY

S3-1.
IRON UPTAKE REGULATION IN THE *Sinorhizobium meliloti* BACTERIUM: FROM THE GENOME TO FUNCTIONS, FROM FUNCTIONS TO THE GENOME

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Sinorhizobium meliloti is a bacterium whose major characteristic is the ability to establish a symbiotic association with alfalfa plants and fix atmospheric nitrogen. It belongs to the group of alpha-rhizobia and like virtually all living organisms needs iron to live. In the interactions between bacterial pathogens and hosts, the expression of high efficient systems for iron acquisition is essential for the pathogen survival. Mechanisms employed by symbiotic bacteria to acquire iron without causing damage to the host, are unknown. Taking into account that rhizobia during it symbiotic state, are located in plant cells where leghemoglobin is abundant, we evaluated its use as a source of nutritional iron. We found that indeed rhizobia are able to use iron present in haem compounds. Further, we identified an outer membrane protein (ShmR) present in *S. meliloti*, necessary for iron acquisition from haeme and leghaemoglobin. In a genetic screen for mutants that displayed aberrant control of *shmR* expression, we identified a small protein (HmuP) that positively affects *shmR* expression. Control of iron homeostasis has been studied in different bacterial genera and Fur has been described as the main regulator. However, our results showed that in *S.meliloti*, iron regulation differs substantially and Fur does not respond to iron and it perceives manganese cellular status. In this bacterium, the RirA protein is responsible to repress *shmR* expression under high iron levels. Our main challenge is to elucidate the mechanism of action of these two regulators.

S3-2.
STRUCTURAL BIOINFORMATICS APPLIED TO STUDIES USING CAROTENOIDS CONTENT IN NATURAL PRODUCTS

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Bioinformatics is defined as the development and implementation of systems and computational techniques for the analysis and management of biological data. Furthermore, the Structural Bioinformatics involves the application of molecular modeling techniques and others for determining the structure and functions of biomolecules. These strategies are a key part of those used in the discovery of bioactive compounds. Basic definitions and concepts on Structural Bioinformatics and it inclusion in the process of design of bioactive compounds will be provided. Two examples will be showed: a. carotenoid content in natural products, biomolecules involved in its metabolic action and possible carotenoid-biomolecule interactions and b. Design of new agonists of α4b2. nicotinic acetylcholine receptors

S3-3.
IONIC EVENTS DURING EPITHELIAL WOUND HEALING

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The major role of epithelial tissues is to constitute protective and diffusional barriers between body compartments. In this way, any epithelial discontinuity represents a hazard to the integrity of the organism and has to be rapidly overcome. This is achieved via a complex response involving dramatic modifications both at the cellular and tissue levels, the wound healing process. A fundamental problem of epithelial physiology is to identify the signals and mechanisms involved in the healing response. In this respect, diverse factors have been recognized to participate in the initiation and development of wound healing. Among them, the ionic changes underwent by the border cells would play a crucial role in this process. Thus, the fast calcium wave developed immediately after wounding activates the transcription of immediate early genes that would control the healing velocity. We have contributed to the study of the ionic events that take place during epithelial wound healing by reporting, for the first time, that the border cells increase the expression of the epithelial sodium channel (ENaC) which, in turn, determines ionic and electrical modifications. Also, we have shown that the inhibition of these phenomena significantly decreases the velocity of healing. The modulation of the ENaC-dependent ionic events may constitute a therapeutic strategy to modify the epithelial healing response in the course of diverse pathological conditions.

S4- SYMPOSIUM OF PLANT BIOTECHNOLOGY

S4-1.
PLANTS ADAPTAION TO ADVERSE ENVIRONMENTAL CHANGES. THE ROLE OF HD-ZIP I TRANSCRIPTION FACTORS

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Transcription factors belonging to the HD-Zip I subfamily bind the same DNA sequence and exhibit similar expression patterns in response to environmental stresses. However, mutant and overexpressor plants in these genes exhibit different phenotypic characteristics. The hypothesis stated is that the conserved motifs outside the HD-Zip determine the interaction with other proteins, interaction which in place is responsible for the functional diversity. The structure and function of different HD-Zip proteins from many species like sunflower, Arabidopsis, Medicago and *Nicotiana attenuata* was analyzed. Overexpressors and mutant plants were obtained presenting a distinctive combination of biotechnological beneficial traits. Among them, tolerance to drought, salinity, freezing, herbivory attack, waterlogging, flooding and high yield. The assays were initially performed in Arabidopsis and then in crops. The results obtained in the model system have been reproduced and validated in crops and in the field. Expression patterns and results from experiments performed in the culture chamber and in the field will be presented as well as the evidence indicating particular molecular and physiological mechanisms triggered by each gene. In conclusion, the conserved motifs outside the HD-Zip are responsible, at least in part, for the functional divergence of these transcription factors and the use of model plants was validated for these genes in crops and in the field.

S4-2.
THE PLANT HEAT SHOCK PROTEIN 90 (HSP90) AS *CARRIER-ADJUVANT* FOR VACCINE DEVELOPMENT

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The family of the heat shock protein 90 (Hsp90) participates in immunological processes. Also, some Hsp90s show immunomodulatory properties. We investigated the role of plant-derived Hsp90 as B-cell mitogens by measuring their proliferative responses. Recombinant plant Hsp90 (rpHsp90) was able to induce a prominent expansion of B cells. Splenocytes from C3H/HeJ mice, with a spontaneous mutation in the TLR4 gene (Tlr4Lps-d), responded poorly to rpHsp90 suggesting that the B-cell mitogenic properties of the protein are dependent of TLR4 pathway. These results show that plant Hsp90 provides a new example of a non-pathogen-derived ligand for TLRs. In order to investigate the capacity of rpHsp90 to induce an immune response against a reporter antigen, BALB/c mice were immunized with maltose binding protein (MBP) or the mixture of rpHsp90+MBP or MBP fused to rpHsp90 (MBP-rpHsp90). Immunization with the fusion MBP-rpHsp90 protein presented the highest titers of anti-MBP IgGt and anti-MBPIgG1/IgG2a, which persisted up to 84 days. The cellular response induced by the MBP-rNbHsp90 immunized mice had a Th1 profile with IFN-β production and the activation of CD8+ cells. Also, a strong and specific linfoproliferation of the splenocytes was observed in MBP-rpHsp90.3 immunized mice. In conclusion the administration of rpHsp90 covalently bonded to an antigen induces a strong and prolonged specific humoral and cellular response suggesting that rpHsp90 could be propose as *carrier-adjuvant* of antigens in the production of subunit vaccines.

S4-3.
POTENTIALITY OF THE NITRIC OXIDE (NO) IN PLANT BIOTECHNOLOGY PROGRAMS

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Nitric Oxide (NO) is a diatomic gas molecule with important functions in animal physiology. In the last 15 years, NO was shown to be a multifunctional regulator in plants too. No modulates the cellular responses to the perception of changes in the levels of plant hormones through precise molecular mechanisms. Thus, NO functions as a second messenger in relevant processes associated to plant growth, development and stress physiology. NO is essential for the control of root growth and architecture determination through the regulation of auxin actions. NO regulates the water vapour loss in plants through the modulation of ion channel activity in guard cells. This occurs through the NO-mediated regulation of stomatal aperture in an ABA-dependent pathway. We have also studied and characterized the gene and the enzyme NO synthase from the unicellular microalgae *Ostreococcus tauri* (OtNOS). We have started the study of the higher plant behaviour expressing *OtNOS*. The biotechnological impact of these findings will be discussed.

1.

AH-26 FIBER AREAS DISTRIBUTION IN COMPARTMENTS OF PIG BICEPS FEMORIS MUSCLE

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Since muscular architectural design is considered, it could be observed, due to the muscle task diversifications, different neuromuscular compartments with particular characteristics in their muscular fibers. The heterogeneousness found among neuromuscular compartments produces biochemical variations that influence in the transformation of the muscle into meat. The aim of this research has been to determine the fiber type percentages, the fiber type areas and fiber type relative areas into pig biceps femoris muscle, for applying in meat quality studies. Muscular samples were obtained from the core of each compartment (BR1, BR2, BR3 and BR4). Were identified by histochemistry the I, IIA, IIX and IIB fiber types and after that, were determined their relative areas. The results showed significant differences in fiber areas between compartments (BR1<BR4); the relative area values indicated an interaction of the fiber types and the region, only in BR4 ($p < 0.05$). The fibers percentages by regions showed that BR1 and BR3 are different to the other regions and different between them (Chi square). These results indicate the importance of relative areas as a more representative parameter than the fibers percentage for measuring the muscular fibers characteristics that influence on the transformation of muscle into meat.

2.

AH-30 RANKL AND OPG DETERMINATION IN FELINE DENTAL TISSUES

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The Nuclear Receptor Activator K Ligand (RANKL) and osteoprotegerin (OPG) are mediators involved in normal bone metabolism. The balance between them regulates the normal bone production and resorption. Recently, its determination via PCR and immunocytochemistry (ICQ) techniques allowed to correlate its concentration with pathologies as osteopenias, periodontal disease (PD) and cancer. Since the lack of studies about feline dental tissues the aim of this paper is to describe the development of the technique of ICQ for determination of RANKL and OPG in feline teeth (*felis silvestres catus*). We analyzed 42 sections from 6 teeth belonging to 8 cats. The teeth were fixed in buffered formalin, decalcified in 10% formic acid and processed according to standard technique (H / E). For ICQ polyclonal primary antibodies were used OPG (N-20) and RANKL (N-19) (Santa Cruz Biotechnology®), both goat IgG isotype, and rabbit anti-goat IgG as secondary antibody. Rat small intestine and bone marrow sections were used as positive controls for OPG and RANKL respectively. Both for OPG to RANKL, the reaction was positive in all cuts observed on dental cement, as well as periodontal ligament and alveolar bone. This study demonstrates that the technique of ICQ allows the detection of primary antibodies in feline dental tissues. We expect to relate the concentration of both molecules in tissues with nomal characteristics and pathological canges, particularly PD.

3.

BM-13 VIRTUAL SCREENING APPLIED TO SEARCHING COMPOUNDS WITH TRYPANOCIDAL ACTIVITY

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The progressive advances in biological knowledge about*Trypanosoma cruzi*, the causative agent of Chagas disease, have allowed the development of new approaches for the rational design of specific chemotherapies. A promising target for such purpose is cruzipain (cysteine protease cathepsin L-type), main proteinase of *T. cruzi*, active in all stages of the parasite. We have performed a virtual screening campaign to identify new cruzipain reversible inhibitors with trypanocidal activity. A discriminant function capable of differentiating inhibitors from non-inhibitors was generated and validated using the software Dragon 4.0 and 6.0 and Statistica 10 (StatSoft, 2011). Subsequently, it was applied in the screening of DrugBank database, which compiles 6516 Food and Drug Administraion (FDA) approved or investigational new drugs (IND). 63 candidates were identified as potential reversible inhibitors of cruzipain; seven of them were selected and its effect on proliferative capacity of *T. cruzi* epimastigotes and on cruzipain *in vitro* activity was evaluated. One of them, bromocriptine, traditionally used in the treatment of Parkinson's disease, had the highest effect, leaving a residual activity of 62% at 50um, with an IC50 = 100uM. The results confirm the value of virtual screening in the rational pursuit of second medical uses of known drugs.

4.

BM-15 ACYL-COA SYNTHETASE 4 (ACSL4): REGULATION OF ITS EXPRESSION BY NATURAL ANTISENSE TRANSCRIPTS

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Natural antisense transcripts (NATs) are endogenous RNAs complementary to mRNA, which may act on transcription, maturation, transport, stability and translation of its counterparts. Acsl4 is crucial in steroidogenesis as well as in pathologic processes such as tumorigenesis. This enzyme appears rapidly in response to the respective trophic hormone and seems to have a short half-life, however the mechanisms of transcription regulation have not been studied. We have identified and sequenced three NATs complementary to Acsl4 mRNA. The aim of this study was to evaluate the physiological role of these NATs in the mechanism of Acsl4 expression and steroidogenesis. For this purpose, three antisense transcripts (NAT-1, 1986 bp; NAT-2, 925 bp and NAT-3, 320 bp) complementary to different regions of Acsl4 mRNA, were validated by sequence specific RT-PCR. NAT-1 is noncoding and complementary to an Acsl4 mRNA alternative splicing variant. Its presence was confirmed in different mouse tissues. Based on these results we studied its hormonal regulation and the effect of its overexpression. hCG increases its expression reaching a maximun level after 5 h of stimulation (control 2.1 ± 0.2 vs 3.3 ± 0.3 hCG $p < 0.001$, arbitrary units). Acsl4 NAT-1 overexpression impacts differentially on Acsl4 transcripts levels, increasing mRNA variant 2 expression, protein synthesis and steroidogenesis. The results demonstrate the existence of Acsl4 NATs that participate in the regulation of ACSL4 mRNA expression and activity, influencing steroidogenesis regulation.

5.

BM-37 NEUROTROPHIN RECEPTORS EXPRESSION IN CAIMAN LATIROSTRIS THYMUS

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Neurotrophins (NTs) are growth factors that act in nervous system and also in lymphatic system of domestic animals. The objective of this work was to determine the location of high affinity receptors for NTs in thymus of *Caiman latirostris* compared with other domestic and wild animals (*Astianux fasciatus*, pigeon, broiler chicken, *Chaetoprhactus vellerosus*, pig, cow, horse, *Lama glama* and human. 14 thymuses were fixed in 10% buffered formalin and processed with histological routine technique, embedded in parafin. On serial sections an indirect immunocytochemical method (ABC) was applied using anti-TrkA, B and C, diluted 1:100. In the negative controls primary antibody incubation was omitted. TrkA expressed in type VI reticular epithelial cells as in *Astianux fasciatus*, broiler chicken, *Chaetophractus* sp, pig, cow, *Lama glama* and human. TrkB was identified in dendritic cells of the cortico-medullary region as in pigeon. TrkC was localized in type VI reticular epithelial cells as in *Astianux fasciatus* and *Chaetoprhactus* sp. As in other vertebrate species, NTs receptors were identified in the thymus cythoreticulum cells, suggesting a possible paracrine role of NGF (TrkA), BDNF (TrkB) and NT3 (TrkC) growth factors on the lymphocyte maturation.

6.

BM-42 MOLECULAR CHARACTERIZATION OF WILD CARROT SPECIES (*Daucus* SP.) FROM ARGENTINA

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Three wild carrot species -closely related to the cultivated carrot *Daucus carota* L. (2n=2x=18)- have been reported in Argentina: *D. montanus* Humb. et Bonpl. ex Schult (2n=6x=66), *D. pusillus* Michx. and *D. montevidensis* Link ex Sprengel (both with 2n=2x=22). According to various authors, the diploid species can be identified by some morphological traits and geographic distribution. Our group has postulated that these two species are synonymous. To obtain more information on the wild carrot germplasm for breeding purposes, 154 genotypes from 12 accessions -collected in sites for which populations of either one or the other "species" had been described- were molecularly characterized using three combinations of AFLP markers, and including one accession of *D. montanus* and one of *D. carota* as outgroups. Data was subjected to Cluster Analysis using the Simple Matching coefficient and Principal Component Analyses. Three distinct groups were formed: one with *D. carota*, the other with *D. montanus*, and the third with the remaining accessions, which exhibited high dissimilarity levels with the former (r=0,996161). These results give further support to the hypothesis that *D. montevidensis* and *D. pusillus* are taxonomic synonymous.

7.

BM-52 GnRH AND GnRHR PRODUCING CELLS IN PORCINE AND BOVINE OVARIES

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The local production of GnRH and GnRHR-I have been determined in ovaries of some species of mammalian. The object of this work was the identification of the cells that synthesized GnRH and GnRHR in porcine and bovine ovaries. 10 samples of ovaries from slaughterers, with or without corpus luteum, were fixed in 10% buffered formalin and conventionally histological processed. Immunocytochemistry indirect method (LSAB2® System, Dako) was applied on serial sections of 5 µm, using polyclonal rabbit anti-LHRH 1:2000 and monoclonal anti-hGnRHR 1:100 (Thermo Scientific) after hot antigen retrieval. Rat hypothalamus and hypophysis were employed as positive controls while the negative ones were omitting the primary antibodies. GnRH positive cells were scarce and isolated in both ovaries. They were localized in: internal theca of medium follicles (5-8 mm), granulosa cells of some atretic follicles, rete ovarii, interstitial interfollicular tissue and sometimes in medullar cells. GnRHR was different location in both species. Bovine ovaries: superficial epithelium, ovocytes of pre-antral and medium follicles, granulose cells of medium follicles, luteal cells. Porcine ovaries: ovocyte of pre-antral and small antral follicles (< 5 mm), scarce granulose cells in medium follicle. The cells which synthesized GnRH and GnRHR were different location in the ovaries of both species. The relationships between animal condition and expression of these molecules must be determined

8.

BT-17 EVALUATION OF THE ANGIOGENIC EFFECTS OF NOVEL BIOACTIVE GLASS-CERAMIC SCAFFOLDS

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The aim of present study was to evaluate the angiogenic potential of the ionic dissolution products (IDPs) from glass-ceramic scaffolds made from 45S5 Bioglass® containing B_2O_3 (2 wt%) (labeled 45S5.2B). The angiogenic response was evaluated using the quail chorioallantoic membrane (CAM) as an *in-vivo* model of angiogenesis. Fertilized eggs of Japanese quail, were incubated *in ovo* at 37ºC, cracked at embryonic day 3 (E3) into 6-well plates, and cultured further *ex ovo* at 37ºC. The extract containing the IDPs was prepared by soaking 1% w/v 45S5.2B scaffolds in DPBS (pH 7) in an orbital shaker at 37ºC for 72 h. The elementary content of Ca, Si, B, P and Na in the extracts were determined by ICP analysis. At E7, 0.5 mL IDPs-conditioned DPBS solution was applied to the surface of each CAM. DPBS without IDPs or bFGF served as control. The embryos were incubated further at 37ºC. At 5d after treatment, IDPs-conditioned DPBS stimulated angiogenesis by increasing 74% the number of blood vessels branch points. This experimental study provides the first evidence that boron-containing IDPs induces a strong angiogenic response making 45S5.2B scaffolds attractive for the regeneration of highly vascularised tissues.

9.

BV-41 COMPARATIVE STUDY OF CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *Eucalyptus grandis* AND *Eucalyptus dunnii* KNOTWOOD

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Current total area of forest in Uruguay has increased significantly in the last two decades. The forested area reached 990000, principally *Eucalyptus* (70%), being *E. globulus* and *E. grandis* the predominant species. Recently *E.dunnii* becomes more relevant since their planted area has increased largely. Knots are considered defects in all structural wood uses, and produce problems in cellulose pulp production. The aim of this work is to analyse the chemical composition of the *E. grandis* and *E .dunnii* knotwood extractives and their antioxidant activity. The samples of 17 years old trees *E. grandis* and *E. dunnii* were obtained from Cerro Largo, Uruguay. The soxhlet extractions were performed in two steps, first using dichloromethane and next using methanol or acetone. Quantification of phytosterols and β-sitosterol over lipophilic extractives were carried out using TLC-scanner and gas chromatography. On the hydrophilic extractives were determined the total phenols by Folin-Ciocalteu, and the antioxidant activity using ABTS and DPPH. *E. grandis* knots lipophilic extract had higher content of total phytosterols and β-sitosterol. The amount of phenols was similar in all hydrophilic extracts. *Eucalyptus dunnii* hydrophilic extracts exhibited the highest antioxidant activity.

10.

BV-46 ESTABLISHMENT OF *IN VITRO* CULTURE FROM SEEDS *Pittosporum tobira Ait-Azarero*

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When it comes to growing plants, we generally refer to grow them in pots or grown in the fields, but at present the use of *in vitro* culture techniques to conserve genetic resources in the short, medium and long term. Generation of *in vitro* culture protocols of native or ornamental micropropagation or as a requirement for other biotechnological applications is very useful scientific, technological and business-seed germination in sterile medium is one possible way to obtain *in vitro* plants that will serve as a starting point (establishment phase) protocols for micropropagation, callus induction and cell suspension cultures. The objective of this work is to use the seeds of Pittosporum tobira *in vitro* culture and be able to develop and propagate it. We require test techniques to achieve the drupe scarifying of this plant, wich gave us better result was chemical scarification consisted of placing the seed in 98% sulfuric acid rinse in sterile water. Then we use the most nutrient MS, inositol, Ana, BAP, vitamins, saccharose and agar, sterile once, using laminar flow chamber. Where we introduce the seeds to that medium. At first exposed to a T° 25"C in darkness and then the growth chamber with a photoperiod of 16 hours light and 8 of darkness, after 3 weeks we observed the process of organogenesis seen. From this point the next step is to the multiplication of the species.

11.

EB-53 VITELLOGENIN INDUCTION IN IMMATURE *Caiman latirostris* FEMALES IN RESPONSE TO EARLY POSTNATAL EXPOSURE TO BISPHENOL A

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Caiman latirostris is an oviparous species widely distributed in South American aquatic ecosystems. Vitellogenin (Vtg) is a hepatic yolk precursor protein synthesized in response to estrogen. Vtg induction in males and immature females has been proposed as biomarker of exposure to environmental estrogens. Bisphenol A (BPA) is a xenoestrogen found in food, freshwater and sediments. The aim of this study was to assess the effects of early postnatal exposure to low doses of BPA on caiman vitellogenesis. Two injections (s.c), 7 days apart from each other, of E2 (1.4 and 0.014 ppm), BPA (140 and 1.4 ppm) or vehicle were administered to one month old female caimans. Liver and plasma samples were obtained 7 days after the last injection. Hepatic Vtg mRNA and protein were assessed by qPCR and IHC, respectively. Vtg plasma levels were measured by ELISA. In caimans treated with E2 1.4 ppm increased Vtg mRNA, hepatic Vtg granules, and Vtg plasma levels were detected. Circulating Vtg was also detected in females exposed to the low dose of BPA. Plasma Vtg detection in immature caiman females might become a useful non-invasive tool to monitor environmental estrogen exposure.

12.

EM-54 KISS1 IN $GABA_{B1}$RKO FEMALE REPRODUCTIVE DISRUPTION

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Kisspeptin, encoded by *Kiss1* and synthesized in the AVPV and ARC hypothalamic nuclei, stimulates GnRH neurons. GABA, through $GABA_A$ and $GABA_B$ receptors ($GABA_B$Rs), also regulates reproduction. Adult female $GABA_{B1}$RKO mice have altered GnRH levels and pulsatility, and compromised fertility. However, the interaction between $GABA_B$Rs and *Kiss1* neurons (Kiss1n) is unknown. We studied Kiss1 participation in the reproductive impairments of $GABA_{B1}$RKOs and the role of sexual steroids therein. We assessed in WT and $GABA_{B1}$RKO adults the $GABA_{B1}$R co-localization in AVPV *Kiss1*n (double-label *in situ* hybridization (ISH), only females), *Kiss1* mRNA in AVPV and ARC (ISH and qPCR), estrogen receptor alpha (ER) mRNA in anterior hypothalamus (AH), including AVPV (qPCR), and estradiol and testosterone serum and gonad contents (RIA). Nearly all *Kiss1* neurons (~98%) co-express $GABA_{B1}$R in the AVPV. *Kiss1* mRNA, by ISH and qPCR, was similar between genotypes in the AVPV and ARC. Sex steroids were similar between genotypes. However, ERα in AH was higher in $GABA_{B1}$RKOs than in WTs. Thus, reproductive disruption in $GABA_{B1}$RKO females is not due to abnormal *Kiss1* expression in AVPV or ARC. However, this phenotype might be influenced by increased sensitivity to estradiol in AH.

(CONICET, UBA, ANPCYT, ISN-CAEN)

13.

EM-55 PROLIFERATION AND GENE EXPRESSION IN ISLETS FROM NEWBORN AND ADULT GABABKO MICE

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We have previously used GABAB knock-out mice (KO) to demonstrate that GABAB receptors participate in the endocrine pancreas function. Here we evaluated cellular proliferation and gene expression in KO Langerhans islets, aiming to better understand the role of said receptors in endocrine pancreas physiology and development. We analyzed expression of the following genes: *Ins1, Ins2, Gcg, Sst, Ppy, Nes, Pdx1*, via qPCR, and cellular proliferation with staining for PCNA, insulin and glucagon in newborn (4 days old) and adult (3-4 month old) mice, both KO and wild-type (WT) and both genders. Newborn KO mice showed a significant decrease in *Ins2*, *Sst* and *Ppy* expression (Two-way ANOVA; $p<0.05$), without differences found for *Nes* or *Pdx1*. In contrast, adult KO females had an increased expression of *Ins1, Ins2* and *Ppy* genes (Two-way ANOVA; interaction: $p<0.05$; $p<0.05$) vs their WT litter-mates. *Pdx1* had a higher expression in adult KO mice of both genders ($p<0.04$). On the other hand, we found that newborn KO mice had diminished proliferation of both alpha and beta cells in endocrine clusters, in contrast to the increased proliferation found in adult KO females. No differences where found in the proliferation indexes of the islet population between all genders and genotypes. We conclude that the absence of functional GABAB receptors in KO mice alters cellular proliferation and gene expression of critical islet genes.

(CONICET, UBA, ANPCYT)

14.

EM-56 SEXUAL DIMORPHISM IN THE TELEOST FISH *Cichlasoma dimerus* SOMATIC GROWTH

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In all vertebrates, growth is regulated by the GH/IGF-I axis. Growth hormone is synthesized and released from the pituitary gland under hypothalamic regulation. In many teleost fish, there is a sexually dimorphic pattern in body growth. In *Cichlasoma dimerus*, we have observed that males grow faster and are usually larger than females when placed in communitary tanks with three pairs but not four pairs or in individual tanks. In this context, the objective of this study was to analyze if this differential growth depends on the differences in food intake. Twenty-four fish (three pairs per tank) were kept for two months in a common tank and the reproductive behavior was analyzed. At the end of the experiment body weight and total length were measured and both were significantly higher in males compared with females ($P < 0,05$). Then, we measured by RT-PCR the mRNA expression levels of IGF-I in males and females. We also analyzed the hepatosomatic index (as an indirect indicator of metabolic status) and the levels of CRH and orexin (known to be anorexigenic and orexigenic peptides respectively). Surprisingly, none of these parameters showed significant differences. The role of sex hormones and the interaction with the growth axis on somatic growth will be analyzed in further experiments.

15.

FA-30 LOW SALINITY EFFECT ON ALKALINE PHOSPHATASES IN PRE/POST-INGESTA HEPATOPANCREAS OF *Neohelice granulata*

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Integrative studies on digestive adjustments at the biochemical level upon hyper-regulation conditions in euryhaline crabs are lacking. The aim was to determine the pre and post-ingesta effect of low salinity (S) on levamisole-insensitive (LI) and levamisole-sensitive (LS) alkaline phosphatase activities (AP) in hepatopancreas (HP) of *N. granulata*. Adult male crabs (Mar Chiquita lagoon, Bs. As. province) maintained for 14 days (feeding regime: 3 times a week) at 35 (osmoregulation) or 10 (hyper-regulation) ‰ S were unfed for 5 days (pre-ingesta=PrI) and then fed. AP were determined at PrI, and at short (2h=t2, 4h=t4) and long-term (12, 23, 46h) post-ingesta (PoI). Supernatant of 10000xg 15min from HP homogenate (0.1MTris-HCl pH 7.4) (4 ml bufferxg tissue^{-1}) was used. LI and LS (µmol pNPxmin^{-1}xmg prot^{-1}) were measured by pNPP hydrolysis (9.5mM) in 0.1M Tris-HCl, 4mM $MgSO_4$ (pH 8.5) with (LI) and without 16mM levamisole. LS: difference between both assays. LI and LS at PrI were higher at 10 (LI=682, LS=210) than in 35 (LI=31, LS=4) ‰S (n=5, ANOVA, $p<0.05$). At short-term PoI, AP were similar to PrI in 35 ‰S, but they were lower in 10‰S (LI: t2=39, t4=43; LS: t2=19, t4=4). At long-term PoI, AP were similar to PrI. The responses of AP in 10‰S suggest its role in digestive adjustments upon hyper-regulation.

16.

FA-31 EFFECT OF AN ABRUPT CHANGE OF SALINITY ON CARBOHYDRASE ACTIVITIES IN THE HEPATOPANCREAS OF CRAB *Neohelice granulata*

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Studies about possible digestive adjustments associated to salinity change in euryhaline crabs are lacking. The aim was to study the effect of an abrupt salinity change on amylase (Amy), maltase (Mal) and sucrase (Sac) activities in hepatopancreas. Male crabs maintained for 10 days in 37‰ salinity (S) (hypo-regulation) were transferred (To) to 10‰S (hyper-regulation). Amy, Mal and Sac were determined at To and at 2 (T1), 4 (T2) and 24 (T3) h after transfer. Controls: Crabs maintained in 37‰S. The supernatant (10000xg 15min) from a hepatopancreas homogenate (0.1M Tris-HCl, pH 7.4) (4ml buffer x g of tissue^{-1}) was used. Amy (µg maltose x min^{-1} x mg protein^{-1}) was assayed by hydrolysis of starch in 50mM phosphate 30ºC and Suc and Mal (µg glucosa x mg prot^{-1} x min^{-1}) by hydrolysis of the corresponding substrate in 0.1 M maleate/OHNa 30ºC. At T1 and T2, Amy (7885± 1442; 9255 ± 1373), Mal (1187±34; 957±177) and Suc (55±15; 43± 3) were similar to To. At T3, Amy (3174±1476), Mal (310±119) and Suc (18±9) were lower than activities at To (n= 5; Anova, $p<0,05$). No differences occurred in control. The modulation of Amy, Mal and Sac in hepatopancreas upon an abrupt salinity change suggests that differential adjustments in digestive capacity could be occurring in relation to changes in osmoregulatory status.

17.

FA-32 *Neohelice granulate* FROM DIFFERENTS HABITATS: ALKALINE PHOSPHATASES AND LIPASE UPON DISTINCT LIPIDIS DIET

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Studies on digestive adjustments of *N. granulata* in relation to contrasting mudflat (MF) and saltmarsh (SM) are lacking. The aim was to study the effect of diets with different lipid ratio on levamisole sensitive (LS) and insensitive (LI) alkaline phosphatases (APs) and lipase (Lip) activities in hepatopancreas of individuals from MF and SM. Adult osmoconforming male crabs were fed with diets with 3.5(D1) or 5% (D2) of lipid. 10 and 30 (t30) days postingesta, APs and Lip were measured in supernatant of 10000xg15min from homogenate (0.1MTris-HCl pH 7.4). Control=values in the field (tF). APs (µmol pNPxmin^{-1}x$mgprot^{-1}$) and Lip (µmol pNPx min^{-1}x $mgprot^{-1}$) were assayed by hydrolysis of pNPP (9.5mM) (0.1M Tris-HCl,pH 8.5/4mM $MgSO_4$ with (LI) and without 16mM Levamisole (LS=difference) or pNPpalmitate (50mM Tris/HCl, pH 8.5), respectively. Only D2 at t30 had effects compared to TF: MF= LS(50±19) was 25 fold higher. LI and Lip were similar. SM:Lip was 1.7 fold lower (71.7±7) (ANOVA, $p<0.05$). LI ans LS did not change. The differential responses suggest the existence of distinct digestive adjustments in relation to habitat characterisitics.

18.

FA-35 PULMONARY LESSIONS IN CADMIUM-INTOXICATED PREGNANT RATS

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This study examined the effects of Cd, a potentially toxic metal, in the lungs of rats at different stages of gestation. Groups of 6 Wistar rats were injected subcutaneously with $CdCl_2$ (10 mg Cd^{+2}/kg body wt) on days 4 (G1), 7 (G2), 10 (G3), and 15 (G4) of pregnancy. G5 dams received an equal volume of saline. Females were sacrificed on day 20 of gestation. Samples of lungs were obtained at necropsy and processed for histopathology or for the determination of Cd concentration by atomic absorption spectrophotometry. Stereological were applied to quantify the alveolar surface. Histopathological examination revealed emphysema and atelectasis in the majority of Cd-treated dams. Cd concentration was higher in all groups than in controls ($p<0.05$). The alveolar surface was significantly smaller in G1, G2, and G3 animals than in controls ($p<0.01$). It was confirmed that Cd is deposited in the lung, causing morphological alterations in the alveoli.

19.

FT-18 HCB AS AN INDUCER OF APOPTOSIS IN AN EXPERIMENTAL ANIMAL MODEL

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Hexachlorobenzene (HCB) is known for its action as a reproductive and endocrine disruptor, its neuro and hepatotoxic properties, and its carcinogenic action. Furthermore, it is known that its toxicity in humans is associated with the production of porphyria. In this study we evaluated the action of HCB in the generation of apoptosis, for which three parameters indicators of apoptosis were evaluated: caspase-3 (CASP3), protease that plays an important role in the execution phase of apoptosis, using as substrate Ac-DEVD-pNA; levels of cardiolipin (CL) in mitochondrial membranes, using orange 10-nonyl-N-acridine, and JNK through the use of a commercial kit. In addition, the generation of porphyria was evaluated through biochemical parameters. During 5 consecutive days an HCB solution (100 mg / kg) dissolved in oil (10 ml / kg) was administered by gavage to female Wistar rats. The rats were euthanized, after fasted on the 2nd, 4th, 6th, 9th and 12th weeks after poisoning. The results showed that as the weeks went by, there was an increase in the activity of CASP3 (12th week: HCB group (T) vs. Controls (C): 1.49, ± 0, 35 vs. 0.28 ± 0.04 µmoles p-NA/mg protein). The relative levels of mitochondrial membrane CL decreased with time, indicating its damage. Furthermore, from time after intoxication, there was an increase in the concentration of JNK. These results suggest that in this model apoptosis is produced. In addition, it also showed that the animals had a biochemically expressed porphyria.

20.

FT-20 ACTIVITY GLUTATHIONE S-TRANSFERASE S-TRANSFERASE IN HELMINTHS EXPOSED ALBENDAZOLE

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The worms are a major medical problem in both pet health and humans being the key tool to combat chemical control. The Ascariasis Fasciolosis and are a major cause of losses in livestock production and its control is mainly based on the use of albendazole (ABZ). All helminths have different biochemical mechanisms for detoxification. This study aimed to evaluate a model "*ex vivo*" effect of ABZ on the activity of the enzyme detoxificativa Phase II, glutathione S-transferase (GST) in Fasciola hepatica and Ascaris suum. Ascaris five flukes and 5 were placed immediately collected with varying concentrations of ABZ in PBS medium at 37ºC. After 4 h, were processed to obtain high speed supernatant evaluated GST activity in the cytosol. This activity was inhibited in a concentration dependent manner. So, 10 nmolesde ABZ caused a decrease in GST activity in Fasciola 40% in Ascaris 25% and 20 nmol of ABZ in decreased 64 in Fasciola and Ascaris 40%. These preliminary results contribute to the understanding of the response in different helminths detoxificativa and understanding ABZ antiparasitic effect.

21.

MI-21 POLYMORPHISM STUDY OF *Streptococcus uberis pauA* GENE -ISOLATED FROM BOVINE MASTITIS

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The genomic variability of *S.uberis* has hindered the development of a vaccine to prevent mastitis by this pathogen. The plasminogen activator (PauA) is involved in the early stages of infection and has been proposed as a candidate vaccine component. However, its genetic variability has not been studied. The aims of this work were: i) to survey the presence of the *pauA* gene, ii) to assess its polymorphism and iii) to study the relationship among the polymorphism, the clones identified by pulsed field gel electrophoresis (PFGE) and the geographical region. 153 isolates of *S. Uberis* from major dairy regions of Argentina were confirmed molecularly by RFLP. Clonality was analyzed by PFGE. The *pauA* gene was analyzed by PCR and the sequences from 20 selected isolates from different geographical regions and pulso types were studied. Sequences were analyzed using the Sequencher, Code Codon Aligner and Blast software. The PFGE identified more than 20 clones with different subtypes among the isolates of *S. uberis*. The presence of *pauA* gene was detected in 100%of them. Moreover, the sequence analysis revealed more than 90% of identity among the isolates and with the available sequences from reference strains in Gen Bank. The variability of the strains together with the identity found for the *pauA* gene make this virulence factor a good candidate for use as a vaccine component.

22.

NQ-22 ONTOGENIC EXPRESSION OF LIVER X RECEPTORS IN FEMALE BRAIN OFFSPRING OF DIABETIC RATS

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Gestational diabetes (GD) produces a diabetogenic effect in the progeny. Liver X receptors (LXRs) are considered as potential targets to treat and prevent diabetes mellitus. Previously we presented the early and late changes of LXRs in hippocampus (HC) and hypothalamus (HT) of the male offspring of diabetic mothers (OD). The aim of this study was to evaluate the changes in the female offspring. We used an experimental model of streptozotocin-induced DG, evaluating the expression of LXR-α and LXR-β by Western blot. Tissues were obtained from female OD and controls (OC) at 1, 10 and 35 days and 11 months old. A decrease in expression LXR- α along ontogeny was observed in HC in both groups ($p < 0.05$). In contrast, LXR-β expression increased along ontogeny with the highest expression observed at 11 months ($p < 0.05$). In HT a slightly variation LXR-α was found with a peak at 35 days of age while LXR-β, behaved like in HC ($p < 0.005$). Differences were observed between OC and OD only at 1 day: LXR-β was increased in the HC while LXR-α was increased in the HT of OD. The regionally differences occurring in female OD are different from those observed previously in male OD, suggesting that females are more resistant to metabolic alteration related to DG.
(PIP860-CONICETy 06CM09-UCCuyo).

23.

NQ-23 ALTERATIONS OF LXR-α AND LXR-β EXPRESSION IN THE HYPOTHALAMUS OF GLUCOSE-INTOLERANT RATS

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Despite the growing importance of LXR in SNC little is known about their localization and functionality. Here we studied the expression of LXR-a and LXR-b in different brain areas and we compared it to an animal model that develops glucose intolerance. A groupof rats receivedonly 10% (w /v) fructose as beverage (GF) while the control group received only water (GC). After 6 weeks the GF presented hypertriglyceridemia, hyperinsulinemia and became glucose intolerant, suggesting a progression towards type II diabetes. In the hypothalamus GF presented a decreased LXR-b expression and an increased LXR-a expression compared to GC while no changes were observed in hippocampus, or cortex. Hypothalamic levels of both receptors correlated negatively with serum insulin and triglycerides. Furthermore, the levels of LXR-b expression negatively correlated with the area under the curve of the glucose tolerance test (AUC) in GC. Conversely, the AUC and LXR-b correlation was positive in GF. Immunocytochemistry revealed that the paraventricular and ventromedial nuclei express mainly LXR-a whereas the arcuate nucleus expresses LXR-b. This is the first study showing a relationship between carbohydrate and lipid homeostasis and LXRs expression in the hypothalamus, suggesting that LXR might modulate neuronal responses related to the control of food intake and energy expenditure.
(PIP860-CONICET).

24.

NQ-69 MICROTUBULE ASSOCIATED PROTEIN (MAP2) AND NEUROFILAMENTS (NFs) CHANGES IN RAT CEREBRAL CORTEX AND HIPPOCAMPUS CULTURES UNDER HYPOXIA

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Previous studies *in vivo* have revealed that hypoxia induced long term neuronal changes. In this work, the effect of an *in vitro* hypoxia on the integrity of the MAP2(b,c) and NFs(200,160) expression in rat cerebral cortex (CC) and hippocampus (HC) was evaluated. Steroid treatments of allopregnanolone (**A**), pregnanolona (**P**) and their synthetic analogues SB11-1 and Ns1 on the changes observed by hypoxia were evaluated. One hour hypoxia significantly decreased cortical MAP2 (b:14%,$p<0.05$; c:13%,$p<0.05$), NFs (200:17%,$p<0.05$; 160:35%,$p<0.05$) and hippocampal MAP2 (b:15%,$p<0.05$; c:12%,$p<0.05$) and NFs (200:22%,$p<0.05$; 160:21.%,$p<0.05$) levels. The steroid treatment (5μM) 24 h prior and during the hypoxia resulted in attenuated MAP2 CC, **A** slightly attenuated MAP2 and NF200 decreased, while others only attenuated MAP2c and Ns1 only NF160 decreased ($p<0.05$). In HC, SB11-1 and Ns1 attenuated the MAP2b decreased, but the others steroids only prevented MAP2c decreased ($p<0.05$). **A** and Ns1 attenuated NFs decreased and **P** only prevented NF160 decreased ($p<0.05$). **A** and Ns1 were the most effective on axonal damage prevention triggered by hypoxia.
(CONICET- PIP860).

25.

NQ-70 SYNTHETIC STEROIDS ACTIVITY ON $GABA_A$ RECEPTOR IN CORONAL RAT BRAIN SLICES

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Previously we evaluated the behavior of synthetic steroids, allopregnanolone (**A**) and pregnanolona (**P**) analogues, like SB11-1, MVD100 and Ns1, in cerebral cortex (CC) and cerebellum rat's synaptosomes, with $GABA_A$ receptor specific ligands (TBPS (TBPS) and flunitrazepam (FLU)). In this work we studied those behaviors in coronal brain slices by autoradiography. We evaluated the ^{35}S-TBPS (10nM) binding displacement and ^{3}H-FLU (10nM) binding stimulation by the various steroids (25nM) addition. Incubations were made at 25ºC by 60 min to TBPS and 4ºC by 90 min to FLU. Picrotoxin (1mM) and diazepam (5µM) were used for the non specific binding determination respectively. Exposure times were 10 and 15 days. Optical densities were measured in CC and hippocampus (HC) and in subareas of these structures. In CC, all steroids reduced TBPS binding and stimulated FLU binding significantly ($p<0.05$). In HC, the same CC behaviors were observed, but SB11-1 and MVD100 had significant differences with **A** ($p<0.05$). Ns1 could be the synthetic steroid with a behavior more closely to **A** and **P**.
(CONICET- PIP860).

26.

NQ-71 SOX2 AND WNT IN OLFACTORY NEURAL STEM CELL PROLIFERATION AND DIFFERENTIATION

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The vertebrate olfactory epithelium (OE) has been known for its remarkable characteristics of actively generating olfactory receptor neurons throughout lifespan of the animal. The OE of larval *Xenopus laevis* shows all the characteristics of an adult vertebrate with a robust regenerative capacity, offering an ideal system for the study of Neural Stem Cells (NSC) in adults and its differentiation in a neuronal lineage. Identity and characteristics of NSC in the OE are still unknown. Previous studies suggest that Wnt signaling is necessary for Sox2 expression, which in turn activates proneural gene expression in the *Xenopus* retina, and block neuronal differentiation through Notch activation. In the present study, we described a significant increase in the number of proliferative basal cells and the number of basal cells expressing Sox2, during regeneration after the extensive damage of the OE by ZnSO4 treatment. Furthermore, we found that Wnt/β-catenin signaling pathway is activated in basal cells in normal condition, while during regeneration Wnt promotes Sox2 expression in basal cells but not through the canonical pathway. These results suggest an important role of Sox2 in the undifferentiated proliferative cells, possibly involve in the maintenance of neural-progenitor cell properties, and Sox2 expression would be regulated by Wnt.

27.

OC-24 ADRENERGIC EFFECTS ON HUMAN BREAST CELL PROLIFERATION, ADHESION AND MIGRATION

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Beta-adrenergic receptors (B-AR) were described in tumor and non tumor breast cell lines. The aim of this work was to study the effect of the endogenous catecholamine epinephrine (Epi), the Beta-agonist isoproterenol (Iso) and the alpha2-agonist (Dex) on cell proliferation (measured by thymidine incorporation and cell count), cell adhesion and migration (transwell assays) on non tumor human breast cell lines MCF-10A and HBL-100 and MCF-7 and MDA-MB-231 tumor cell lines. Epi decreased cell proliferation, increased cell adhesion and reduced migration on non tumor cells. Similar results were obtained when the cells were incubated with Iso, suggesting the role of B-AR as a mediator of stress response in non tumor cells. In MCF-7, Epi increased cell proliferation and migration. Similar results were observed with Dex. Adhesion on tumor cells was not modified by either Epi or Dex. MCF-10A and MCF-7 cells were transfected in order to overexpress the B-AR or knock-down the receptor expression using siRNAs. In both cell lines, receptor over-expression caused a decrease in cell proliferation and an increase of basal cell adhesion. Knock-down of B-RA caused an augment on cell proliferation and a decrease on basal cell adhesion. These results describe the role of AR as mediators of stress response in human tumor and non tumor breast cells.

28.

PV-38 NUTRITIONAL SUPPLEMENTATION FOR GOATS WITH SORGHUM SILO

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One of the limiting factors of the goat farmers in the Taco Ralo area (Tucumán) arethe availability and quality of forages during dry season (winter-spring). The forages of the scrubland are insufficient to cover the nutrients requirements.The objective wasgenerated information ofthe supplementation with sorghum silo in order to improve the nutritionof the goats´flocks of smallholder producers: Atamisqui (*Caparis_atamisquea*), Chañar (*Geoggroeadecorticans*), Mistol (*Ziziphus_mistol*), Algarrobo (*Prosopispallida*) and sorghum silo were analyzed Crude proteins (% CP) by AOAC, neutral detergent fiber (% NDF) and acid detergent fiber (% ADF) by Van Soest and % of digestibility calculated (% D). August: Atamisqui: %CPx= 5,62±1,9; %NDFx = 33, 5±0,5; %ADFx = 18, 34±0,78; %D=74, 0; Sorghum silo: %CPx = 5,82±0,63; NDFx =28,24 ±2,07; ADFx =18,22±0,62; %D=74,0. December: Mistol: %CPx= 4,49±0,13; NDFx =13,32±0,39; ADFx = 14,08±0,5; %D=78,4; Chañar: CPx % =3,62±0,11; NDFx=27,47±0,54; ADFx= 18,33±0,09; %D=74,63; Algarrobo: CPx % = 3,62±0,39; NDFx= 19,67±0,4; ADFx =14,1±0,3; %D = 77,92; Sorghumsilo: %CPx= 4,76±0,5; NDFx= 24,71±0,13; ADFx=16,41±0,05, %D = 76,14. The improvement of the protein and energy contribution given by the sorghum contributed the rumen' substrate in order to increase the syntheses of the microbial proteins.

29.

RE-04 MITOCHONDRIAL AND OXIDATIVE ACTIVITIES IN PORCINE OOCYTES MATURED *IN VITRO* WITH PENTOSE PHOSPHATE PATHWAY MODULATORS

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We have previously observed the inhibition of the pentose phosphate pathway (PPP) in porcine oocytes by 6-aminonicotinamide (6-AN) and NADPH, but NADP showed no effect. Our aim was to determine the influence of PPP modulation on mitochondrial activity (MA) and oxidative activity (OA) of porcine oocytes during *in vitro* maturation (IVM). Cumulus- oocyte complexes (COCs) were recovered by aspiration of antral follicles (3-8mm) from ovaries of slaughtered gilts. IVM was carried out during 48 h at 39ºC in medium 199 with gonadotropins (control) + 12,5 mM NADP (PPP stimulator), 0,125 mM NADPH or 0,025 mM 6-AN (PPP inhibitors). MA and OA were determined at times associated with maturational events (0, 24, 32, 40 y 44 h) by fluorescent stains of Mito Tracker Green FM and RedoxSensor Red CC-1, respectively. Data were analysed by ANOVA. In control group, MA and OA decreased until 32 h of IVM and then increased again ($P<0.05$). MA and OA lowered in the presence of 6-AN, NADPH or NADP ($P<0.05$). MA and OA would decrease until metaphase I and augment again in 1º polar body extrusion and metaphase II to reach the level observed in germinal vesicle breakdown. PPP inhibition would affect MA and OA in porcine oocytes, but NADP could influence them without modification of PPP.

30.

RE-05 NOTCH AND PROGESTERONE REGULATE THE CORPUS LUTEUM FUNCTION OF SUPEROVULATED RATS

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The Notch signaling pathway regulates cell fate decisions, including proliferation, differentiation and apoptosis. We have previously shown that blocking ovarian Notch system with the inhibitor DAPT decreases progesterone (P4) serum levels and increases pro-apoptotic protein expression in the CL. Our hypothesis is that P4 plays a key role in regulating the Notch pathway and CL function of the superovulated rats. In this study CL´s were isolated from ovaries and cultured for 4 hours in the presence of P4 (500ng/ml), aminoglutethimide (inhibitor of cytochrome P450scc 0.15 mM), DAPT (20 uM), and both inhibitors with the addition of P4 (500ng/ml). We determined P4 levels in the culture medium, and CL levels of PCNA and the phosphorylation of ERK and AKT by Western blot. The treatment of CL´s with each inhibitor significantly decreased P4 levels ($P <0.05$, $n = 4$). The culture with P4 significantly increased the protein levels of PCNA. This effect was reverted in the presence of DAPT. AKT phosphorylation significantly decreased with the addition of aminoglutethimide or/and DAPT, while ERK phosphorylation only decreased in the presence of aminoglutethimide. These results suggest the existence of a regulatory mechanism between progesterone and the Notch signaling pathway in the CL of superovulated rats.

31.

RE-06 PRESENCE OF FIBROBLAST GROWTH FACTOR RECEPTORS (FGFRs) IN HUMAN SPERM AND THEIR PARTICIPATION IN ACROSOMAL EXOCYTOSIS

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**equal contribution to the work.*

FGFRs are present in several cell types and their interaction with FGF2 activates numerous signaling pathways; however, there is no evidence of their expression and function in human sperm. The aim of the study was to evaluate the presence of FGFRs in human sperm and their participation in acrosomal exocytosis (AE). Western immunoblotting and immunocytochemistry were performed with anti FGFR1, 2, 3 and 4 (Santa Cruz Biotech). The effect of FGF2 on basal AE and progesterone (Prg)-induced AE was analyzed using *Pisum sativum* agglutinin-FITC. Protein forms of the expected molecular weight were detected in human sperm extracts, similar to those of MCF7 and T-47D cells (controls). The FGFRs were localized in the flagellum and the acrosomal region of non-capacitated and 22-h capacitated sperm. Sperm incubation with FGF2 (1, 10 or 100 ng/ml) for 22 h led to a significant inhibition ($P<0.01$) of Prg-induced AE, without affecting basal AE ($n=5$). The addition of FGF2 to capacitated sperm also inhibited Prg-induced AE, but it significantly increased the basal AE ($n=8$). These effects were blocked by a FGFR inhibitor (PD173074, 0.1 μM). In conclusion, FGFRs are present in human sperm and have a modulatory effect on the AE.

32.

RE-07 EFFECT OF ENDOMETRIOSIS (EDT) ON FOLLICLE DEVELOPMENT AND APOPTOSIS IN THE OVARY, IN A RAT MODEL

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Different mechanisms have been proposed to explain EDT associated infertility including alterations in follicular and peritoneal environment, in folliculogenesis and in granulosa cells function. The objective of this work was to evaluate the effect of EDT in follicle development and in apoptosis in the ovary. EDT was surgically induced in female Sprague Dawley rats. One month after surgery animals were sacrificed at proestrus. EDT-like lesions were removed, fixed, sectioned and stained to confirm the development of EDT. Both ovaries were excised. One was fixed, sectioned at 50 um intervals and stained, and follicles at different stages were counted. Total protein extracts from the other ovary were used to determine the expression of pro- and anti-apoptotic proteins by Western blot. The number of total ovarian structures was reduced in EDT animals compared to the Sham group ($p<0,01$). EDT females showed a reduced number of primordial, primary and preantral follicles ($p<0,05$) and a decrease in the percentage of primary follicles besides an increase in the percentage of atresic follicles ($p<0,05$). There were no changes in Bax and Bcl-2 expression between EDT and Sham ovaries, however the ratio Bcl-Xs/Bcl-Xl was increased in ovaries from EDT rats ($p<0,05$). These results suggest that EDT causes a reduction in ovarian reserve and an increase follicular atresia and Bcl-Xs/Bcl-Xl would be involved in this process.

33.

RE-08 RELEVANCE OF GALECTIN-1 FOR SPERM FERTILIZING ABILITY

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Galectin-1 (Gal-1) is an animal lectin that activates different cell signaling pathways by crosslinking surface glycoproteins. Based on our previous results showing the presence of Gal-1 in both mouse sperm and cumulus-oocyte complex (COC), the aim of this study was to investigate the participation of Gal-1 in the fertilization process. *In vitro* fertilization assays in which wild-type (WT) and knockout (KO) COCs were inseminated with epididymal sperm of both genotypes were conducted. Results showed significantly ($p<0.01$) lower percentages of fertilization for KO sperm regardless of the COCs genotypes. To analyze the mechanisms involved in this reduction of fertilizing capacity, WT and KO sperm were *in vitro* capacitated and protein tyrosine phosphorylation (Western blot), sperm motility and progesterone-induced acrosome reaction (AR) (Coomassie Blue staining) were evaluated. While no differences were detected in either protein tyrosine phospohorylation or sperm motility between the two genotypes, a higher ($p<0.02$) percentage of angulated tails was observed in KO sperm. This defect could be overcome by increasing the osmolarity of the media. While KO spermatozoa also exhibited lower ($p<0.01$) levels of progesterone-induced AR, previous exposure of the cells to exogenous Gal-1 reverted this effect. Together, these results suggest that Gal-1 would be involved in the regulation of sperm cellular volume and AR supporting the relevance of this protein for the fertilization process.

34.

RE-09 DIFFERENTIAL ACTIVITIES OF ISOENZYMES OF ISOCITRATE DEHIDROGENASE IN PORCINE SPERM

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The objective of this work was to determine the activity of the NAD and NADP dependent isoenzymes of isocitrate dehydrogenase (IDH) in porcine spermatozoa and to study its relation with sperm quality parameters. The activity of the isoenzimes of IDH was determined spectrophotometrically at 340 ηm, during 10 minutes, in sperm extracts. Enzime unit (U) was defined as the amount of IDH (NAD or NADP) that catalyzes the reduction of 1 µmol of NAD or NADP/min. Motility was evaluated by optic microscopy, viability by the eosin-nigrosin technique and acrosome integrity was evaluated by trypan blue combined with DIC. The results were expressed as mean ± SD and were analysed by ANOVA. The Bonferroni test was used as post-ANOVA. The activity of IDH (NAD) was 0,56±0,50 $U/10^{10}$ spermatozoa and the activity of (NADP) IDH was 8,43±3,89 $U/10^{10}$spermatozoa. In both cases, the activity did not show any correlation with any of the seminal parameters studied. In previous research, the boars were categorized according to their freezability. The activity of IDH (NAD) did not show significant differences between the animals. However, IDH (NADP) activity was significantly higher in the boars that showed "good" freezability and lower in the boars of "regular" or "bad" freezability, indicating that the activity of this enzyme could be a good predictor of the freezability of the porcine sperm.

35.

RE-10 COMPARED GLYCOLITIC AND PENTHOSE PHOSPHATE PATHWAY ACTIVITIES BETWEEN BOVINE AND PORCINE COCS

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The *in vitro* maturation (IVM) rate is higher in bovine (B) than in porcine (P) cumulus-oocyte complexes (COCs). These results could be partially explained by a differential metabolic activity involved in this process in COCs from both species. Glycolisis (G) and penthose phosphate pathway (PPP) activities were studied in B and P COCs during the IVM. The IVM was performed in medium 199 supplemented with gonadotropins. Glucose uptake and lactate production by COC (glycolitic activity) were evaluated by spectrophotometry. The percentage of COCs with high PPP activity was evaluated by brilliant cresyl blue stain. The activity of the key enzymes of G and PPP, phosphofructokinase (PFK) and glucose 6-phosphate dehydrogenase (G6PDH) respectively, was determined in immature COCs. The glucose uptake was 1.52±0.07 and 0.62±0.1 nmol/h ($p<0.05$) and lactate production was 6.08±0.29 and 1.68±0.27 nmol/h ($p<0.05$) in B and P, respectively. The percentage of COCs with high activity of PPP was 95.5 and 89.9 % ($p<0,05$) in B and P, respectively. The PFK activity was 5.77±2.69 and 1.94±0.89 $x10^{-5}$ IU/COC ($p<0.05$) and for G6PDH was 5.07±1.71 and 1.52±0.52 $x10^{-5}$ IU/COC ($p<0.05$) in B and P, respectively. Our results indicate a higher metabolic activity of the studied pathways in the B rather than the P.

36.

RE-12 ENDOCANNABINOID SYSTEM PARTICIPATION IN THE REGULATORY VASOACTIVE MOLECULES IN HUMAN PLACENTA

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Anandamide (AEA), one of the most important endocannabinoid, is produced by the human placenta. At term, the levels of AEA significantly increase during labor. In other tissues, it was found that this endocannabinoid could regulate the production of prostaglandins (PG) and nitric oxide (NO), important molecules involved in the regulation of placental blood flow. Therefore, the aim of this study was evaluate the role of AEA in the regulation of vasoactive molecules in human placenta. Methodology: we use term placentas (38-40 weeks) obtained from women with uncomplicated pregnancies after elective cesarean sections (without labor, STP) or vaginal delivery (with labor, CTP). We performed RT-PCR and western blot, radioimmunoassay (PGs) and Bredt & Snyder technique (NOS activity). Results: We found that oxytocin (2.5-5 mUI / ml) diminished FAAH protein expression, the principal enzyme involved in the catabolism of AEA, in placentas STP. Additionally, we observed that the AEA increased NOS activity ($P<0.01$) and inhibited the production of PGE2 and PGF2alpha in placentas without labor. On the other hand, AEA decreased ($p<0.01$) NOS activity without affecting the synthesis of PGs in placentas obtained from vaginal deliveries. Taken together these results suggest that AEA, through its effect on NO synthesis and prostaglandins production, would be a necessary signal in the regulation of progression and the onset of labor at term.

37.

RE-36 MORPHOLOGICAL AND HISTOCHEMICAL ASPECTS OF THE DIGESTIVE SYSTEM DURING PRENATAL ONTOGENY OF ALPACA (*Lama pacos*)

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Observations on prenatal ontogeny of alpaca (Lama Pacos) are scarce. This study analyzes the characteristics of the abdominal portion of the digestive system of this species using macroscopic observation, magnifying glass observation, conventional light microscopy, immunohistochemistry and lectinhistochemistry in embryos and fetuses of different development stages. Differentiation processes are observed in the stomach and intestine including modifications in the epithelium (changing of stratified to simple and showing changes in the carbohydrates pattern) and in the mesenchyme (with cell differentiation to muscle in the further tunica muscularis). The stomach is compartmentalized and glandular sectors appear in them. In the liver, hematopoietic sectors are observed in early stages of development. This study shows some aspects similar with other species but other own related to the compartmentalization gastric characteristic of this species.

38.

RE-47 PARTICIPATION OF CUMULUS CRISP1 IN THE FERTILIZATION PROCESS

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Recent results from our laboratory indicate that CRISP1 is present in cumulus oophorus that surrounds the egg and that its lack in knockout (KO) mice produces a significant decrease in the percentage of fertilization. In view of this, the aim of this work was to investigate the mechanism by which cumulus CRISP1 participates in the fertilization process. Cumulus penetration assays using cumulus-oocyte complexes (COCs) from KO and wildtype (WT) mice for CRISP1 revealed that the KO COCs were penetrated by a sperm number significantly lower than that for control COCs. Subsequent studies aimed to evaluate both spontaneous and hyaluronidase-induced cumulus cell dispersion did not reveal differences between KO and WT COCs, suggesting that the absence of cumulus CRISP1 would not affect the extracellular matrix. In view of this, the possibility that CRISP1 acts as a chemoatractant was evaluated. Videomicroscopy studies using different concentrations of either native or bacterially- expressed recombinant CRISP1 as well as heat-denatured or heat -denatured and DTT-reduced CRISP1, revealed that the protein exhibits a conformation-dependent quemoatractant activity. Together, these results confirm the participation of cumulus CRISP1 in the fertilization process through a mechanism of chemotaxis of sperm towards the egg.

39.

RE-48 CONSECUENCES OF OBESITY ON THE FOLLICULOGENESIS

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Obesity is the most important non transmissible disease worldwide. It affects the reproductive health since obese women have high rates of menstrual dysfunction, anovulation, infertility, etc. The aim was to study whether obesity alters the follicular development. Female Wistar rats were fed *ad libitum* with three different diets: A) control group was fed with a standard diet. B) The DC group, received a high caloric diet with a pellet made with cookies, peanuts, butter, etc. C) The DCI group was offered with food directly that was periodically changed: chocolate chip cookies, peanuts, sausages, etc. In the DC and DCI groups, we detected an increase in the caloric intake and in the rates of weight gain, as well as in the glucose serum levels, respect to the control group. Cholesterol and triglyceride serum levels were normal in all groups. Respect to the ovaries, obesity did not alter the number of follicles in different stages of development, although we detected the presence of cystic follicles and follicles showing multiple oocytes in the DCI group. We conclude that the diet-induced obesity alters follicular development and those changes depends on the type of diet.

40.

RE-49 HYALURONIC ACID AND HEPARIN EFFECT ON OXIDATIVE METABOLISM IN CRYOPRESERVED BOVINE SPERMATOZOA CAPACITATION

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Hyaluronic acid (HA) and heparin (H) are glycosaminoglycans present in genital tract of bovine female. Capacitation of bovine sperm requires oxidative substrates in order to obtain energy, as lactate and pyruvate. The aim of this study was to evaluate oxidative metabolism variation and cryopreserved bovine spermatozoa capacitation through oxygen consumption, lactate dehydrogenase (LDH) activity and variation of lactate concentration in incubation medium, in capacitation induced by HA and H. Capacitation was evaluated by the chlortetracycline epifluorescent technique and viability and acrosome integrity by Trypan blue stain/DIC. LDH activity and lactate concentration in incubation medium were determined spectrophotometrically at 37°C. Oxygen consumption was measured polarographically with an oxygen electrode modified Clark type. Data was analyzed by ANOVA and Tukey test ($P < 0.05$). Oxygen consumption was significantly higher with H (14.76±2.89 $\mu LO_2/h/10^8$ sperm) than HA (7.29±1.86 $\mu LO_2/h/10^8$ sperm) ($P < 0.05$). LDH activity was 0.39±0.24 x 10^8 sperm with H and 0.48±0.19 x 10^8 sperm for HA. Lactate concentration presented an increase in samples treated with HA, respect to control ($P < 0.05$). Heparin and hyaluronic acid are capable to induce sperm capacitation, promoting differential metabolic changes where heparin presents a higher oxidative level than hyaluronic acid.

41.

RE-50 FIBRONECTIN (FN) INDUCES HUMAN SPERM CAPACITATION MODULATING THE ENDOCANNABINOID SYSTEM

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There are molecules present in the oviductal fluid such as Fn, extracellular matrix glycoprotein that binds to $\alpha_5\beta_1$ integrin, and anandamide (AEA), main endocannabinoid which acts through cannabonoid receptors (CB1 and CB2) and vanilloid receptor type 1 (TRPV1), involved in the modulation of sperm function and fertilizing ability. We have previously demonstrated that Fn increases and accelerates human sperm capacitation. In the present study we investigated whether Fn modulates the endocannabinoid system during human sperm capacitation. The presence of the antibody reverted the capacitating effect.Sperm from healthy donors were pre-incubated with an anti-α5 antibody and *in vitro* capacitation was assessed to determine the specificity of Fn effect. The presence of the antibody reverted the capacitating effect. Also, co-incubation of Fn with CB1 or TRPV1 antagonists significantly reduced Fn-induced capacitation. Capacitation status was evaluated by chlortetracycline assay. Then, sperm were incubated with and without capacitating medium plus Fn, and FAAH activity (the main enzyme that regulates AEA levels) was determined. We observed a 66% decrease in FAAH activity of capacitated sperm and the presence of Fn increased the activity of this enzyme. These findings suggest that interaction of Fn with its corresponding integrin induces capacitation via the endocannabinoid system, possibly by regulating the tone of AEA in human sperm.

42.

RE-59 TRANSMISSION AND SCANNING ELECTRON MICROSCOPY OF THE FEMALE PLAINS VISCACHA (*Lagostomus maximus* RODENTIA CHINCHILLIDAE)

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The female prostate gland (paraurethral or Skene's gland), appears in all adult female plains viscacha. We have previously shown that the gland surrounds the urethra and provides a lobular pattern with adenomeres lined by a pseudostratified epithelium, with basal cells and secretory cells of cylindrical appearance. Here we analyzed the structure of the gland using scanning and transmission electron microscopy. Samples from six adult females were fixed with glutaraldehyde and osmic acid. Three samples were analyzed by optical microscopy using semithin sections and transmission electron microscopy using ultrathin sections. The other three samples were analyzed by scanning electron microscopy. The results confirm the heterogeneity in size and electrodensity of the granules of secreting cells. The existence of clear cells was established. The ultrastructural features of the surface and the adenomeres support our previous histochemical studies that consider this gland as functional and not vestigial.

2st Joint Meeting of the Biology Societies from Argentina

San Juan, Argentina. August 17-19, 2011

E.1.

AH16 - CELLULAR ASSOCIATIONS OF LH-GONADOTROPHS IN PARS DITALIS OF VISCACHA. EFFECT OF PHOTOPERIOD

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Gonadotrophs are main cells in the hypothalamic-pituitary gonadal axis to regulate reproduction. The aims of this study were to study the cellular associations of the LH-gonadotrophs with other cellular types in adult male viscacha PD during long and short photoperiods. The pituitaries used were obtained from vizcachas captured during long (n=4) and short (n=4) photoperiods. Double inmunohistochemistry allowed to locate the LH-gonadotrophs with DAB, then other endocine cells (FSH, PRL, GH, ACTH, TSH) and folliculostellated cells (FSC or S-100-ir) using New Fuchsin. Bihormonal gonadotrophs were isolated and interspersed with monohormonal cells. The FSH-gonadotrophs, PRL, ACTH and TSH cells were surrounded by LH-gonadotrophs. In the dorsal region, isolated gonadotrophs were enclosed by GH cells. Irregular ACTH cells were associated with gonadotrophs. The cytoplasmic processes of FSC were contacting with gonadotrophs. During short photoperiod, a decrease of LH-FSH, LH-ACTH and LH-FSC associations, and a increase of LH-PRL association were observed. These results demonstrate the presence of intrapituitary communication between the LH-gonadotroph and other cell types, suggesting that the specific associations are necessary to achieve an integrated response of gonadotrophs. The associations vary with changes in photoperiod, according to paracrine mechanisms that regulate the activity of these cells during the reproductive cycle of the viscacha.

E.2.

BT22 - IGY ENCAPSULATION BY HYDROGELS AND ASSESSMENT OF ITS STABILITY IN SIMULATED GASTRIC FLUID

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Egg yolk antibodies (IgY) are a novel alternative for the treatment and prevention of enteric diseases. However, the activity of IgY can be reduced or destroyed by gastric conditions. Hydrogels are polymeric networks that can protect a protein from a hostile environment and make a controlled release changing its structure in response to stimuli. The aim of this study was to evaluate pH-sensitive hydrogels as a method of oral delivery of IgY antibodies. Hens were immunized with *Escherichia coli* to obtain specific IgY. Hydrogels were synthesized with acrylic acid and acrylamide. The effect of encapsulation medium pH on Encapsulation Efficiency (EE) and loading capacity (LC) for IgY were evaluated. The stability of encapsulated IgY in simulated gastric fluids (FGS, pH 1.2) was also assessed. Statistical analysis was performed using ANOVA with INFOSTAT software. EE and LC for IgY were significantly altered by pH of the encapsulation medium, and pH 5 was the optimum pH for the evaluated parameters ($p<0.05$). Encapsulated IgY remain intact during incubation in FGS as found in PAGE. PH-sensitive hydrogels effectively incorporated IgY and protect it from acid and enzymatic hydrolysis, suggesting that pH-responsive hydrogels could be used for protection of IgY during gastric passage.

E.3.

MI41 - COMPARISON OF COLONY MORPHOLOGY CHARACTERISTICS AND THEIR TOXINS IN STRAINS OF *Clostridium botulinum* MENDOZA OF TYPE A ISOLATED FROM INFANT BOTULISM AND SOILS

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Clostridium botulinum (Cb), agent of botulism, is an organism sporulated anaerobic, widely distributed, being its main reservoir floor. The most common form of this pathology is infant botulism (BL) (intestinal-borne). In the isolation of the strains was observed morphological heterogeneity of the colonies. The objective was to compare between strains of different origin, colony morphology and characteristics of the toxins. Strains were selected from BL: BLMz84 / 3 BLMz99 / 5 BLMz32 / 4, and Soil: Sumz-641 / 3 and Sumz-635 / 3. Toxigenicity was controlled by IP inoculation in white mice in CCM subculture (34ºC, 96 h). The colony morphology was studied in cultured on agar plates 1.5% (film formation) and 4% (circumscribed colonies), incubated under anaerobic conditions (34ºC, 24-72 h). Neurotoxins (BoNT) generated in 200 ml of toxin production medium under anaerobic conditions (34ºC, 96 h), centrifuged and the supernatants were purified by salting ($(NH_4)_2SO4$ 60% saturation). We estimated the power (DL50/ml) by titration (Reed and Muench) by IP inoculation in mice. Was determined: protein concentration in mg / ml (Lowry), specific activity (AE) (DL50/mg protein) and haemagglutinating activity (HA) with human red blood cells. Molecular characterization was performed by non-denaturing electrophoresis (native) polyacrylamide gel. Results: 1.5% agar, strains of soil formed film, not the case for BL. In 4% agar soil strains dominated colonies with ground glass appearance of fine, dark center, and BL strains produced colonies with ground glass appearance of thick, clear center. For all toxins, AH was negative and the PM was ≈ 300 kDa. The AE was higher in strains of soil. Conclusion: No difference in the molecular type of NTBo for different morphotypes of colony. Most AE toxin from strain of soil indicating a greater toxic potency. The film formation and greater AE of toxin in strain of soil indicate different infectivity. The last two features might be lost in strain of BL during intestinal transit.

E.4.

NQ17 - KETAMINE DISRUPTS ACQUISITON BUT NOT CONSOLIDATION IN A PASSIVE AVOIDANCE TASK

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Ketamine is an anesthetic that has been referred to show psychotogenic effects under determined conditions. Its therapeutical and collateral actions are mediated by NMDA-glutamatergic antagonism. Blockade of these receptors is studied as a model of schizophrenia. In present study the effect of systemic administration ketamine (15 mg/Kg ip) was evaluated in a memory passive avoidance task. Male rats derived from a Holtzman colony were used weighing 240-270 g, n=10-20). Latency to head (Latency 1) and body entrance to aversive camera of the apparatus (latency 2), and expelled fecal boli were measured. A clear disruption was observed in acquisition in latency to body entrance (latency 2, $p<0.01$ versus saline control). Additionally, fecal boli were decreased by treatment in this condition ($p<0.01$). No effects were observed in consolidation. We conclude that Ketamine disrupts acquisition but not consolidation in a passive avoidance task.

E.5.

RE43 - THE INHIBITON OF PHOSPHO-FRUCTOKINASE 1 AND MALATE DEHYDROGENASE AFFECTS 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 phosphofructokinase 1 and malate dehydrogenase enzymes 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 carried out during 48 h, 39ºC, 5% CO2 in medium 199 supplemented with gonadotropins (control) and different concentrations of oxoglutarate (5,10, 20 mM, inhibitor of phosphofructokinase 1) or malonic acid (1, 5, 10 mM, inhibitor of malate dehydrogenase). *In vitro* fertilization was performed in mTBM with fresh semen by 18 h. Nuclear and cytoplasmic maturation were evaluated by presence of metaphase II or decondensed sperm heads and/or pronuclei, respectively. Vitality was evaluated by the diacetate fluorescein stain. The percentages were analized by the Chi-square test. Nuclear and cytoplasmic maturation diminished with 20 mM of oxoglutarate ($p<0.05$) and with 10 mM of malonic acid ($p<0.05$). Vitality wans´t affected by any concentration of inhibitors. The inhibition of the enzymes of glycolysis and Krebs cycle impairs *in vitro* maturation of porcine oocytes.