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C1.
OPENING CONFERENCE: PRIMARY PRODUCTIVITY AND ACCLIMATION OF PHYTOPLANKTON IN MID-LATITUDES IN A SCENARIO OF CLIMATE CHANGE

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Climate change includes many variables mostly driven by incident irradiance, and the emissions of greenhouse gases (GHGs) by humans. Infrared radiation is responsible for the increase in temperature; while UV-B is increasing as a result of ozone depletion. Gas emissions, include CO_2, CH_4, N_2O and CFCs, being these latter associated with both global warming and ozone depletion. Increased GHGs result in an increase of global temperature in the troposphere, with their concomitant decrease in the stratosphere. The stratospheric cooling will result in a slower recovery of the ozone layer and thus organisms and ecosystems might be exposed to higher UV-B. Other outcomes of global change are the increase of stratification in the water column and changes in ocean circulation patterns and mixing rates. These changes would induce a higher stress in phytoplankton cells by exposing them to higher UVR due to their circulation within a shallower upper mixed layer. Instead, an increase of CO_2 will favor some phytoplankton species by enhancing their growth rates, while changing the pH will harm others. In the high productive area of the Argentinean Sea, wind and solar radiation, play a significant role in determining the production and the inhibition of photosynthesis due to UVR. Repair of UVR-induced damage to the DNA or photosystem are temperature-dependent and therefore higher temperatures might benefit some species. It is evident that the effects of global change on the aquatic biota are very complex and so, the future behavior of the system lie in a narrow line of balance between damage and repair.

C2.
CLOSING CONFERENCE: MOLECULAR MECHANISMS OF DEVELOPMENT AND REGENERATION IN THE MECHANOSENSORY LATERAL LINE SYSTEM OF ZEBRAFISH

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The lateral line system in zebrafish is under intense study due to its spectacular developmental features, which include complex migratory behaviors, coordination of cell division and cell specification, and differentiation of a functional sensory organ, all in a matter of hours. The neuromasts, individual sensory patches distributed over the body of the fish, contain sensory hair cells, which are able to regenerate when destroyed by trauma or toxicity. In adult fish, entire regions of the lateral line system can regenerate as well, after amputation of the tail, for example. This includes sets of neuromasts and their corresponding innervations. We have undertaken the task of identifying genes that are involved in these regenerative responses as well as to clarify the nature of the progenitor cell pool that is present in this system. New genes expressed in the progenitor cells have been found through a combination of cell sorting and microarray analysis. We show that some of these possess roles in progenitor cell migration and sensory cell differentiation. Other genes have been identified through genetic screens and candidate gene analysis. Several of these have become useful markers for the different cell types present in the lateral line system. We have examined the proliferative behavior of cells within neuromasts during the regeneration process and we find evidence for mitotic and non-mitotic modes of regeneration of hair cells. Combining immunostaining methods and transgenic zebrafish lines, we characterize the cell population which could give rise to new hair cells through cell division and possibly by direct transdifferentiation. In a related line of inquiry, we show that damage to hair cells and other lateral line components elicits a potent innate immune response, which may play a role in regeneration. Furthermore, regeneration of entire neuromasts can be achieved by de novo migration of cells from existing lateral line components after tail amputation in adult fish. This feature underscores the regenerative capacity and plasticity of the lateral line system in the zebrafish. Finally, the relationship between hair cells and their innervating neurons has been studied by severing the neural connection through laser axotomy and by ablating hair cells and observing the behavior of the neural component. These experiments show that, while survival of hair cells and neurons are independent of each other, regeneration of the lateral line nerve is partially dependent on an intact set of sensory cells.
ICM P06-039F; Fondecyt 1070867, UNAB DI-01-09/I and DI-07-09/R.

C3.
ATTENUATION OF TRAUMATIC MEMORIES BY INTERFERENCE OF FEAR MEMORY RECONSOLIDATION

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Under certain boundary conditions, the retrieval of a stable consolidated memory results into a labile one followed by a stabilization period termed reconsolidation. During the instability phase, memory trace can be disrupted by a number of pharmacological agents. We study the effectiveness of different doses of midazolam (MDZ), a short acting benzodiazepine ligand, in preventing reconsolidation of different fear memory ages. The results showed an amnesic action of MDZ on fear memory reconsolidation within a time-limited window. Such effect was evident on recent but not on remote memories. An additional goal of the present study was to evaluate the vulnerability of recent and remote fear memories in animals that have experienced a stressful situation prior to fear acquisition. The results show that stressed animals were less vulnerable to MDZ´s disruptive effect. We investigated whether activating NMDA sites prior to reactivation by D-cycloserine (DCS), a partial NMDA agonist, promotes the destabilization of resistant memories such as those of stressed rats. Our findings indicate that DCS promotes retrieval-induced lability in a resistant memory since when DCS was administered, MDZ impaired memory reconsolidation in stressed animals. In sum, MDZ could be potentially used as a pharmacological intervention to interfere with traumatic memories.

C4.
OVIDUCTAL SPERM RESERVOIRS IN LLAMAS
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Ovulation in South American Camelids occurs 36 h after mating. The question was whether the oviduct could maintain spermatozoa until ovulation. Several studies performed with anatomical, histological, histochemical and electron microscopical methods showed differences between the different oviductal segments, which might be associated with the aptitude to form sperm reservoirs. This hypothesis was then demonstrated by *in vitro* assays. By means of different studies we demonstrate that llama oviduct forms sperm reservoirs in the utero-tubal junction (UTJ) by sperm adhesion to the oviductal mucose for at least 28 hours after mating. The anatomical design of the UUT, the surface features of the epithelial cells, the oviductal carbohydrates recognition (Galactose and N-Acetyl galactosamine) by the sperm and the involvement of seminal plasma elements contribute this process. 35 hours after mating, when ovulation is about to happen, no sperm adhered to the oviduct were observed, hereby we suggest a possible participation of the oviduct in sperm liberation.

C5.
BOTULISM IN ARGENTINA
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Botulism is a highly lethal paralyzing disease caused by the botulinum neurotoxins (BoNT) produced by Clostridium botulinum (A, B, C, D, E, F, Ab, Af, Ba, and Bf), and other species: C. argentinense (G), C. baratii (F), and C. butyricum (E). Physiopathogenic forms are: poisoning, by food (FB), iatrogenic (IatB), accidental (AB), and intentional (IntB); toxi-infection by wounds (WB), and intestinal toxemia (IT), in infants under 1 year old (InfB), and cryptic (CB) or indeterminate in older people. BoNT inhibits the release of acetylcholine at myoneural junction, causing symmetric, descending flaccid paralysis and death from respiratory arrest. FB is caused by intake of previously synthesized BoNT present in a foodstuff. In WB and IT, colonization and production of BoNT occur *in situ* in a wound or the intestine. AB refers to accidents in a laboratory setting. IatB are associated with the medical use of BoNT as a drug. Cases in which the BoNT source cannot be traced are labeled CB. The source of contamination with Clostridia is mainly the soil. Of 2009 soil samples of Argentina, 23.5 % were positive (A 57 %, B 15 %, F 4 %, G 0.4 %, Af 4 %, A+B 3 %, A+F 0.2 %, B+F 0.2 %). InfB is currently a cause of concern because of its high incidence, its difficult diagnosis and its probable relationship with infant sudden death syndrome. Between 1982 and 2006, 507 InfB cases were recorded in Argentina. Although transmission mechanisms are not fully elucidated, spores have been detected in honey, corn syrup, formula milks and herbal teas. According to our records, 95 FB outbreaks (including 294 cases) occurred in Argentina until 2006.

C6.
HOW TO APPROACH POSTDOCTORAL STUDIES ABROAD
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For most graduate students in biomedical sciences aiming to do scientific research the enrolment in a Ph. D. program is a necessary and unavoidable step. In contrast, after obtaining the Ph. D. degree several options seems equally valid. Prominent among these options is the decision of performing postdoctoral studies either in the country or abroad. During the talk, we will discuss the advantages and disadvantages of each alternative and which would be more appropriate for each individual.
The seminar will be of particular interest fort those interested in perform postdoctoral studies abroad. This stage is critical for an adequate development as investigator and therefore requires that the laboratory of choice will be selected carefully. During the talk we will discuss aspects related to the search of laboratories, the CV and interview preparation, etc. We will also discuss aspects related to the postdoctoral life such as economic and migratory situation. Finally, we will discuss the alternatives available upon finishing the postdoc including returning to the country. It is important to note that this seminar is not the result of a scientific or statistic analysis. It represent a personal view of the postdoctoral experience that will attempt to share positive and negative experiences that might be useful for those entering this path.

C7.
SCIENTIFIC, MEDICAL AND ENVIRONMENTAL COMMUNICATION IN AUDIOVISUAL SUPPORT
Lobo G.
TN Ciencia.

The dynamism of knowledge in the society of information forces us to adapt very quickly to changes and opportunities. The expertise in this adaptation will condition the opportunity to benefit from communication. This expertise will be useful to handle pitfalls present in the world of disclosure. If we take into account that the worst communication is the one that is not released, we can question: What is the need of massive news transmission from the scientific, environmental or medical fields? And, in any case: Which are the aims of this communication? For those who believe, me included, that one of the aims of the generation of knowledge is to transmit it, another question arises: Am I a good communicator? The view from which these questions are tackled in the TV program TN Science will be commented. This program has been emitted by Todo Noticias Channel for sixteen years and is one of those with the longest history in the transmission of what is new, surprising, and polemic and motivator of knowledge. The opportunities and difficulties of a program on audiovisual support will be addressed. The technical and discursive complexities which take place behind the screen will be analyzed. Scientists are invited to participate in the fabulous and unique opportunity which is offered by the massive means of communication.

S1.
HOW TO START A START UP AND NOT DIE IN THE TRIAL
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Inmunova constitutes the first spin-off of the Instituto Leloir, a private not for profit research organization dedicated to advancing knowledge in the fields of biochemistry, molecular and cellular biology. Inmunova develops a proprietary platform for vaccine design and has state of the art capabilities for single domain antibody generation and selection. The BLS technology represents an innovative and versatile molecular display system endowed with intrinsic adjuvant properties, allowing optimal antigen presentation to the immune system. Its application in subunit vaccines is suitable for prophylactic and therapeutic medicine. BLS vaccine technology can also be used for immunization against new diseases or pathogens for which vaccines are not available, while substantially improving the currently existing vaccines. This proprietary technology platform is based on the decameric (highly stable) enzyme lumazine synthase from Brucella spp . (BLS), established as a modular system decorated with up to 10 copies of desired target antigens. The BLS platform was efficiently proven to possess potent adjuvant properties in different animal disease models. Inoculation of BLS chimeras induces high levels of specific antibodies against self or foreign molecules. Generation of an antigen-specific cellular immune response and protection against subsequent challenges is also observed, independently of the adjuvant formulation used or even in its absence. The BLS carrier is also able to elicit an immune response when orally administered, showing a clear ability to induce mucosal immunity. Immune protection generated by this strategy was shown to be significantly more efficient than that developed utilizing other well known vaccine carriers. BLS technology represents a new molecular scaffold for multiple display of selected antigens and a suitable vaccine for prophylactic and therapeutic medicine. BLS technology enables the induction of potent innate and adaptive immune responses to co-administered antigens, breaking immunologic tolerance of self antigens. BLS presents a large and versatile carrying capacity, able to accommodate protein domains, full proteins, peptides, polysaccharides, or other chemical entities. Is also a multivalent system that allows for design of multiepitope vaccines. BLS presents the advantage of potential inoculation of vaccines via several routes of administration such as nasal, high-pressure injection, or oral. BLS is effective when used both as a protein and as a DNA vaccine, inducing high titers of specific antibodies and effectively priming cell-mediated immune responses.

S2.
APPLIED REPRODUCTIVE BIOTECHNOLOGY IN EQUINES
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Argentina is one of the 3 most important embryo transfer (ET) producers of horses in the world. This biotechnology has permitted the distribution of our breed of polo ponies and has contributed to increase reproductive efficiency in the mare. Last season there were 6,500 births of ET polo ponies, with a record of 12 live births from the same mare in one season. These biotechnologies are used in old mares, unable to carry a gestation to term. The OPU (ovum pick-up) technique is used to produce embryos *in vitro* using ICSI (intracitoplasmatic sperm injection). Currently we obtain early embryos using unconventional protocols of equine sperm preservation (dehydration and desiccation). Cloning has also given its results: Dr. Gordon Woods (Idaho, USA) obtained the first cloned mule in 2002, using oocytes obtained by OPU and nuclear transfer of fetal fibroblasts. In 2003 Dr. Galli (Italy) reported the birth of the first equine clone. Other research groups have been working to produce animals of high genetic value that can no longer be reproduced. Such is the case of geldings of superior sporting abilities that cannot produce descendents. Here nuclear transfer acquires unusual dimensions as we have the case of the birth of the clone of the endurance world champion whose semen is currently being cryopreserved and commercialized. Twenty one births using this technique have already been reported and our research group started a trial that resulted in the birth of the clone of a high performance polo mare (Polo Open player).

S3.
TRANSGENIC PLANTS: VIRUS RESISTANCE AND MOLECULAR FARMING
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Solanum tuberosum cv. Spunta was transformed with a construct containing the Potato virus Y (PVY) coat protein. After screening for PVY resistance under greenhouse and field conditions, we selected several genetically stable resistant lines. Parallel studies performed in virus-free environments showed that agronomical performance, agricultural traits and biochemical composition of the selected lines were indistinguishable from those of the traditional Spunta cultivar. The final aim of this work is to achieve the commercial release of a transgenic potato plant resistant to PVY.
Plant based expression systems offer advantages over traditional host in terms of cost efficiency, product safety and scalability.
However, in nuclear transgenic plants, levels of recombinant protein expression could be relatively low. The transformation of chloroplasts represents an attractive alternative as it allows high expression of the recombinant protein. In our laboratory we are using nuclear transgenic, viral vectors and transplastomic plants for the production of molecules of interest for the pharmaceutical industry and the development of vaccines for use in veterinary medicine.

S4.
THE BIOTECHNOLOGIST, A NEW SUBSPECIES OF BIOLOGIST?
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We, as biologists, acquire a solid training that allows us to play very dissimilar tasks.
Nevertheless, under some circumstances, when we leave the academic area, we face problems that are not simple to overcome.
The incorporation of biologists in the pharmaceutical industry, especially in the area of Biotechnology became a real challenge.
The tranformation to become a "Biotechnologist" goes beyond applying our knowledge in this area. The incorporation of new concepts from fields as different as law, economy, engineering, etc. is required. In addition a change in the way we face the problems and projects is crucial.
Taking into account the experience of a local group with 25 years of experience in the field, we will try to obtain experience and conclusions in the fields of establishing new companies in the area, different ways of association, driving the products to the market, valuation of technologies and in the advantages and real difficulties that a Biologist can face if he takes this challenge.

S5.
MOLECULAR BASES OF PROGESTERONE-INDUCED IMMUNESUPPRESSION IN BREAST CANCER
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Compelling data support the view that the immune system is essential for the control of tumor development and growth. However, tumor cells employ a diversity of mechanisms that circumvent antitumor responses. These mechanisms include the secretion of immunosuppressive factors as galectin-1 and TGF-β and the expansion and/or recruitment of suppressor cells as CD4+-CD25+-Foxp3+ regulatory T (Tregs) cells. Based on the tolerogenic properties of progesterone, and its promoting role in breast cancer, we investigated whether progesterone may create an immune privileged microenvironment in breast cancer, either by regulating galectin-1 expression or controlling Tregs differentiation. The progesterone analogue medroxiprogesterone acetate (MPA) was able of inducing galectin-1 expression in two hormone-dependent human breast cancer cell lines and in a mouse mammary tumor. Interestingly, *in vitro* MPA-treatment of mouse splenocytes or human PBMC induced a significant increase in the frequency of Tregs and skewed the balance toward a Th2-type cytokine profile. *In vivo* MPA-treatment increased the frequency of Tregs in tumor-draining lymph nodes and within the tumor. MPA-induction of Tregs was associated with an effect both on CCL22 chemokine and on TGF-β pathway. Our results showed that progesterone fosters an immunosuppressive tumor stroma by regulating galectin-1 expression and augmenting the frequency of Tregs in breast cancer.

S6.
SOUTH AMERICAN TREEFROGS: DIVERSITY AND PHYLOGENY
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Treefrogs (Hylidae) are the most speciose family of extant amphibians, with more than 870 species. Among the huge diversity in shapes, colors, and reproductive modes, the general public is most familiarized with some members of the subfamily Phyllomedusinae, like the monkey frogs and the red-eye treefrogs. In this talk we will present results of ongoing research regarding treefrog diversification in general, and in particular we will focus on the phylogenetic relationships of phyllomedusinae, including its implications for our understanding of the evolution of some character systems associated with reproductive biology, behavior, and physiology.

S7.
IMMUNOMODULTATORY OLIGODEOXINUCLEOTIDES AS POTENTIAL TOOLS FOR TISSUE REGENERATION
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Synthetic ODNs containing CpG motifs are known to be effective as adjuvants in vaccines and cancer therapies. We found that strong immune stimulation could also be achieved with non-CpG ODNs containing a PyNTTTTGT motif. Moreover, we proved that its protpotype, IMT504, stimulates Bone Marrow derived adult Mesenchymal Stem Cells *in vivo*. We subsequently found that IMT504 accelerated the reparation of bone defects, osteoporosis with progressive trabecular bone loss and neuropatic pain induced by sciatic nerve crush in rats. Recently, we studied the effect of s.c. administration of IMT504 on blood glucose and pancreatic islet morphology in streptozotocin-induced diabetes in rats. Animals with glycaemia between 11-20 mM on day 4, were injected 10 consecutive days with 4 mg IMT504. ODN treatment greatly improved blood glucose and food and water intakes by day 8. Intraperitoneal glucose tolerance tests were improved on day 30 together with islet number and beta cell content. Moreover, after 2-5 IMT504 injections we found an increase in pancreatic nestin (mainly in endothelial cells), proliferating cell nuclear antigen (PCNA) and neurogenin 3 (Ngn3, unambiguous marker of islet progenitors) expression. In sum, IMT504 induced a marked recovery which correlated with early expression of progenitor cell markers. Clinical trials will soon asses the possibility of its medical use.

1.

GENE EXPRESSION OF KISSPEPTIN-1 RECEPTOR (KiSS1R) IN PEJERREY, *Odontesthes bonariensis*

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Kisspeptin (KiSS-1), also known as metastin, is the natural ligand of the protein G-coupled receptor GPR54, called now as kisspeptin-1 receptor (KiSS1R). It is well known that the KiSS-1/KiSS1R system plays a key role in the onset of puberty in mammals and fish. The study of KISS-1 and its receptor will allow us to understand the molecular mechanisms involved in the sexual development.
From the analysis of the open reading frame (ORF) of KiSS1R cDNA sequences of different fish species we designed consensus primers and a 916 bp fragment was obtained from pejerrey brain cDNA. This sequence, called pjKiSS1R, showed high similarity to other teleost KiSS1R. The expression pattern of KiSSR1 was performed using different pejerrey brain areas but not in the pituitary gland.

2.

EXPRESION DIFFERENTIAL MICROSOMAL AND CYTOSOLIC OF GLUTATHION-S-TRANSFERASE IN THE FLUKE *Fasciola hepatica* SUSCEPTIBLE AND RESISTANT AT TRICLABENDAZOLE

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The parasites fluke (*Fasciola hepatica*) produce the fasciolosis, a zoonotic liver disease. In our country this disease is principally treated with triclabendazole (TCBZ), a anthelmintic halogenated benzimidazole thiol derivative. The helminth parasites possess different biochemical mechanisms for detoxification and the overexpression of metabolic enzymatic systems it can be one of them. Glutathion-S-Transferase (GST) catalyses nucleophilic attack by reduced glutathione to a wide array of compounds, including toxic products of lipid peroxidation. The resistance of *F. hepatica* to TCBZ is growing worldwide. Hence, the knowledge of detoxification and resistance mechanisms in *F. hepatica* is needed. This work was aimed to assess the microsomal and cytosolic enzymatic activity of GST in adult *F. hepatica* specimens, susceptible (Cullomptom strain) and resistant (Sligo and Oberon strains) at TCBZ. Both resistant strains expressed significant major metabolic activity compared to that measured in the cytosolic and microsomal fractions obtained from susceptibles flukes. TCBZ action may induce secondary oxidative stress in *F. hepatica*, which may explain the observed increment in GST activities as a defensive mechanism. These preliminary results may be useful to further understand the mechanisms underlying the drug metabolism/disposition and activity in target helminth parasites.

3.

STUDY ABOUT THE α SUBUNIT IN SPECIFIC CELLS OF THE PARS TUBERALIS (PTesp) OF THE RAT

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The α chain is a component of the glycoprotein hormones in PTesp cells and it is suspected that they would be part of their secretion. The aim of the present study was to evaluate the presence of α subunit (subα) in PTesp of rats in two different experimental models. Moreover, the use of the specific antibody (FB02) allowed to visualize the secretory product of PTesp cells and to compare the different degree of immunostaining. The first experiment consisted in three groups exposed to extreme photoperiods (n=4, continue dark, continue light and control). The second experiment were formed by three groups (n=3, poisoned orally with albendazole (ABZ) 0.5 and 1.5 g/kg and control group). Animals were slaughtered five weeks after the beginning of the study for the first experiment and 48 h post treatment for experiment 2. PTesp were extracted, fixed in Bouin´s liquid and processed with routine histological techniques. An immunohistochemical technique (peroxidase – antiperoxidase) was used to visualize the subα and the cells secretion. In experiment one, when the product secretion was evaluated, it was observed paranuclear immunostaining, during the continue dark, while it was not detected immunostaining with continue light. Subα was detected difuse in the three groups. In the second experiment, it was not observed immunostaining to subα while FB02 allowed observe paranuclear immunostaining. Results would indicate that the product secretion is photoperiod dependent and that the secretion of subα would be part of a component not detected by FB02. The presence of subα just in the control groups in the second experiment (poisoned with ABZ) would suggest that the alteration of the microtubules by ABZ would cause a variation of its presence.

4.

METHODOLOGY OF MONITORING: THE GROWTH OF THE TARSUS OF CHICKS OF WILSON'S PETREL (OCEANITES OCEANICUS) IN ANTARCTICA

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Wilson's Petrel Oceanites oceanicus is a pelagic bird widely distributed in Antarctica and subantarctica. It nests in caves among the rocks of up to a meter of depth. The aim of this work was to develop a methodology of monitoring using the rate of growth of the tarsus of chicks of O. oceanicus in the 25 Mayo Island, South Shetland Island, Antarctica. The capture of measures was realized in the reproductive seasons 2007 (n = 21) and 2009 (n = 15). As soon as the chicks were found alone inside the nest, the length of the tarsus was registered every 3 days by a digital caliper (precision 0,01). The chicks of every season were grouped by age (5, 10, 15, 20 and 25 days of age), for which calculated the rates of average growth. The rates of growth didn't show significant differences on having compared each of 5 groups of ages in both seasons (Student's Test). Using the calculation of the rate of growth of the tarsus for the zone of study, indirect information linked to the food availability in the sea, changes in the Antarctic ecosystem product of anthropic activities or the climatic global change. It was chosen for the length of the tarsus as variable due to the fact that the margin of mistake on having taken this measure diminishes with regard to such others as, body mass, length of the head or chord of the wing. With this methodology, we propose a protocol for monitoring to this so abundant and widely distributed species.

5.

IMMUNODETECTION OF NEURAL CADHERIN IN BULL SPERMATOZOA

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Introduction: Epithelial (E-cad) and neural (N-cad) cadherin proteins participate in cell-cell adhesion. Our group has characterized the expression of E-cad in bull sperm and its involvement in fertilization. However, no studies have been reported on the presence of N-cad in bull sperm. Aims: to immunodetect N-cad in frozen-thawed bull sperm. Methods: 1) N-cad protein sperm forms were identified; 2) N-cad localization patterns were characterized in non-capacitated (NC), heparin-capacitated (C-HEP) and its control (heparin + glucose: C-HGLU), and in A23187 Ca2+ ionophore-acrosome reacted sperm (R). To detect N-cad, the H-63 antibody (Sta Cruz Biotech) was used. Results & Discussion: In protein sperm extracts, the 135 kDa N-cad form was identified. In NC sperm, the main pattern (P1) (84±6; mean±SDM; n=36) showed a signal in the acrosomal and postacrosomal regions. Similar results were obtained with sperm incubated with H-63 prior to fixation. In C-HEP sperm, P1 was preponderant (63±9; n=21) but lower, with more cells with staining only in the acrosomal (P2) or postacrosomal (P3) regions. This shift was not found in C-HGLU sperm (P1: 82±18). In R-sperm suspensions, a staining pattern in the equatorial segment (P4) was observed (R: 22±6; control: 1±1; n=21). Over 90% of P4 cells were classified as R after co-localization with Pisum sativum agglutinin. This is the first report on bull sperm N-cad expression and its localization in cell regions involved in gamete interaction.

6.

AMYLASE AND MALTASE ACTIVITIES IN HEPATOPANCREAS OF *Cyrtograpsus angulatus*: RESPONSE TO ENVIRONMENTAL SALINITY

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Little is known about biochemical digestive physiology in eurihaline crabs. The aim of this work was to study biochemical characteristics and the response to environmental salinity of amylase (Amy) and maltase (Mal) activities in hepatopancreas (H) of *C. angulatus*. Adult males were acclimated 10 days in 35‰ salinity (S) (characterization) or 35‰ and 10‰S (salinity effect). The supernatant (10000xg 15min) from an hepatopancreas homogenate (0.1M Tris-HCl, pH 7.4) (4ml buffer x g of tissue-1) was used. Amy activity (µgmaltose x min-1 x mg protein-1) was assayed by hydrolysis of starch (St) in 50mM phosphate 30ºC (pH curve: 5.2-7.0, St=15mg/ml; substrate curve: St=0.06-17.97mg/ml, pH=5.2; salinity: St=15mg/ml, pH=5.2). Mal activity (µgglucose x min-1 x mgprotein-1) was assayed by hydrolysis of maltose (M) 0.1 M maleate/OHNa 30ºC (pH curve: 3.5-8.3, M=28mM; substrate curve: M=2.8-42mM, pH=5.2; salinity: M=42mM, pH=5.2). Both activities were high over a wide range of pH and exhibited Michaelis-Menten kinetics (Amy: Km(mg/ml)=0.11±0.04; Mal: Km(mM)=8.08±3.96). In 35‰ S, Amy (6,619.25±976.25) and Mal (595.57±110.87) activities were high. In 10‰ S, both activities were lower (about 49%) (Amy: ANOVA, F=7.58, p=0.025; Mal: ANOVA, F=6.66, p=0.033). The results suggest the role of amylases and maltase activities in mechanisms of adjustment related to osmorregulatory status.

7.

RAC/β2-CHIMAERIN REGULATION OF mRNA

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The small GTPase Rac1 controls different cellular processes including; cell movement, cell cycle regulation, proliferation, differentiation and the remodeling of the acting cytoskeleton. The post-transcriptional regulation of mRNAs is a crucial event directing these processes. β2-chimaerin is a multi-domain protein regulating Rac activity. Using a proteomic approach we identified G3BP (Ras-GAP SH3-domain binding protein-1) as a β2- chimaerin interacting protein. G3BP is an RNA binding protein with endonuclease activity known to bind and regulate the stability and localization of mRNAs. Several lines of evidences suggest that G3BP is involved in both mRNA regulation and small GTPase signaling.
In the present work, we evaluate the role of β2- chimaerin and G3BP in linking small GTPase signaling and the regulation of different mRNAs using a variety of reporter constructs. These reporters codify for chimeric mRNAs with a target 3'UTR region downstream a reporter gene under the control of a Tet-responsive promoter. As models to study different aspects of this regulation, we chose the 3'UTR regions of three different genes known to be regulated by G3BP: β-actin whose mRNA is localize in the actin polymerization sites; c-Myc, an early responsive gene that shows transient expression; and Cyclin D1, a crucial protein for cell cycle progression.

8.

LARVICIDE ACTIVITY OF *Larrea cuneifolia* (Zygophyllaceae) ON *Aedes aegypti* (Diptera: *Culicidae*)

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Aedes aegypti is the main vector of virus Dengue with a wide distribution in Argentina. Due to the re emergence of this virus in our region, is necessary to find out new strategies of vector's control. Previous studies have demonstrated insecticide properties of *Larrea cuneifolia* and *L. divaricata* on *Culex quinquefasciatus*, therefore the larvicide activity of *L. cuneifolia* on *Ae. aegypti* was evaluated. Three concentrations of extracts of different polarities obtained with chloroform, methanol and hot water were tested (250, 100 and 50 ppm). The extracts were applied in trays containing 30 III stadium larvae; larvae mortality was registered every 24 h. In order to detect differences between the treatments an ANOVA was performed. LC_{50} and LC_{90} values were estimated by Probit regression. The chloroformic extract showed the biggest larvicide activity causing 100, 82 and 31% of mortality at 250, 100 and 50 ppm respectively. DL50 and DL90 estimated for this extract were 69 and 110 ppm respectively. Methanolic and hot aqueous extracts caused low mortalities (<10%). This study reveals a great larvicidal effect of the chloroform extract; field studies should be performed to confirm these results.

9.

ULTRASTRUCTURE OF HUMAN TOOTH ENAMEL IN LOWER PREMOLARS AND MOLARS

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According to Koenigswald and Clemens's hierarchical classification of enamel microstructure, enamel types comprise radial enamel, with higher abrasion resistance, enamel with Hunter Schreger bands and irregular enamel that prevent fracture propagation. The aim of this work was to identify and relate the microstructure of human tooth enamel with biomechanics. Twelve pieces of each group were longitudinally cut, embedded in acrylic resin and prepared for observation under SEM. Micrographs of free faces and cusps were identified. In premolars, irregular (inner) enamel and radial (outer) enamel were seen in the occlusal, medial and cervical thirds of the free faces. In the inner third of the cusps irregular enamel compatible with knot-like enamel and radial enamel was observed in the outer zone. In molars, in the occlusal and medial third of the free faces the enamel type was radial in the outer zone and with bands in the inner zone; in the cervical third the only enamel type present was the radial type. In the lingual cusp, inner enamel was irregular with a marked prism intercrossing and radial enamel as far as the outer surface. In the vestibular cusp the inner enamel evidenced bands and the outer enamel was of the radial type. We conclude that the presence of different types of enamel and their combination in each tooth group would constitute a biomechanical adaptation of the functional areas.

10.

OLIGONUCLEOTIDE IMT504 DOES NOT REVERT LOW-PROTEIN DIET INDUCED DIABETES BUT ALTERS BODY WEIGHT IN MALES

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We have previously shown that the oligonucleotide IMT504 improves streptozotocin-induced diabetes in rats, in addition of having an anorexigenic effect. Here we studied the effect of IMT504 on diabetes induced in rats by low protein diet given to dams during pregnancy and lactation. Wistar rats were fed with an 8% protein diet (LP) or control diet (C: 20%) during pregnancy and lactation. Starting on the day of weaning (day 22) pups were injected daily with IMT504 (4 mg/0.2 ml saline) for 10 days (BP-IMT504 and C-IMT504) or with saline (BP-SAL y C-SAL). Body weight (BW) and glycemia evolution were studied. Glucose and insulin tolerance tests were performed in adulthood. Blood glucose was similar in all groups. BWs in LP females were lower than in C females, without differences due to IMT504. In males, LP diet induced a reduction in BW which was aggravated by IMT504 treatment [BW at 60 days (g): C-SAL: 348.1±7.55 (3), C-IMT504: 349.6±13.82 (4), BP-SAL: 282.7±8.55 (4), BP-IMT504: 243.8±8.46 (4), two way-ANOVA: interaction: $p<0.05$, BP-IMT504 different from all: $p<0.01$]. Although IMT504 did not improve the diabetic condition in this model of diabetes, it had a clear effect on BW in males.
(CONICET-ANPCYT-UBA).

11.

ANDROGEN SYNTHESIS IN GONADS DURING MORPHOLOGICAL SEX DIFFERENTIATION IN PEJERREY *Odontesthes bonariensis*

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The steroids were subjected to intensive studies in relation to their pharmacological role during fish gonadogenesis. Studies in this area pointed out the clear effect of estrogens in female differentiation, whereas the androgens were related to maleness. Furthermore, it was observed that in the gonochoristic fish, the steroid sensitivity was greatest around the sex differentiation time point. In this context, the hypothesis of a physiological role of the steroids in sex fate is plausible. This work deals with the testing of the gonad 11-oxygenated androgen behalf of this hypothesis. The approach adopted consisted in the qPCR analysis of 11β-hydroxylase (P45011b) in isolated gonads around the time of sex differentiation for male- and female-producing temperatures (MPT and FPT). Furthermore, the enzyme activity was assessed in MPT treated fish prediferentiated gonads by using biochemical techniques. The results obtained indicated that P45011b was active at least in predifferentiated MPT treated fish, although P45011b mRNAs levels were not clearly differential until morfological sex differentiation were they positively correlated to morphological differentiation in males. These and previous results suggested an 11-oxygenated androgen role in the initiation of the morphological testicular differentiation processes.

12.

NEONATAL EXPOSURE TO BISPHENOL A PRODUCED OVARIAN MORPHOLOGY ALTERATIONS IN ADULT RATS

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In previous studies we have demonstrated that animals neonatally exposed to Bisphenol A (BPA) showed an increase in GnRH pulse frequency, alterations in the serum sexual hormones and lower ovarian weight in adulthood. Aim: study of the effects of neonatal exposure to BPA on adult ovarian morphology and histology. Methods: Sprague-Dawley females were injected sc from postnatal day 1 to 10 with BPA [500µg/50µl (B500) or 50µg/50µl, (B50) in oil], or vehicle (C) and sacrificed in adulthood in estrus. Ovarian morphology was analyzed in Hematoxylin-eosin-stained slides. In addition, the level of cellular proliferation in early antral follicles was evaluated by PCNA staining. Results: Animals neonatally exposed to BPA500 had altered ovarian morphology, with a large number of cystic follicles and a lower total number of ovarian structures, many of which were atretic follicles. In addition, in B500 the level of granulosa cells and theca cells proliferation of early antral follicles was decreased: PCNA (% positive cells /total granulosa cells): C 51.4±3.56, B50 39.7± 6.9, B500 17.2±3.65, $p<0.001$ vs. C, $p< 0.02$ vs. B50. Discussion: high-dose BPA generated cystic follicles, lower number of ovarian structures and a decrease in proliferation. The alterations in the serum sexual hormones and GnRH pulsatiliy previously described, and the morphologic alterations in the ovary presented in this study, suggest that BPA induces a Polycystic Ovarian Syndrome-like condition in the rat.
(CONICET-UBA-ANPCYT).

13.

DIET GOATS OF GRAZING IN EXTENSIVE RANGELAND AT THE MONTE REGION IN TWO SEASON OF YEAR IN LA RIOJA

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In La Rioja, the extensive goat for meat are a very importante resource in the region because of their good adaptation. The base of the feeding is the resource forrage native. The rainfall are seasonal, dry during the winter-spring and wet during summer-fall. The aim of this work was to evaluate the botanical composition of the goats diets grazing in extensive system rangeland in two season. In winter/2008 (Inv) and summer/2009 (Ver), in a private country (province fitogeográfica of the monte, 29°05'00.02"S and 67°37'59.97"W, Chilecito), goats feces samples were collected from the rectum of 20 ramdomly-selected. The botanical composition was determine for microhistological analysis (5 compound samples), quantifying gramíneas (Gram-C3, C4), shrubs (Arbu), forbs (Lath).
The results show that do not there was differences ($p \geq 0,05$) among the groups (Gram Inv=47,6 vs. Est=56,1%, Arbu Inv=38,2 vs. Est=29,7%, Arbo Inv=7,5 vs. Est=5,9%, Lath Inv=6,7 vs. Est=8,3%) and if ($p < 0,05$) between Gram (C3 Inv=28,6 vs. C3 Est=9,3%, C4 Inv=18,9 vs. C4 Est=46,8%). In the trial, the diet goats seasonal consists principally of Gram and Arbu. In Inv the Gram C3 they are in stage of growth, join to major quality, in C4 to happen in summer, this would explain because the C3 are consumed in Inv and the C4 in summer. The goats in grazing intake trash duff, the most of the Arbu are decidous in Inv, this would explain the bearing of Arbu in the diet de Inv.

14.

DIETARY SUPPLEMENTATION OF EXTRA VIRGIN OLIVE OIL AS A PREVENTIVE AGENT IN ALZHEIMER'S DISEASE: BRAIN ANTIOXIDANT EFFECTS IN A TRANSGENIC MOUSE MODEL

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Introduction: A number of theories involving various risk factors such as diet, lifestyle, socioeconomic status, genetic predisposition and head injury have been proposed, but the degree of contribution of each factor is still controversial. However, there are two factors that appear to be widely acknowledged: along with age, chronic inflammatory reactions seem to be a mayor risk factor for the development of AD. **Objective:** Epidemiological studies indicate that anti-inflammatory, antioxidant and neuroprotective agents present in health promoting foods including those in extra virgin olive oil (EVO) may protect against age-related cognitive decline and AD, possibly through scavenging of reactive oxygen species (ROS), interleukin downregulation (i.e. IL-1β and IL-6) and strengthening the neurons antioxidant defence. **Methods:** Our pilot experiment was aimed to investigate whether a dietary supplementation of EVO during 30 days, could ameliorate or reverse the cognitive deficits and the brain oxidative damage displayed by transgenic mice overexpressing familial forms of the Amyloid Precursor Protein (APP). **Results:** We have found a 20% reduction in the total content of cortical nitrated protein and a 50% reduction in the level of IL-1β in EVO treated transgenic mice compared to corn oil treated transgenic mice. **Discussion:** The *in vivo* brain antioxidant and anti-inflammatory properties of virgin olive oil diet has been confirmed.

15.

GHRELIN AND LEPTIN ROLE IN FRUCTOSE-INDUCED INSULIN–RESISTANT RATS

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Leptin (Lp) and Ghrelin (Gh) have been reported to play a role in the interaction among the metabolic syndrome (MS), insulin resistance (IR), and testicular function. Fructose-rich diet (FRD) is a good experimental model of MS and IR. We have previously observed lower *in vitro* steroidogenic response in FRD than in Control(C) rats, and an inhibitory effect of Lp and Gh on hCG-stimulated testosterone production by both FRD and C Leydig cells. However Gh inhibitory effect was higher in FRD group. Aim: Assessment of eventual differences in the expression of Gh, Lp and its specific receptor GHSr-1a, and Ob-Rb in testicular tissue from FRD and C rats. Methodology: Normal male rats were fed with a standard commercial diet and water without (C) or with 10% fructose (FRD) for three weeks. Blood glucose, triglycerid and insulin levels were measured at sacrifice. Gh, GHSr-1a, Lp and Ob-Rb expression (relative to β actina expression) was evaluated by RT-PCR real time in testicular tissue aliquots from both groups. Results: FRD rats showed normoglycemia but impaired glucose tolerance, hypertriglyceridemia, and hyperinsulinemia. GHSr-1a expression was higher in FRD than in C testicular tissue and the opposite was observed for Ob-Rb expression ($p<0.05$). Gh and Lp expression showed low decrease and increase respectively in FRD. Discussion: Present results confirm the significant role played by Lep and Gh in testicular dysfunction observed during SM and IR. Changes in Ob-Rb and GHSr-1a expression in testicular tissue may reflect hyperleptinemia characteristic of FRD and could explain the high Gh inhibition on hCG-stimulated testosterone production found *in vitro*.

16.

MITOCHONDRIAL INSULIN DEGRADATION REGULATES ITS TRANSPORT

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Objetives- We studied if insulin degradation participates in insulin transport to mitochondria. Methods- Insulin-degrading enzyme (IDE) was extracted from rat muscles by successive chromatographic steps. Insulin degradation (gel filtration in Sephadex G50 or 5% TCA pre-cipitation) was studied in liver mitochondria at 37ºC, 30ºC and 25ºC. Mitochondria were recovered with 100% oxygen and studied with 1 ng/tube insulin and 105 c/m 125I-insulin. To make confocal studies, in-sulin and IDE were identified with specific antibodies. Results- Con-focal studies showed that IDE increased insulin in mitochondria. At 25ºC insulin degradation was decreased and IDE increased insulin accumulation in mitoplasts. At 30ºC this accumulation was less vi-sible and insulin degradation was almost instantaneous. Insulin dose/ response studies (1 ng to 10 µg) showed partial saturation of insulin transport and degradation. N-ethylmaleimide 0.1 mM increased insulin internalization (mitochondria, control: NS, IDE: $P<0.00005$; mitoplasts, control: $P<0.02$, IDE: $P<0.002$) and partially inhibits insulin degradation. Conclusion: The mitochondrial decrease in insulin degradation induced an increment in insulin transport to mitoplasts.

17.

APOPTOSIS REGULATION ON A BOVINE OVARIAN GRANULOSA ESTABLISHED CELL LINE (BGC-1)

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Gonadotrophin releasing hormone receptors (GnRHr) have been identified on ovarian granulosa (GCs) and luteal cells. The activation of GnRHr on GCs regulates the gonadal function. "*In vivo*" and "*In vitro*" hormonal regulation experiences with GnRH agonists on rat ovaries demonstrated an increase of follicular atresia. Our experimental results show a significative increment of apoptotic BGC-1 induced for 24 and 48 hours with 100 nM of Leuprolide Acetate (LA), a GnRH agonist. This was revealed with morphological techniques, TUNEL, Annexin V-FITC and PI signaling detected by flow citometry (FACS) and the results analyzed through a two-way ANOVA and means comparisons. The induction of apoptosis was partially inhibited by the addition of a GnRH antagonist (ANTIDE). The morphological analysis evaluated through DAPI, hematoxylin and Giemsa stains determined the existence of apoptotic bodies phagocyted by viable BGC-1, and this was later confirmed with TUNEL. The apoptotic activation index for the BGC-1 treated with LA was 2.16 (p=0.035), while the previous exposure to an equal concentration of ANTIDE lowered it to 1.48. Given the results we assume that the antagonist "Per Se" does not alter the control's behavior significatively, though necrosis figures where observed. The data obtained by flow citometry substantiate the morphological observations. Thus, LA augment apoptosis on BGC-1 incubated for 24 and 48 hs and this effect is partially inhibited by its competitive antagonist.

18.

TRYPTHOPAN HYDROXYLASE, ITS RELEVANCE IN EXPERIMENTAL ACUTE POPHYRIA

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Human acute porphyrias are diseases caused by alterations in heme synthesis. Their symptoms are characterized by the appearance of neurological disorders. The study of tryptophan serotoninergic pathway was carried out by determining their levels, as well as its intermediate metabolites; we also measured the activity of the rate-limiting enzyme of this pathway, tryptophan hydroxylase (TRH), which converts tryptophan to 5-hydroxytryptophan, the precursor of serotonin, whose activity is modulated by phosphorylation through a protein kinase A. Female Wistar rats were treated with 2-allyl-2-isopropylacetamide and 3,5-diethoxycarbonyl-1, 4 -dihydrocollidine. We measured the activity of 5-aminolevulinic acid synthase (ALA-S) as parameter indicator of porphyria. The levels of tryptophan, serotonin and the activity of TRH were evaluated in brain and liver by HPLC, we observed decreased values for serotonin in both tissues, an increases of tryptophan in the liver, and decreased values for the activity of TRH in both tissues (60% for brain, 30% for liver). The results suggest that increased hepatic tryptophan could be related to decreased enzyme activity, as well as observed decreased levels of serotonin. In brain, the minor level of serotonin could be related to the symptoms manifested by patients, explaining in part the neurological disorders that they present in attacks. The alteration of enzyme activity could be due to changes in its phosphorylation.

19.

XANTHINE-XANTHINE OXIDASE/ CATALASE SYSTEM IN THE METABOLISM OF CAPACITED BOVINE SPERMATOZOA

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In bovine spermatozoa, in the presence of heparin (H) or xanthine-xanthine oxidase / catalase system (X-XO-C) (superoxide anion source), capacitation, the tyrosine kinase (TK) involvement, oxygen uptake and creatine kinase B (CK-B) activity were studied. Genistein (G) was used as specific TK inhibitor. CK-B activity and lipoperoxidation (MDA) were registered spectrophotometrically at 340 and 534 nm respectively. Oxygen uptake was measured polarographically. Capacitation was evaluated by chlortetracycline technique and viability by trypan blue stain. Data were analizad by ANOVA and Tukey test ($P<0.05$). The viability was not modified by different treatments ($p>0.05$). MDA was modified by treatments. In capacitated spermatozoa with H/G, CK-B activity, oxygen uptake and capacitation decreased vs. H-treatment ($p<0.05$). Capacitation and oxygen uptake in the presence of X-XO-C at 15 min (7.38 ± 1.34 µLO2/h /) and 45 min (7.38 ± 1.5 µLO2/h/) decreased vs. H-treatment but CK-B only decreased after X-XO-C treatment at 45 min ($10.45 \pm 0.4Ux10^{-2}/10^{8}$esp) ($p<0.05$). The level of superoxide anion induces sperm capacitation, modifies MDA, without provoking respiratory burst and modulates CK-B activity, both processes dependent on tyrosine kinase phosphorylation.

20.

EVALUATION OF THE HORMETIC RESPONSE INDUCED BY COPPER IONS RELEASE IN THREE CELL LINES

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Metallic copper ions released to the medium have been shown to induce hormesis in CHO-K1 cell line. In order to evaluate if this response is cell-type specific, the toxicity of metallic copper conditioned media was studied in CHO-K1 (Cricetidae), CHE214 (*Salmonidae*), and *Aedes albopictus* (*Culicidae*) cell lines. Medium with 10% fetal bovine serum was incubated with metallic disks during different periods from 1 to 72 h. Cells were grown into medium for 24 h. As expected, lysosomal activity (estimated by Neutral Red assay) and mitochondrial activity (evaluated by MTT assay) showed a two-phase (hormetic) dose response in CHO-K1 cell line, whereas toxicity in CHE214 (*Salmonidae*) and *Aedes albopictus* (*Culicidae*) cell lines showed a linear dose response.

21.

EFECTS OF OREXIN A AND B ON THE EXPRESSION OF ITS RECEPTORS OX1 AND OX2 IN SUPEROVULATED RAT OVARIAN CELLS

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In previous works we described changes in the orexinergic hypothalamus-pituitary-ovarian system in adult rats (AJP-EM 292: E820-E828, 2007; AJP-EM, 293: E977-85. 2007). Here we continue to study *in vitro* in ovaries of superovulated prepubertal rats (SPO) the presence of OX1 and OX2 receptors and the effects of orexin A (OXA) and B (OXB) on their expression. SPO rat ovarian cells were cultured and stimulated for 48 h with OXA (10-9M) and OXB (10-9M) in the presence or absence of their specific antagonists. We evaluated the secretion of progesterone released into the medium by RIA. The quantification of the receptors was determined by real-time RT-PCR as in AJP-EM 293: E977-85. 2007.
Results: OXA stimulated OX1 [ΔΔCt OX1 C: 1.00 ± 0.00 (n = 8) vs. OXA: 2.31 ± 0.21 (n = 5), p <0.05] and OX2 receptor expression [ΔΔCt OX1 C: 1.00 ± 0.00 (n = 8) vs. OXA: 2.13 ± 0.42 (n = 5) p <0.05]. The presence of selective antagonists abolished these increases of OX1 and OX2, respectively. OXB stimulation only increased the expression of OX2 [ΔΔCt OX1 C: 1.00 ± 0.00 (n = 6) vs. OXB: 1.64 ± 0.15 (n = 6), p <0.05] without significant changes in OX1. Both OXA and OXB decrease the release of progesterone to the medium.
OXA increases the expression of OX1 and OX2. OXB increases the expression of OX2. Both decreased the release of progesterone. These findings reinforce the concept of the involvement of OX1 and OX2 receptors in ovarian function.
(ANPCYT, CONICET, UBA).

22.

IS THE AMOUNT OF UROPYGIAL GLAND´S SECRETION THE SAME FOR BIRDS OF DIFFERENT ENVIRONMENTS?

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The uropygial gland is a sebaceous glandular complex. The uropygial secretion protects the physical structure of feathers and helps with the waterproof function of plumage. Taking into account the different environments we assume that aquatic birds would produce a large amount of secretion than terrestrial birds to keep them isolated from a hostile environment. The purpose of our study was to analyze the mass of the uropygial secretion relative to the body and glandular masses in birds of different environments. Glands were removed, weighed and squeezed to obtain the secretion and then we calculated the percentage of the body and glandular masses represented by the secretion mass. We noticed that the glandular mass relative to body mass was greater in aquatic birds. The amount of secretion relative to body mass was also greater in aquatic birds, however, when considering secretion mass relative to glandular mass no differences were found between terrestrial and aquatic birds. Therefore, we state that the amount of uropygial secretion relative to body mass is related to the environment, being greater in aquatic birds, but when we considered the glandular mass we found that birds with similar glandular mass will produce the same amount of secretion, independently of the environment.

23.

PILOT STUDY: TWO YEARS EVALUATION OF TREATMENT WITH ACETYL-L-CARNITINE AND NICOTINAMIDE

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Objectives- Our purpose was to evaluate the treatment with acetyl-L-carnitine (50 mg/Kg/day) and nicotinamide (25 mg/Kg/day) in chil-dren with high risk for Type 1 Diabetes. **Methods**- Children consan-guineous of type 1 diabetic patients were studied with HLA-DQB1, antibodies (GABA, PAA, IA2A), IVGTT and serum carnitine. **Treated Patients-** Children with positive antibodies, first insulin peak (FIP)<49 μU and $\frac{\log_n (\text{ins. area}) \times K_g}{(Eq)}$<15 were treated. In the study participates 5 children (one girl developed Clinical Diabetes and could not be treated). Times of treatment are different because all patients have been included in different moments. All of them had specific antibodies, except one (RU: 6 y) still without detectable antibodies. Then, except the two girls with big differences in times of treatment (BL: 5 years and RP: 2 months), the other are close: 29, 23 and 12 months. BL, treated during 5 years without signs of intole-rance, normalized hers values long ago. The children PVTS (23 months; FIP: 85; Eq: 39) and RU (12 months; FIP: 64; Eq: 22,36) normalized their values. The other two children are in favorable evo-lution although they did not yet reach normal values. **Conclusion**- The treatment has been successful during the short time elapsed, still in children at very high diabetic risk.

24.

PARTICIPATION OF GABA AND GABAB RECEPTOS ON PANCREAS ISLET CELL PROLIFERATION

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We have previously shown that GABA and its GABAB receptors participate in the regulation of glucose homeostasis in mice with a deletion of the GABAB receptor (KO), presenting a prediabetic condition, with insulin resistance (AJP-EM 294:E157-67:2008). In addition, KO animals present very large islets not observed in wild-types (WT) pancreas. GABA, through GABAB receptors, has been demonstrated to regulate cell proliferation (Eur J Neurosci. 19:2641-9:2004). Here we studied islet cell composition (α and β cells) and proliferation indexes by immunohistochemistry (PCNA, insulin and glucagon expression) in pancreas of adult WT and KO animals.
In adult females, β cell area per pancreas area was similar between genotypes. In KO we observed a significant decrease in the % of α area/total pancreas area (WT: 0.11±0.02 vs KO: 0.06±0.01, p<0.05). In addition, a significant increase in the % of PCNA-positive α cells was observed in islets of KO mice (%PCNA+: WT: 4.1±0.5 vs KO: 12.1± 2.1, $\chi 2$: p<0.001). while the % of β PCNA-positive was similar between genotypes.
We conclude that the absence of functional GABAB receptors alters the islet cell composition, with a decrease in the area of α cells, in the presence of an increase of α cell proliferation.
(ANPCYT, CONICET, UBA).

25.

FERTILIZATION INDUCES A TRANSIENT EXPOSURE OF PHOSPHATIDYLSERINE IN MOUSE EGGS

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Phosphatidylserine (PS) is a phospholipid localized in the inner leaflet of the plasma membrane, and its exposure is a marker for apoptosis. However, recent evidence suggests that this exposure could also be associated with non-apoptotic events as well as to viral fusion. Considering the similarities between this fusion event and sperm-egg fusion, our aim was to evaluate the involvement of PS in gamete interaction. First, we observed that the addition of fluoresceinated annexin 5 (ANX5, a protein that specifically binds PS) during gamete co-incubation, did not affect the percentage of penetrated eggs at any of the concentrations tested. Surprisingly, fertilized eggs presented a positive labelling for ANX5 on their surface that was observed in intact and zona pellucida-free eggs. Non-fertilized oocytes, eggs not exposed to sperm, and eggs activated with Ca^{2+} ionophore did not present labelling, suggesting that the exposure of PS would be mediated by sperm. The follow-up of the labelling showed that it disappeared from the sperm-entry site in the decondensed head stage, it is faint in the 2-pronuclei stage and, finally, non-detectable in 2-cell embryos. Altogether, results show for the first time the existence of a transient exposure of PS in fertilized eggs not associated with apoptosis and that would be induced by sperm.

26.

PARTICIPATION OF NEURAL CADHERIN IN HUMAN GAMETE INTERACTION

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Introduction: Previous studies from our laboratory have described the localization of the adhesion molecule neural cadherin (N-cad) in the acrosomal region of intact human spermatozoa and in the equatorial segment of reacted cells. *Aims:* To evaluate the participation of N-cad in sperm interaction with the *zona pellucida* (ZP) and the oolemma. *Methods:* The hemizona and ZP-free hamster oocyte sperm penetration assays were carried out, in which gametes were preincubated with anti N-cad antibodies directed towards different extracellular domains of the protein (clone GC-4, Sigma, domain 1; H-63, Santa Cruz Biotech., domain 3-4). *Results and Discussion:* Sperm preincubation with either H-63 (10 and 100 µg/ml) or GC-4 (20 and 200 µg/ml) did not affect their ability to bind to the homologous ZP in the hemizona assay (data not shown). Contrasting, preincubation of both gametes with anti N-cad antibodies led to a significant decrease ($P<0.01$) in the number of sperm penetrations per oocyte in comparison with controls (% inhibition for H-63 20 µg/ml: 57±12%, mean±SEM, n=3 assays; GC-4 200 µg/ml: 51±11%, n=8 assays). A similar effect was found when only oocytes were preincubated with the antibodies. N-cad was immunodetected in human and hamster oocytes. The results suggest that N-cad has a role in gamete adhesion/fusion but would not participate in sperm-ZP interaction.

27.

GnRH EXPRESSION AT HYPOTHALAMUS OF *LAGOSTOMUS MAXIMUS* (PLAINS VIZCACHA)

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Gonadotropin-releasing hormone (GnRH) is synthesized in a pulsatil manner from puberty to menopause, except during pregnancy, to regulate folliculogenesis. Plains Vizcachas show natural polyovulation with corpus luteum abundance and ovulation during pregnancy. To understand the modulation of hypothalamus-pituitary-gonadal axis (HPG), the aim of this work was to characterize hypothalamic GnRH expression in the vizcacha. Vizcachas of both sexes (n=5 each), captured at Estación de Cría de Animales Silvestres (ECAS), were anesthetized with ketamin-xilacin and sacrificed with Eutanyl®. Coronal brain slices were dyed with Hematoxylin and the hypothalamic regions Preoptic Area (POA), Ventromedial Nucleus (VMN), Medial Eminence (ME) and Arcuato Nucleus (AN) were localized by comparison with histological brain atlas of rat and guinea pig. Specific immunolocalization of GnRH was observed in the cytoplasm of neurons at POA and AN (at both, somas y ramifications), and at varicosities of POA, VMN and ME. Similar GnRH distribution was detected in animals of both sexes. GnRH expression level was studied in male and female plains vizcachas and no significant differences were determined between sexes. Tissue GnRH localization and its description at hypothalamic regions involved on HPG axis in this animal would allow the comprehension of HPG axis modulation with extrapolation into human fertility pathologies.

28.

LOCALIZATION OF PITUITARY AND EXTRAPITUITARY GONADOTROPINS BY *IN SITU* HIBRIDIZATION IN PEJERREY *Odontesthes bonariensis*

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In a previous study by RT-PCR it was demonstrated the presence of α and β gonadotropins (GtHs) subunits in pejerrey brain and gonads. In this work, using *in situ* hybridization, it was possible to identify these transcripts in the brain, pituitary, and gonads of adult pejerrey of both sexes. As it was expected, the three GtHs subunits RNAm were detected in cells of pituitary *pars distalis proximalis* and *pars intermedia* and conspicuously in neurons of *nucleous lateralis lemniscus*. The three GtHs subunits were identified in testicular spermatogonia and spermatocytes, whereas in the ovary they were observed in oocytes at different developmental stages. In spite of, extrapituitary GtHs function are not known yet, these results suggest that they can play novel roles acting as brain neuromodulator and as regulators of the gonads.

29.

IDENTIFICATION OF CRISP1 PROTEIN IN THE FEMALE REPRODUCTIVE TRACT

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CRISP1, CRISP2, CRISP3 and CRISP4 are members of the CRISP (Cystein RIch Secretory Proteins) family which have been mainly characterized in the male reproductive tract. As little information exists on these proteins in females, in the present work we investigated the expression of CRISP1 protein in the female reproductive tract. The presence of CRISP1 mRNA was evaluated in uterus, ovary, oviduct and cumulus cells from adult rats by RT-PCR followed by sequencing of the PCR products. Results showed the presence of CRISP1 mRNA in all the samples tested. To study the expression of these messengers, protein extracts from uterus, ovary and oviduct were analyzed by Western blot using a specific antibody against CRISP1. In agreement with our observations, CRISP1 protein was detected in the three tissues evaluated. In addition to this, cumulus cells incubated with anti-CRISP1 and analyzed by indirect immunofluorescence exhibited a clear fluorescent labeling not observed in cells incubated with a control antibody. Together, these results confirm the expression of CRISP1 in the female reproductive tract opening the possibility of new roles for this protein within this tract.

30.

SEMEN QUALITY OF *(Dama dama)* DEER IN CAPTIVITY

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Dama dama deer was used like a model of endangered cervids for a preservation plan. The aim of the study was to determine the frozen-thawed semen quality and sperm response to heparin capacitation induction. Deers were isolated during 4-5 days before semen collection which was made by electroejaculation. Heparin induces capacitation in bovine spermatozoa. Capacitation was evaluated by chlorotetracycline epifluorescence technique. Data was analyzed by ANOVA and Tukey Test ($p<0.05$). The progressive motility percentage of ejaculated spermatozoa was 88.33±6.23% and sperm viability (78.33±9.28%) was determined by eosin-nigrosin stain. Sperm concentration mean of ejaculates was $192.83\pm8.50x10^6$ esp/ml. Two diluents were used to cryopreserve deer semen: Fructose-Tris-Glicine (FTG) and Fructose-Tris (FT); frozen-thawed semen which was diluted with FTG presented a progressive motility of 52.8±5.2% and a vigor of 3-4. From cryopreserved sperm samples with FTG diluent, the percentage of heparin capacitated spermatozoa at 15 and 45 minutes incubation were 11.43±1.90% and 19.0±2.58%, respectively showing CTC bovine sperm patterns. Semen of *Dama dama* deer maintains its quality better when it is cryopreserved using FTG diluent compared to FT diluent; heparin may be considered a capacitation inducer of cryopreserved deer spermatozoa.

31.

ANALYSIS OF SEXUALLY DIMORPHIC GENE EXPRESSION AT EARLY GONADOGENESIS OF PEJERREY *Odontesthes bonariensis* USING A HETEROLOGOUS cDNA MICROARRAY

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Gonadogenesis is the process of morphological development of the gonads. Studies on gonadal gene expression with sex-related dimorphism are important to increase our understanding on gonadogenesis and to investigate how environmental factors such as temperature can regulate or even direct development of the testes or the ovaries. We have used an EST microarray derived from medaka (*Oryzias latipes*) larval gonads, to hybridize a cDNA obtained of gonad of pejerrey larvae. Pejerrey larvae were reared at male- and female-producing temperatures (MPT and FPT respectively) during the gonadal differentiation period. A total of 94 different transcripts were identified to be induced at MPT, whereas only 30 were found at FPT. Three differentially expressed genes were selected for validation by cDNA sequencing, real-time PCR and *in situ* hybridization. The cross-species hybridization correctly identified several robustly expressed genes, providing a powerful tool to further studies in this species. Our results indicate that temperature regulates the expression of apoptotic genes especially related to testicular development.

32.

SPATIAL AND TEMPORAL EXPRESSION OF THE MUC5AC MUCIN DURING RAT DEVELOPMENT.

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Introduction: Human MUC5AC is a secreted high molecular weight mucin that polymerizes on the epithelia cell surfaces forming a mucus gel network. In adult tissues, it is synthesized by the goblet cells of the respiratory epithelia, the mucous cells of the gastric epithelia and the goblet cells of the conjunctiva. The onset of MUC5AC expression as well as its specific localization at various stages of development would provide useful information about the differentiation of the epithelia and cells involved in its synthesis. Objective: To analyze MUC5AC expression at various stages of rat development. Methodology: Eight fetuses of each stage (E) E12-E20 (12 to 20 days of gestation) were included, n=8 from each stages. The monoclonal antibody 45M1, directed against the highly conserved Cys9 domain of the human MUC5AC, was used. Inmunohistochemistry (IHC) and Western blot (WB) methodologies were employed. Results: By IHC, MUC5AC expression was observed in lung and gastric epithelia; the intensity of reaction varied from low to strong, the expression was detected from E14 in lung tissue and from E18 in gastric epithelium. Interestingly, MUC5AC was detected in the epidermis from E12 to E20. The IHC results were validated by WB; an increase in expression of the mucin in correlation with the development progress was observed. Conclusions: MUC5AC expression during rat development is temporally and spatially determined by the tissue which expresses this mucin and by the differentiation grade.

33.

IN SITU EXPRESSION PATTERNS AND MMPs ACTIVITY IN THE TROPHOBLAST-DECIDUA INTERPHASE IN MURINE MEDIAL GESTATION. POTENTIAL ROL ON VEGF EXPRESSION

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The objective is to study the localization, distribution of metalloproteinases (MMPs) and growth factor VEGF on the implantation site (IS) and MMPs activity in decidua (D) and its culture, in murine medial gestation, because no description is available so far. From superovulated female mice, mated and sacrificed at day 10 of gestation, IS were fixed for MMP-9 and VEGF immunohistochemistry and zymograms were performed using conditioned media (CM) and homogenates from D cultured for 24 h. Immunostaining was observed for MMP-9 in fetal placenta (FP) (chorionic trophoblast, spongiotrophoblast and trophoblast giant cells (TGC)). MMP-9 was expressed in decidual cells and extracellular matrix (ECM) but not in maternal endothelium. VEGF was localized in TGC while positive staining was observed in endothelium and decidual cells next to maternal vessels of the vascular mesometrial deciduas (vMD). MMP-9 activity was detected only in its active form in CM and tissue. Pro-MMP2 and MMP-2 showed activity in CM and D. These results show, for the first time, presence of MMPs and activity in maternal and fetal compartments of murine placenta at day 10 of gestation, playing a potential rol in VEGF expression during remodelling, vascularization and angiogenesis in early murine placentation.

34.

MEIOTIC RECOMBINATION PATTERNS IN A MAMMAL WITH AN ACHIASMATIC XY PAIR

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The species Meriones unguiculatus (Gerbillinae) is used as a model in basic and clinical research. This gerbid is useful for meiotic studies as it has an achiasmatic XY pair, differently from most eutherian mammals. Frequencies of total recombination, bivalent recombination rate and intrachromosomal localization will be described as well as the behaviour of the XY pair through the fluorescent immunolocalization of the proteins γ-H2AX, BRCA1, SYCP3, SYCP1, CREST and MLH1 and by basic cytogenetic techniques.

The formation of the XY body follows the same pattern as in most eutherian mammals but no synaptic junction was observed in the XY pair. BRCA1 is located on the X and Y axes. γ-H2AX differentially decorates the chromatin of the X and Y chromosomes before they are joined to each other, similarly to the pattern observed in marsupials. An end-to-end joining associates the X and Y chromosomes and these ends show only chromatin. MLH1 foci (23,47± 1,16, n= 43) are equal in number to the chiasmata at MI (22,74 ±1,29, n=31). The distribution of these foci is not random ($p<0.001$). Each bivalent shows a single MLH1 focus/chiasma –most often interstitial– and only large bivalents may occasionally show a pair of foci. The XY pair does not show MLH1 foci.

M. unguiculatus shows simultaneously the ordinary segregation mechanism for autosomes (chiasmatic) and an alternative mechanism for the XY pair. The sex chromosomes could be associated by the "stickiness" of heterochromatin, which forms a large part of the X and Y, in a way similar to that of other rodent species (Psammomys obesus, Microtus agrestis and other).

MJF is recipient of a fellowship from UBACYT.

35.

HISTO-MORPHOLOGY OF THE PLACENTA OF *LAGOSTOMUS MAXIMUS*

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Six to 10 embryos are implanted in *L. maximus* but only one or two are gestated to term (those nearest to the cervix). This selective embryo resorption could relate to a functional placenta deficiency, sustained through the prevalence of a massive abundance of secondary corpora lutea throughout gestation. The aim was to characterize the histo-morphology of the placenta of *L. maximus* throughout gestation. Five placenta belonging to early- (n=2), mid- (n=2) and late- (n=2) developing L.maximus embryos. Placenta were perfused and fixed with 4% paraformaldehide. Sagital sections were stained with haematoxylin and eosin, Masson´s trichrome and PAS. *L. maximus* showed a labyrinth placenta divided into lobes separated by interlobular trophoblast. In the lobes the maternal blood formed spaces surrounded by syncytial trophoblast. In interlobular regions, the vascular maternal channels converge into bigger channels these receiving blood from two or more adjacent lobes. The subplacenta is organized as lamellae of cytotrophoblast over a layer of mesenchymal tissue. In conclusion, the *L. maximus´* placenta is a lobulated one, with maternal-fetal exchange occuring in the labyrinths. It also shows a typical histricognatha subplacenta.

36.

EFFECT OF PREGNANCY ON EQUINE OOCYTES MATURATION

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The mare's reproductive anatomy does not allow obtaining high rates of oocytes collection; therefore it is important to recognize the limiting factors which affect the oocyte viability. The aim of this work was to assess one of them: the effect of the mare's gestation state on *in vitro* maturation of collected oocytes, after slaughter. The ovaries were collected and separated in two groups: group I: non pregnant or under 60-day pregnant mares; and group II: over 60-day pregnancies. The collection, maturation, and denudation of oocytes were carried out by standard procedures. Those who presented first polar body were considered matured. Out of a total of (I, 329 y II, 146), the number of mature, immature and degenerated oocytes was I, 177, 39 y 113; II, 53, 11 y 82 respectively. The collection rate (oocytes obtained per ovary) was I 2,7 y II 1,3. It was possible to notice a significant difference on the degenerated oocytes as well as a clear tendency on the matured ones (Chi-square $p<0,05$ test). These results suggest that both, the collection rate and *in vitro* meiotic oocytes competence were not successful on over three-month pregnant mares, which was possibly due to the endocrine changes of gestation.

37.

ENDOGENOUS HORMONAL LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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ALS is a neurodegenerative disease. Older age, shorter time to diagnosis (TD), bulbar onset and rapid progression are factors of worse prognosis. We determined serum levels of progesterone (PROG), cortisol, total testosterone and oestradiol, and correlated them with prognostic factors and survival. We measured hormones in 27 patients and 21 controls by radioimmunoassay. Hormonal levels: in ALS were 1.09 to 1.4 fold higher than controls: PROG [ALS (mean ±SEM): 0.55±0.06ng/ml vs control: 0.38±0.04 ($p<0.05$)], cortisol [17.02±1.60µg/dl vs 11.83±1.38 ($p<0.05$)], testosterone [306.15±55.94pg/ml vs 213.06±45.52 (p=ns)] and oestradiol [12.22±1.72pg/ml vs 11.15±2.50 (p=ns)]. Prognostic factors: PROG showed a negative correlation with age (R_{Rho}= -0.48, p=0.01), positive with TD (R_{Rho}=0.36, p=0.06), lower levels in bulbar onset patients (p=0.05) and rapid progressors (p=0.008). Survival: positive correlation with PROG (R_{Rho}=0.43, p=0.04). No association with prognostic factors was demonstrated among the other hormones. Discussion: increased hormonal levels in ALS can be due to activation of the hypothalamic-pituitary-adrenal axis. Nevertheless, only PROG showed an association with good prognostic factors and survival. We hypothesize endogenous PROG might display a differential role in neuroprotection.

38.

MORPHOLOGY OF PIG SUPERFICAL CERVICAL LYMPHOCENTER INO (Sus scrofa domesticus)

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The lymphatic system of the pig has been studied by several authors, however it appears that there are differences in the presence of lymphnodes and nomenclature used. The aim of this study is to describe superficial cervical lymphocenter with their dorsal, middle, ventral lymph nodes . 8 Landrace breed samples 2 to 3 months of age were used, injected with Gerota paste and fixed with 10% formalin. We observed only one superficial dorsal cervical lymph node. craniodorsal to shoulder joint, covered by the trapezius and omotransversus muscle. The ventral lymph nodes represent a chain of lymph nodes in relation to the brachiocephalic muscle, covered by the parotid gland. Regard to middle lymph nodes , are smaller than previous ones, located on the side of the external jugular vein and covered by the brachiocephalic muscle. With MO was determined that the core region is inverted as was described by other authors. With transmission electron microscopy verified that the ultrestructure is similar to parotid, mandibular and retropharyngeal lymph nodes analyzed in this species.

39.

NEUROTROPHIN RECEPTOR EXPRESSION IN ASTIANUX FASCIATUS 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 Astianux fasciatus compared with other domestic and wild animals (Caiman latirostris, pigeon, broiler chicken, Chaethofractus vellerosus vellorosus, pig, cow, horse, Lama glama, and human). 12 thymuses were fixed in 10% buffered formalin and processed with histological routine technique, embedded in paraffin. On serial sections an indirect immunocytochemical method (ABC) was applied using anti-Trks A, 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 Caiman latirostris, broiler chicken, Chaethofractus sp, pig, cow, Lama glama and human. TrkB was identified in dendritic cells of the cortico-medullary region as in pigeon. TrkC was found in type VI reticular epithelial cells as in Caiman latirostris and Chaethofractus sp. As it was observed in other vertebrate species, NTs receptors were expressed in the thymic cythoreticulum cells, suggesting a possible paracrine role of NGF (TrkA), BDNF (TrkB) and NT3 (TrkC) growth factors in the lymphocyte maturation.

40.

LABELING THE EXTRACELLULAR DOMAIN OF INSULIN RECEPTOR SPLICE VARIANTS IN LIVING CELLS

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Insulin regulates a variety of cellular processes such as transport and metabolism of glucose, lipids and proteins, nucleic acid synthesis and expression of certain genes. Two splice variants of insulin receptor (IR) exist in mammalian cells: IR-A lacking 12 aminoacids coded by exon 11, and the full length IR-B. Both isoforms behave differently in the presence of ligands and cannot be distinguished by antibodies.

Aim and methods. In order to study the dynamics of the IR in living cells we applied a labeling scheme based on the covalent modification of cell surface proteins making use of the post-translational modification of the acyl carrier protein by a phosphopantetheinyl transferase (ACPwt-S) which transfers the 4'-phosphopantetheine from coenzyme A (CoA) to a conserved serine residue of ACP.

Results and discussion. We cloned the IR-A and IR-B with an small tag (12 or 36 aminoacids) inserted in the position 1878 (pair of bases) of IR. In this way we could label extracellular domain of IR in living cells for the first time using ACPwt-S and fluorescent or biotinylated CoA. In addition, these chimeras bind biotinylated insulin revealed by confocal microscopy using streptavidin quantum dots or fluorescent streptavidin.

41.

DIFFERENTIAL CELLULAR DYNAMICS OF INSULIN RECEPTOR A AND B

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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 disregulation of its signalling pathway was described in many cancer models. Insulin receptor (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.

Aim and methods. In order to study the dynamics of the activation and internalization of the IR isoforms by microscopy in living cells we combined 2 powerful labelling techniques: streptavidin-QDs conjugated with biotinylated ligands; and visible fluorescent proteins (VFPs).

***Results and discussion*.** We generated IR fused to eGFP, CFP and YFP without affecting functionality. We demonstrated that biotin amido caproyl bovine insulin was able to bind IR and IR-VFPs (A and B) in living cells, to activate it and to induce internalization which could be imaged by confocal and programmable array microscopy (PAM). Determination of IR internalization levels in a cell by cell basis showed an early differential rate for IR-A-GFP (65±11%) and for IR-B-GFP (47±13%). These tools will allow us to study interactions between insulin and IR, binding, endocytosis and recycling processes.

42.

DEVELOPMENT AND STUDYING OF A SOLUBLE TRUNCATED RECEPTOR OF EPHRINB2, POSSIBLE PRO-ANGIOGENIC ACTIVITY

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Introduction: Eph receptors and Ephrin ligands are transmembrane proteins that interact by cell-cell contact and regulate diverse biological activities such as migration, adhesion and cell proliferation in most cell types. Recent studies have shown that the system EphB4/EphrinB2 receptor/ligand is involved in adult angiogenesis and may be involved in arteriovenous identity associated with tumorigenesis. It has been shown that both EphrinB2 expression in tumor cells and the endothelium will be associated with high vascularity. Objective: The aim of this work was to study the effect of complex EphB4/EphrinB2 in the proliferation of endothelial cells and human melanoma using truncated soluble receptors. Methodology: We designed expression vectors encoding the extracellular region fused to a portion EphrinB2 human IgG Fc (EphrinB2-Fc) and the generated recombinant proteins were purified from supernatant of transfected HEK293 cells. Results: We studied the effect of EphrinB2-Fc on *in vitro* proliferation by ^{3}H-thymidine incorporation of endothelial cells (HUVEC) and three human melanoma lines (Mel-J IBB, A-375 and M8). We have been shown that the recombinant version stimulates proliferation of endothelial and melanoma cell lines compared with untreated cells ($p<0.001$). Moreover, we determined the levels of VEGF in culture supernatants by ELISA, observing an increase in melanoma cells treated with EphrinB2-Fc. Western blot analysis also reveals an increase in VEGF expression in A375 and IBB Mel-J cells treated whit EphrinB2-Fc. Discussion: These results suggest that the truncated version of EphrinB2 acts as a pro-angiogenic molecule to two levels: by stimulating endothelial cell proliferation and inducing the expression of VEGF in melanoma cells, indicating that EphrinB2 could be a target drug in melanoma.

43.

GENOMIC INSTABILITY INDUCED BY THE HERBICIDE DICAMBA AND ITS TECHNICAL FORMULATION BANVEL®

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We have previously used different biomarkers, i.e., the comet and micronuclei assays, to demonstrate the genotoxic and cytotoxic potencial of the herbicide dicamba and its formulated product Banvel® (dicamba, 57,70%) in several cellular systems. In this communication we extend our research by analyzing nucleoplasmic bridges and nuclear buds induced *in vitro*. Exponentially growing CHO-K1 cells culture were treated with 0, 50, 100, 200 y 300 µg/ml of both compounds in the presence of cytochalasin B (3,0 µg/ml) during 24 h. The nucleoplasmic bridges and nuclear buds presence were monitored in 1000 binucleated cells. The statistical analysis was performed by the χ^2 test. The results showed: 1) an increased frequency of nucleoplasmic bridges frequency induced only by Banvel® ($P\leq$ 0,001), and 2) a highly significant nuclear bud frequency induced by both chemicals ($P\leq$ 0,001). These results confirmed our previous investigations on both compounds' deleterious ability whereas the greater genomic instability induced by Banvel® suggested the presence of excipients, in this commercial formulation, that would enhance the active principle dicamba effects.

44.

MORPHOLOGIC CHARACTERISTICS RELATED TO THE FLIGHT EFFICIENCY ON DOVES OF COMPETITION

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The level of exercise developed by birds influence the determination of characteristics as the heart size. Other morphologic characteristics are involved in the maximization of the flight efficiency. The objective was to compare the heart mass relative to the body mass and other morphologic characteristics that are involved in the flight efficiency between Rock Doves *Columba livia* selected for competition and those from the city. Data of body and heart mass, body, tail and wing length, wing chord and span from adult doves of competition (n = 12) and of the city (n = 74) were obtained. The heart mass, calculated as percentage of the body mass, was higher in doves of competition, which also presented lower body mass and length. Neither wing span nor wing length differences were found, but doves of competition presented greater wing chord and tail length. Higher heart's relative mass could directly influence the resistance and speed of flight. A greater wing chord, maintaining the same length and span indicates a greater proportion of the wing formed by the remiges, structures of low weight that produce the impulse for flight, and the larger tail would improve the sustentation. Lesser body size and mass, accompanied of higher heart mass, tail length and wing chord are characters that being selected would improve the flight efficiency.

45.

EFFECT OF THE EXTRACT OF *Calendula officinalis* L- ON OXIDATIVE DAMAGE TO BRAIN

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The production of reactive oxygen species in the brain has been implicated as a common factor in the etiology of a number of neurodegenerative diseases. *Calendula officinalis* extract (CO) rich in flavonoids, terpenoids and lutein has antioxidant activity and antiinflamatoria and has been linked with reduced risk of chronic diseases including macular degeneration, cancer and cardiovascular and neurodegenerative diseases. We studied the antioxidant effect of an aqueous extract of CO on brain mitochondria and microsomes of Wistar rats subjected to nonenzymatic peroxidation, Fe^{2+}-ascorbate-induced. The extract was added in different concentrations: 0.1 to 0.4 mg / mg of protein fractions in the study, in all cases controls were performed without the addition of the extract. The lipid peroxidation was monitored by chemiluminescence, and changes in fatty acids composition was determined by GLC. After incubation of brain mitochondria and microsomes (0.5 mg protein) in an ascorbate-Fe^{2+} system at 37ºC for 180 minutes, it was observed that the extract was reduced, concentration dependent, of chemiluminescence, measured as total cpm. The values were from 2,476,853 ± 312,987 to 1,462,044 ± 142,044 cpm and from 847,994 ± 164,551 to 561,968 ± 48.308 cpm, with the addition of 0.4 mg extract / mg prot. in mitochondria and microsomes respectively. Polyunsaturated fatty acids ranged from 29.32% in native mitochondria, 8.3% in peroxidized and 24.58% in peroxidized + 0.4 mgextrac/mg prot. This shows that protection is dose dependent.

46.

HIGH RESISTANCE FROM DIFFERENT ORGANELAS OF CHIKEN BROWN NICK OVARY AND LIVER TO THE LIPID PEROXIDATION

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Previous studies have demonstrated that birds have low degree of unsaturation of fatty acids (FA), when compared to mammals of similar body size. The aim of this study was to evaluate de FA composition and lipid peroxidation sensitivity of mitochondria and microsomes obtained from ovary and liver of young poultry hens. The FA composition of mitochondria and microsomes was obtained by gas chromatography. Lipid peroxidation (LP) was quantified by means of chemiluminescence (Q). In mitochondria and microsomes of both organelles there prevailed AG C16:0 and C18:0, AG not saturated more relevant was C18:1 and C18:2. In mitochondria of both organs we do not find C22:6. Light emission equal to chemiluminescence originated from liver and heart organelles was not statistically significant when control and peroxidized samples were compared .The AG profiles, were not modified after LP. Our results indicate that in the organelles of ovary and liver of the chicken predominantly unsaturated fatty acids with low number of double bonds. This and other factors might be the cause of the low sensitivity to LP observed in these organs, protecting them against oxidative damage.

47.

CONTROL OF PENTOSE PHOSPHATE PATHWAY DURING BOVINE OOCYTE *IN VITRO* MATURATION

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The glycolytic pathway is the main fate of the glucose uptake by bovine cumulus oocyte complexes (COCs) during *in vitro* maturation, however a fraction of glucose can lead to the pentose phosphate pathway (PPP). The Dehydrogenases of the PPP are regulated by the NADP/NADPH ratio and can be inhibited by 6-aminonicotinamide (6-AN). This work studied the effect of the inhibition by NADPH and 6-AN and the stimulation by NADP on the PPP activity (studied by Brilliant Cresyl Blue stain) and maturation (presence of metaphase II configuration) of the oocyte, and glucose uptake (GU) and lactate production (LP) (studied by spectrophotometric assays) of the COCs. Maturation was carried out in medium 199 supplemented with 5% FBS, FSH + LH (Control), NADP, NADPH and 6-AN at different concentrations. Oocytes matured in the presence of NADPH and 6-AN showed a dose dependant inhibition in PPP activity and in their progression to metaphase II ($p<0.05$). GU and LP of the COCs were not modified in the presence of NADPH, however decreased with the addition of 6-AN ($p<0.05$). None of the studied parameters changed in the presence of NADP. These results demonstrate that PPP activity is essential for bovine oocyte *in vitro* maturation. The 6-AN may be inhibiting not only PPP but also indirectly the glycolytic pathway of COCs.

48.

FIRE TEMPERATURE EFFECTS ON REGROWTH OF COOL-SEASON GRASSES

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Poa ligularis y *Amelichloa ambigua* are two cool-season perennial grass species which differ in animal preference. Fire and/or grazing history determine abundance of this species in the temperate, semiarid rangelands of central Argentina. The effect of fire temperature was evaluated at the plant center and periphery on regrowth capacity of individually-burned plants of *P. ligularis* (palatable) and *A. ambigua* (unpalatable). Burning was conducted on 23 June 2009 within an exclosure, in the Chacra Experimental de Patagones, using a portable burner. Randomly chosen, similar-size plants were burned (n=10). Fire temperature was monitored using 2 thermocouples connected to a data logger placed at the center and periphery of each plant. Burning was conducted in such a way that maximum temperature was maintained at 300 to 500 ºC at the plant center. After plant regrowth, on 07 August 2009, the number of green tillers was determined at the center and periphery of each plant. Maximum temperature was similar ($p>0.05$) in both species. However, temperature was higher ($p<0.01$) and green tiller number was lower ($p<0.01$) at the center than at the plant periphery in both species. Regrowth was greater ($p<0.05$) in *P. ligularis* than in *A. ambigua* after burning. Results suggest that low- to intermediate-intensity burnings could be used as a management tool for improving temperate, semiarid rangelands because palatable species would be favored as a result.

49.

THIOL REDUCTION AND DECONDENSATION OF SPERM NUCLEI *IN VIVO:* COOPERATIVE EFFECT OF HEPARIN AND GSH?

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Sperm chromatin decondensation requires a thiol reducing agent and a protamine acceptor. The aim of this study was to analyze the interplay between both agents during thiol reduction and decondensation of human sperm nuclei *in vitro*. Semen specimens were obtained from normozoospermic (WHO criteria) volunteers. Isolated sperm nuclei were resuspended in HTF-BSA medium and incubated with heparin (Hep) + GSH or DTT at 37°C, adding both reagents either simultaneously (30') or sequentially: thiol reducer (15') + wash + heparin (15') and viceversa. The % decondensed spermatozoa (%Des) was determined by phase contrast microscopy and thiol reduced status of chromatin was evaluated with Acridine Orange. %Des was significantly lower when Hep and GSH were used sequentially, regardless of which reagent was added first. DesR (%Des relative to %Des obtained with Hep + thiol reducer 30') 27±13% (Hep first) and 40±19% (GSH first) (n=3, ANOVA + Tukey, $p<0.05$). %Des was not affected by sequential use of reactants when DTT was used as thiol reducer: DesR 82±13% (Hep first) and 97±7% (DTT first) (NS). These results were mimicked by % nuclei with native (low thiol content, green) or denatured (high thiol content, yellow to red) chromatin. We propose the existence of a cooperative effect between protamine acceptor and thiol reducing agent *in vivo*, where GSH is known to function as thiol reducing agent.

50.

EXPRESSION OF HIPOTHALAMIC GROWTH HORMONE REGULATING FACTORS IN PEJERREY (*Odontesthes bonariensis*)

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Pejerrey represents an important resource from Argentina's ichtyofauna but the growth rates obtained in intensive culture are too low. Thus, it is important to study the endocrine control of growth in this species. As a consequence, it is necessary to establish the mechanisms that control the expression and secretion of the growth hormone (GH). In vertebrates, the hypothalamus exerts influences both inhibitory and stimulatory on GH through peptides such as somatostatin (SS), GH releasing hormone (GHRH) and the pituitary adenylate cyclase activating polypeptide (PACAP), among others. In this work, primers were designed using the medaka (Oryzias latipes) nucleotide sequences due to their filogenetic proximity. With these primers, partial sequences of pejerrey SSII, PACAP and GHRH were obtained. Furthermore, mRNA levels in different tissues and brain areas were analyzed using specific primers for pejerrey sequences. A high similarity (80-90%) was observed when compared to sequences from other fish species. The distribution pattern of PACAP mRNA was similar to that from other fish species being mainly expressed in the brain, particularly in telencephalon and hypothalamus. No expression was detected in the pituitary and cerebellum.

51.

P-AKT/AKT INCREASE IN THE CERVICAL SPINAL CORD (CSC) OF THE WOBBLER MOUSE. EFFECTS OF PROGESTERONE (PROG) AND TETRAHYDROPROGESTERONE (THP)

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive death of motoneurons. The wobbler mouse (Wr) is a model of selective motor neuron degeneration associated with astrogliosis in CSC. The Wr model is a reliable tool to understand some of the basic cellular mechanisms of motoneuron diseases such as ALS. Recent studies show that PROG and THP are neuroprotective in Wr. As the survival pathway is compromised in Wr, here we studied the relationhip P-AKT/AKT in CSC of Wr by immunoblot and the effect of different steroid treatments. Wr or control mice were treated with PROG pellet 20 mg/ 60 days or THP 3.3 mg/kg/ 30 days or vehicle. P-AKT/AKT was increased in Wr compared to control mice (Wr: 0.87±0.09 vs control: 0.37±0.017, $p=0.02$). PROG treatment increased P-AKT/AKT in wild type animals (PROG: 0.61 ± 0.03 vs controles, $p<0.01$) while THP treatment did not show any effect. In Wr animals PROG and THP treatments reduced the P-AKT/AKT compare to non-treated Wr (Wr PROG: 0.44 ± 0.08 vs Wr, $p=0.02$; Wr THP: 0.38 ± 0.04, $p<0.01$), with similar values to control animals (p=NS). Conclusions: The P-AKT/AKT increase in CSC of Wr could be related to the neurodegeneration process and gliosis, in accordance to ALS studies. PROG and THP regulate the P-AKT/AKT probably by acting on glia cells.

52.

HYPOXIA IMPAIRS THE MORPHOLOGY OF NEURONS IN CORTEX AND HIPPOCAMPUS ORGANOTYPIC CULTURES

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In vivo studies have revealed that hypoxia induced long term neuronal and glial changes. Here we studied the effect of *in vitro* hypoxia on the integrity of the 160 and 200 kDa neurofilament isoforms (NF-160, NF-200) by immunoblot. We also evaluated the effect of steroid treatments (allopregnanolone (Allo) or its synthetic analogue, Ns1) on the changes observed by hypoxia.
Results: 1 h hypoxia significantly decreased cortical NF-160 (42.3% $p<0.005$) and hippocampal NF-200 (31.3% $p<0.05$). NF-160 had an slight non-significant decreased in hippocampus (18.2%) while there were no changes in cortical NF-200 by hypoxia. To evaluate the neuroprotection properties of Allo or Ns1, the cultures were treated with these steroids 24 h prior to hypoxia and during the time of hypoxic insult. In cortex, Allo (5 μM) slightly attenuated NF-160 decreased induced by hypoxia, while Ns1 (5 μM) completly reverted NF-160 decreased ($p<0.05$). In hippocampus both Allo and Ns1 attenuated the NF-200 hypoxic decreased but the effect was not significant. These results suggest that Allo and Ns1 could prevent or attenuate the axonal damage triggered by hypoxia.
(UBACYT M012 y PICT727).

53.

MODULATION OF cAMP IN BOVINE OOCYTE MATURATION *IN VITRO*

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The meiotic arrest is regulated by cAMP levels in the oocyte. These levels are modulated by the balance between its production by adenylate cyclase (AC) and its degradation by phosphodiesterase (PDE). Forskolin (FSK) stimulates AC increasing cAMP production while hypoxanthine (HX) inhibits PDE preventing its degradation. The aim of this study was to determine the influence of the modulation of cAMP during *in vitro* maturation with FSK, HX or FSK+HX in the nuclear maturation percentage and embryo cleavage rate. Maturation was carried out in medium 199 supplemented with 5% FBS (Control), FSK, HX or FSK+HX. The oocytes' nuclear maturation was evaluated by the presence of metaphase II chromosome configuration. Fertilization took place in IVF-SOFm supplemented with BSA and heparin. The cleavage rate was determined by evaluating the number of embryos that presented 2 or more blastomeres. The oocytes supplemented with FSK, HX or FSK+HX showed a decrease in the percentage of nuclear maturation ($p<0.05$), but their cleavage was not significantly different from the control, except in the presence of FSK+HX ($p<0.05$). High levels of cAMP would be delaying the oocytes' nuclear maturation. When cAMP modulators are removed by transferring the oocytes to the medium of fertilization, the oocytes would complete the maturation, acquiring developmental competence.

54.

OCT4 EXPRESSION DURING *LAGOSTOMUS MAXIMUS* FEMALE GERM CELL DIFFERENTIATION

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Primordial Germ Cell (PGC) originates in the proximal epiblast, proliferate and migrate reaching the gonadal ridges. This process is regulated by the sequential genes expression. We analyzed the expression of the transcription factor Oct4 that has an essential role in maintaining pluripotency and in germ cell maturation during *L. maximus* germ cell differentiation. We studied 10 embryos from different post-implantation periods by immunohistochemistry. OCT4 expression was confined to the epiblast compartment in presomite embryos. At the beginning of gastrulation, OCT4 was expressed in PGGs (n <90) located at the base of the allantois. They then migrated along the dorsal mesentery and proliferated (n >180 PGCs) by mitosis with a nuclear expression. In the ovaries, OCT4 was expressed in oogonia (n>600 PGCs), but down-regulated in oocytes forming primordial follicles. Two groups of cells were evident. Germ cells showing an immature phenotype, mitotically active, and expressing OCT4, and follicle'enclosed germ cells. The pattern of expression was quite different in both groups and correlated to proliferation, profase I, and primordial folliculogenesis.

55.

DEGRADATION OF INDUSTRIAL DYES BY PURIFIED LACCASE OF THE LIGNINOLYTIC FUNGUS *TRAMETES TROGII*

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Introduction: Ligninolytic enzymes produced by white rot fungi due to their low specificity and high redox potential are able to degrade a wide range of environmental pollutants, among them industrial dyes. The potential of synthetic dye decolorization by the ligninolytic enzyme laccase has been described in several species. However, most dyes are only transformed in the presence of redox mediators. The basidiomycete *Trametes trogii* in a synthetic medium with glucose and asparagine as carbon and nitrogen sources, respectively, with the addition of copper as inducer of laccase activity, produces high titers of this enzyme (110 U/ml). Objective/methodology: In this study we investigated the ability of *T. trogii* purified laccase to decolorize dyes of different chemical structure (anthraquinonic, indigoid, heterocyclic, azo and triphenylmethane dyes). Results: Purified *T. trogii* laccase was able to effective decolorize 9 different dyes in the absence of mediators in 24 hs (95-100% of Bromphenol Blue, Indigo Carmine, Remazol Brilliant Blue R, Malachite Green, Gentian Violet and Xylidine, 65% of Fast Blue RR and around 30% of Azure B and Methylene Blue). With the addition of the mediator HBT it decolorize 100% of this three last dyes after 24 hs. In conclusion: Dye decolorization rates compare favourably with those cited for other ligninolytic fungi and demonstrated that *T. trogii* laccase may be a good tool for the bioremediation of textile processing effluents.

56.

PARTICIPATION OF HUMAN EPIDIDYMAL PROTEIN hCRISP1 IN SPERM-ZONA PELLUCIDA INTERACTION

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Human epididymal hCRISP1, as its rodent counterpart, associates with sperm during maturation and participates in gamete fusion through complementary sites in the oolema. Based on recent observations showing that rodent CRISP1 is also involved in the previous stage of sperm-zona pellucida (ZP) interaction, in the present work we investigated the participation of hCRISP1 in this specific step of the fertilization process. Human hemizona (HZ) tests showed that the presence of bacterially-expressed hCRISP1 (coupled to maltose binding protein, MBP) during gamete co-incubation produced a significant reduction in the number of sperm bound per HZ compared to HZ incubated in medium alone ($p<0.005$) or containing MBP ($p<0.005$). Indirect immunofluorescence experiments using human ZP-intact eggs confirmed the ability of hCRISP1 to bind to the ZP in addition to the reported binding of the protein to the oolema. Finally, with the aim of identifying the ZP ligand of hCRISP1, human recombinant proteins ZP2, ZP3 and ZP4 were co-incubated with hCRISP1 or MBP, and their interaction analyzed by ELISA. Results revealed that hCRISP1 specifically interacted with ZP3 in a dose-dependent manner. Together, these results support the involvement of hCRISP1 in the first stage of sperm-ZP interaction, through its specific binding to ZP3.

57.

EXPRESSION OF NEURAL CADHERIN IN HUMAN MALE REPRODUCTIVE TRACT AND IN SPERMATOZOA

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Introduction: Neural cadherin (N-cad) is a glycoprotein involved in calcium-dependent cell-cell adhesion. Our group has described its localization in human spermatozoa in different functional states *Aims:* To evaluate the expression of N-cad transcript and protein in tissues from the human male reproductive tract and in human spermatozoa. *Methods*: 1) Levels of N-cad mRNA expression in human testis and epididymis were quantitated and presence of the transcript in ejaculated spermatozoa was determined; 2) Testicular and sperm N-cad protein forms were identified and N-cad association to the male gamete was analyzed; 3) Presence and localization of N-cad protein in spermatozoa recovered from the testis were evaluated. *Results and Discussion:* N-cad transcript was detected in sperm and testicular RNA samples; transcript levels in the testis were 99.95 times higher than those in the epididymis. The complete N-cad form of 135 kDa was identified in testicular and sperm extracts, but it was not detected in the epididymis. Sperm N-cad was extracted with detergents, confirming the transmembrane nature of this protein. N-cad was mainly localized in the acrosomal region of human testicular spermatozoa (64±6%, mean±SEM; n=4), as described in ejaculated cells. The studies showed the expression of N-cad transcript and protein in human testis, and detection of the later in testicular spermatozoa, suggesting the testicular origin of the sperm N-cad.

58.

A COMMERCIAL GLYPHOSATE FORMULATION WAS ABLE TO INHIBIT CELLULAR PROLIFERATION AND INCREASE CELL DEATH OF 3T3-L1 FIBROBLASTS

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In 1996, the use of genetically modified soybean seeds was approved by the argentinian government. This glyphosate-resistant soybean and the herbicide Roundup based on glyphosate are manufactured by Monsanto. In the last years, the use of this genetically modified plant and the herbicide was dramatically increased and glyphosate was found as a contaminant in rivers and soil in areas of Buenos Aires where this soybean is extensively used. In our laboratory, we used 3T3-L1 fibroblast, this cell line proliferates previous to differentiate to adipocytes by addition of a mixture containing insulin, dexamethasone, and methylisobutylxanthine (MIX). We wanted to investigate the effect of glyphosate on the proliferation of these cells which was evaluated by cell counting in a Neubauer chamber. We found that treatment of these cells with a glyphosate-based formulation from ATANOR was able to inhibit the proliferation that takes place after the addition of the differentiation mixture in a dose-dependent manner. In addition, treatment of exponencially growing cells with this formulation was able to inhibit cell proliferation and to induce cell death. More studies are in progress to elucidate the molecular mechanism involved in these effects of glyphosate-based formulation.

59.

IVERMECTIN: CITOGENETICS STUDIES ON MOSQUITO CELLS *IN VITRO*

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Ivermectin (IVM) is a semisintetic lactone belongs to a family of avermectins isolated from the actinomycete *Streptomyces avermectinius*. It is an endectocide drug employed in both animal and human health for filariasis and onchocerciosis treatment. The effects of IVM and its formulation Ivomec® (IVO, IVM 1%) were evaluated on *Aedes albopictus* (CCL-126) cells by several genotoxicity [single cell gel electrophoresis (SCGE) and sister chromatid exchange (SCE)] and cytotoxicity [cell-cycle progression (CCP), mitotic index (MI) and MTT and Neutral Red (NR) assays] end-points. The cell cultures were treated within the 1-250 µg/ml concentration-range for 24h. The results showed that: 1) none of the compounds modified SCE frequencies, but they induced single DNA-strand breaks revealed by SCGE in 25-50 µg/ml and 5-50 µg/ml IVM- and IVO-treated cells, respectively; 2) both anthelmintics modify the PCC when 10 µg/ml was employed ($P<0.001$); 3) both IVM and IVO exerted a significant delay in MI from 25 µg/ml treatments ($P<0.001$) ; 4) a significant cellular growing inhibition was revealed by MTT and NR assays when 1-250 µg/ml were employed ($P<0.01$- $P<0.001$). These results highlight that IVM and ivomec® may induce geno- and cytotoxicity effects on *A. albopictus* cells.

60.

REGULATION OF INFLAMMATORY MEDIATORS BY POLYPHENOLS OF ROSMARINUS OFFICINALIS L. IN HUMAN COLON CARCINOMA CELL LINES

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Polyphenols appear to be important metabolic modulators due to their ability to influence several cellular signal transduction pathways. They are able to interact with various intracellular targets such as cyclooxygenase-2 (Cox-2) and several pivotal cytokines, mainly by acting through transcriptional activator protein nuclear factors and mitogen-activated protein kinase signalling. Rosemary polyphenols showed anti-inflammatory antioxidant and antitumor effects, but their mechanisms of action have not been fully investigated. We study the effect of polyphenols of rosemary on proliferation of human adenocarcinoma cell lines: Caco-2, LoVo and HT-29 cells and examined potential target signals. Here we analyzed the regulation of Cox-2 gene expression using the plasmid P2-1900, containing the luciferase reported gene under the control of the -1796 bp to +104 bp sequence of the human Cox-2 promoter. We found that the treatment with both rosemary extracts and pure carnosic acid that inhibited in a dose-dependent manner cell proliferation of Caco-2 cells is able to inhibit the phorbol ester-mediated induction of Cox-2 expression in colon carcinoma cells. Nuclear factor NF kB activation in a phorbol ester-induced system is also modulated by these compounds. This work is crucial in the valuation of these molecules as potential prophylactic and therapeutic agents.

61.
CHEMICALLY ASSISTED CLONING IN BOVINE
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Cloning is a powerful scientific and economical tool, though inefficient. Many factors affect the cloned embryos development, one of them is the oocyte enucleation. The aim of this study was to produce bovine cloned embryos using the microtubule inhibitor demecolcine (DMC), which permit the production of a cytoplasmic protrusion containing the oocyte nucleus making its identification and removal easier with no need of UV light exposure. Oocytes were *in-vitro* matured in standard conditions for 21h, when 0,4 ug/ml of DMC was added until 24h. The nucleus was removed by the extraction of the protrusion formed. Each enucleated and zona free oocyte was electro-fused with a cell from a two day FIV embryo, chemically activated and cultured individually for 7 days (n=40). Other group was not exposed to DMC and UV light was used for the enucleation procedure (n=34). Cleavage rates (67% vs 76%) and blastocyst rates (5% vs 3%) were statistically similar in both groups. The enucleation was technically easier and the cytoplasmic integrity was less compromised when DMC was used, so the cloning technology could be improved by its employment.

62.
OCT-4 EXPRESSION DURING THE EMBRYONIC DEVELOPMENT OF *LAGOSTOMUS MAXIMUS* MALE GERMLINE
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OCT-4 is a transcriptional factor associated to the maintenance of totipotenciallity and germline establishment. We studied OCT-4 expression during the embryonic development of *Lagostomus maximu*s male germline. Pregnant females in different gestational stages were captured at the ECAS. 17 male embryos in different stages of development: early (n=3), mid (n=4) and late (10) were collected. Testes were fixed 4% PFA and analyzed by immunohistochemistry for OCT-4. In early development, OCT-4 expression was weak with nuclear localization, exclusively in spermatogonia. In mid and late development, expression was also nuclear and restricted to spermatogonia, although it was remarkably increased in contrast to the previous stage. The number of seminiferous tubules with at least one positive cell increased with developmental degree. These results show a uniform and strict nuclear localization of OCT-4 throughout development and an increase in the number of positive cells as gestation progresses. This pattern is quite different to that observed in the developing ovary in where OCT-4 expression is initially high and nuclear and it decreases and translocates to the cytoplasm by the end of gestation.

63.
PPARγ AND COX-2: NOVEL THERAPEUTIC TARGETS FOR ENDOMETRIOSIS (EDT)?
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EDT is a common benign illness that affects 10% of women in reproductive age. There is constant need to better understand this affection and to improve the current medical therapies available. Previous reports demonstrated that inhibiting cyclooxygenase (COX)-2 and activating peroxisome proliferator-activated receptor (PPAR)γ have antiproliferative, proapoptotic and antiangiogenic effects in different *in vivo* and *in vitro* cancer models. In this study, we examined the effects of targeting both these molecules in a murine model of EDT. For this purpose, female BALB/c mice underwent EDT induction surgery. Treatment groups were: Control (0.5% carboxymethylcellulose, CMC, in distilled water); Celecoxib, a selective COX-2 inhibitor, (200 mg/kg in 0.5% CMC in distilled water); Rosiglitazone (0.16 mg/kg in distilled water) and Celecoxib + Rosiglitazone (combinational therapy). All treatments started the day after surgery and were administered daily by esophageal gavage for 28 days. Then animals were sacrificed and the number of implants established was counted and measured. Celecoxib alone and the combinational treatment significantly reduced the number of implants established, whereas all treatments significantly diminished their volume compared to the Control group. Cell proliferation and vascularization were assessed and all treatments were effective reducing both theses parameters, as well as enhancing apoptosis within the implants compared to the Control group. These results are encouraging to further investigate these new targets for EDT treatment.

64.
CINGULUM BUNDLE: CONSTITUTION AND FUNCTIONAL ROLE
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Introduction: The following study was designed to find out the fiber constitution of the cingulum bundle (CB) and the possible role it might play.
Objetives: a-To describe the fiber constitution of the cingulum bundle.
b- To propose a functional role of the system, considering the cortical area connectivity.
Material and methods: Ten adult human hemispheres (5 brains) were examined. Each human cadaver was fixed injecting a 10% formalin solution by common carotid artery and common femoral artery. After a month each brain was removed from the cranium and was preserved in a 50% formalin solution for at least one week. The dissection was made using wooden spatulas.
Results: The white matter of the limbic lobe (gyrus cinguli and gyrus parahippocampalis) was isolated by progressive removal of the gray matter. The core of the cingulum is seen as a circle within the limbic lobe. Along its course the cingulum projects to and receives fibers from the cingulate gyrus, frontal lobe, parietal lobe, occipital lobe, hippocampus and parahippocampal gyrus.
Discussion: We suggest the hypothesis that, the cingulum bundle might be implicated in: a- motivation and drive, b- monitoring psychosocial behaviour, c- spatial working memory, d- visual working memory, e- attention and f- learning.

65.
INFERIOR FRONTO-OCCIPITAL FASCICULUS: CONSTITUTION AND FUNCTIONAL ROLE

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Introduction: The following study was designed to find out the fiber constitution of the inferior fronto-occipital fasciculus and the possible role it might play.
Objetives: a-To describe the fiber constitution of the inferior fronto-occipital fasciculus.
b- To propose a functional role of the system, considering the cortical area connectivity.
Material and methods: Ten adult human hemispheres (5 brains) were examined. Each human cadaver was fixed injecting a 10% formalin solution by common carotid artery and common femoral artery. After a month each brain was removed from the cranium and was preserved in a 50% formalin solution for at least one week. The dissection was made using wooden spatulas.
Results: After dissecting the superior, middle and inferior temporal lobe gyri and removing insular cortex, the extreme and external capsule was exposed, and the inferior fronto-occipital fasciculus (IFOF) was identified at the level of the extreme and external capsule. The IFOF is located dorsal to the uncinate fasciculus and inferior longitudinal fasciculus and lateral to fibers of Meyer´s loop and tapetum.

66.
NTRAHEMISPHERIC ASSOCIATION TRACTS OF THE OCCIPITAL LOBE

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Introduction: The following study was designed to find out the fiber constitution of the intrahemisferic long association tracts (ILAT) of the occipital lobe and the possible role they might play.
Objetives: a-To describe the fiber constitution of the intra-hemispheric long association tracts of the occipital lobe.
b- To propose a functional role of the system, considering the cortical area connectivity.
Material and methods: Ten adult human hemispheres (5 brains) were examined. Each human cadaver was fixed injecting a 10% formalin solution by common carotid artery and common femoral artery. After a month each brain was removed from the cranium and was preserved in a 50% formalin solution for at least one week. The dissection was made using wooden spatulas.
Results: The principal ILAT of occipital lobe are: a- inferior occipitofrontal fasciculus (IOF), b- inferior longitudinal fasciculus (ILF), c- cingulum bundle (CB), d- superior longitudinal fasciculus (SLF). The detailed understanding of the superior occipitofrontal fasciculus remains to be ascertained.
Discussion: We suggest for: a- SLF: constructing visual objet in temporal-spatial contexts and language, b- ILF: recognition familiar complex objects, c- IOF: pragmatic and semantic valuation of visual objects, d- CB: emotional valuation and memorization of visual objects.
Discussion: We suggest the hypothesis that, the inferior fronto-occipital fasciculus might be implicated in: a- semantic categorization task, b- working memory, and c- attention and movement planning.

67.
EFFECTS OF Zn ON ENZYME ACTIVITIES OF *Neohelice granulata* FROM MAR CHIQUITA LAGOON (BS. AS)

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In Mar chiquita coastal lagoon (Bs. As.) zinc (Zn) levels up to 1224ug/L has been detected. The aim of this work was to study the effects of Zn exposure on Na^+K^+ATPase activity (NKA) in anterior and posterior gills (AG, PG) and maltase (Mal) and sucrase (Suc) activity in hepatopancreas (H) of the euryhaline crab *N. granulata*. Adult males acclimated for 10 days in 35‰ (osmoconformation) and 10‰ (hyperregulation) salinity (S) were exposed for 96 h in the absence or presence of Zn 1224ug/L. The supernatant (10000xg) from AG and PG (0.25 M sucrose /EGTA Tris pH7.4) and H (Tris-HCl 0.1M pH7.4) homogenates were used. NKA was determined by measuring ATP hydrolysis in the presence of (mM): 20 Imidazole (pH 7.4)/ 100 NaCl/ 30KCl/ 0.5 EGTA (Control: without KCl, with 1mM ouabain). Mal and Suc (µgglucose x min^{-1} x $mgprotein^{-1}$) was assayed by hydrolysis of maltose or sucrose in 0.1 M maleate/OHNa. In the absence of Zn, NAK in BP was higher in 10‰ S (t-test, p=0.03); in the presence of Zn was similar in 35 and 10‰ S (p>0.05). NAK in BA in the absence of Zn was similar in both S (p>0.05); in the presence of Zn was higher in 10‰ S (t-test, p=0.04). Mal and Suc both in the absence and the presence of Zn were similar in both S (p>0.05). The response of NAK in gills to Zn and the maintenance of Mal and Suc in hepatopancreas suggest a differential effect of Zn at the biochemical-physiological level and in relation to salinity.

68.
EFFECT OF METALLIC AND METAL SALT COPPER IONS ON CHE214 (*SALMONIDAE*) AND *AEDES ALBOPICTUS* (*Culicidae*) CELL LINES

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Copper is an essential element for cell metabolism. However, copper ion levels higher than basal values can induce adverse effects related to oxidative stress causing cell death. Cytotoxicity analyses were performed in order to evaluate the tolerance range to metallic and metal salt Cu of CHSE214 (*Salmonidae*) and *Aedes albopictus* (*Culicidae*) established cell lines from different taxa. Cells were exposed to increasing Cu ion concentrations in order to assess lysosomal (RN) and mitochondrial (RN) activity. Dose-response curves showed that CHSE214 cell line is more tolerant to copper ions than the other cell lines analyzed in our study. This difference might be associated to the different taxonomic origin of cells and suggests that cell lines from different species may be used as effect indicators (centinell organisms) instead of animals.

69.

EFFECTS OF ETHINYLESTRADIOL ON THE EXPRESSION OF 11β HYDROXYLASE AND GONADAL AROMATASE IN PEJERREY, *Odontesthes bonariensis*

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Endocrine disrupters are environmental contaminants that interfere with the endocrine system. The xenoestrogen ethinylestradiol (EE_2), used in birth control pills, is one of the most commonly found in aquatic ecosystems. The aim of this study was to evaluate the effect of EE_2 on gene expression of two key enzymes in gonadal steroidogenesis in teleost fish. Five month old male juvenile pejerrey fish were exposed for 10 days to environmentally relevant concentrations of EE_2 in the water (15 ng / L) or an equivalent volume of ethanol as a negative control. The expression of 11β hydroxylase (*cyp11β*) and gonadal aromatase (*cyp19a1a*) was evaluated by real time PCR in the gonads. Gonadal histology was also performed in both experimental groups. Despite no morphological differences between treated and control, EE_2 inhibited *cyp11β* and stimulated *cyp19a1a* expression, involved in the synthesis of the bioactive androgen and estrogen in teleosts: 11-ketotestosterone and estradiol, possible early molecular markers of the beginning of a feminizing process.

70.

ROLE OF MCH IN SOMATIC GROWTH IN THE CICHLID FISH *Cichlasoma dimerus*

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Fish growth is regulated by different environmental factors and this process is stimulated by growth hormone (GH), whose neuroendocrine regulation is multifactorial. Melanin concentrating hormone (MCH) is a hypothalamic hormone that innervates the adenohypophisis and, together with other hormones, participates in the endocrine control of body colour in fish. In *C. dimerus,* like in mammals, MCH could be involved in food intake. In human it has been observed that MCH may regulate pituitary GH secretion. The aim of this work was to analyze if MCH acts on the GH secretion in *C. dimerus*.

The experimental approach was performed at different levels: 1) double label immunohistochemistry (IHC) to MCH and GH, 2) analysis of GH release in pituitary cultures stimulated with MCH, and 3) quantification of somatic growth in fish maintained in white or black background (high or low MCH levels).

By double label IHC we observed MCH fibers in close contact with GH cells in the ADH. Also we observed that MCH stimulates GH secretion. Finally we observed an increase in somatic growth in fish mantained in white background.

These results suggest that MCH participes in GH secretion in *C. dimerus* and therefore, somatic growth is affected by background colour.

71.

CHROMOSOME REARRANGEMENTS IN AN *ORYCTOLAGUS CUNICULUS* DERIVED-CELL LINE

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One of the remarkable events that take place during the establishment of a cell line is the appearance of numerical and/or structural chromosome abnormalities. In the present study we evaluated the changes at chromosomal level that occur during this event in a cellular line derived from skin of rabbit in subcultures (SC) 5-25. The analysis included the karotyping of 20 R-banded metaphases by SC. The results showed that the alterations analyzed not remained stable from early SC (SC5) to late ones (SC25), varying not only the type of chromosomal alterations but also the chromosomes involved in their formation. The appearance of chromosome markers (CM) was manifested all along the scanned SCs, some in a stochstic way, and others involving long lapses (SC15-25). Some chromosomes were observed more prone to be involved in the formation of CM (chromosomes 7 and 14), reaching a frequency of up to 75-90% in SC15-25. Similar results were observed in the occurrence of aneuploidies. Among these may be noted a tendency to -19 from SC5, and -18 and -Y from SC 20. These results would evidentiate a marked chromosomal instability in this cell line mostly due to both a low frequency and to the non-stability of appearance of most of the alterations founded during the maintenance of cell culture.

72.

COMPARISON OF KEY ENZYME ACTIVITIES IN HEPATOPANCREAS OF *Neohelice granulata* FROM MUDFLAT AND SALTMARSH OF MAR CHIQUITA COSTAL LAGOON (BS. AS. PROVINCE)

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Biochemical-physiological adjustments allowing distribution of the euryhaline semiterrestrial crab *N. granulata* in microhabitats with distinct characteristics are unknown. The aim of this work was to carry out a comparative study of proteolytic (Pr), α-amylase (Am) and alkaline phosphatases (AP) activities in the hepatopancreas (HP) of adult males from mudflat (M) and saltmarsh (SM). The supernatant of 10000xg 15 min from a HP homogenate (0.1MTris-HCl pH 7.4) (4 ml buffer x g of tissue-1) was used. Pr activity (units x h-1xmg prot-1 U) was assayed by measuring azocasein hydrolysis (1 % p/v) in 0.1M Tris-HCl, pH 7.5. Am activity was determined by maltose formation from starch (15mgxml-1) (0.05M phosphate buffer, pH 5.2) with DNS reactive. AP activities (µmoles pNPxmin-1xmg prot-1) were determined by measuring pNPP hydrolisis (9.5mM) in 0.1M Tris-HCl, pHs 7.7 and 8.5 (4mM MgSO4) with (LI) and without 16 mM levamisole. LS: difference between both assays. Activities in M were: Pr 2.7±0.6U; Am: 1.2±0.26 mg maltosexmin-1xmg prot-1; AP LI: 68± 31U, y AP LS: 10.2±3.7U. Am and AP LS activities were similar, whereas Pr and AP LI were higher in SM (5.4±0.8 y 351±149 U respectively) than M (n=10, t-test, $p<0.05$). This differential flexibility at the biochemical level could be related with the distribution of *N. granulata* in distinct microhabitats of Mar Chiquita lagoon.

73.

ANTIGENOTOXIC EFFECTS OF THREE SPECIES OF *GRIFOLA* GENUS

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G. gargal, G. frondosa and *G. sordulenta* are native edible polypore mushrooms of Argentina with attributed antioxidant properties. They were evaluated for their potential antigenotoxic effects using the eye-SMART assay in *D. melanogaster* and 7-12-dimethylbenz(α)anthracene (DMBA; 25µmol/ vial) as promutagen/ procarcinogen.
Heterozygote larvae (*white/white*$^{+}$) were grown in media with colonized wheat flour (cWF) and the DMBA solution. Wheat flour, the solvent or water were used as negative controls. The induction of a mutational or recombinational event in the eye larval imaginal discs is expressed as a white spot in the red eye of adults since it causes loss of heterozygosity. The addition to the culture media of cWF with any of the three species increased survival of larvae. Spots per 100 eyes in controls were: 21 (water), 27 (solvent), 20 (wheat flour), 88 (DMBA), 79 (wheat flour+DMBA). In all combined treatments (DMBA plus *G. gargal*, *G. frondosa* or *G. sordulenta* cWF) the frequency of spots/100 eyes decreased in 30%, 25% and 20% respectively ($p<0.05$, $\chi 2$ test). The corresponding values were 57 (*G. gargal*), 62 (*G.frondosa*) and 65 (*G sordulenta*) spots/100 eyes. Conclusions: all three mushroom colonized wheat flours were non toxic, antigenotoxic and increased survival of treated larvae. b) these protective effects could be attributed to their content in antioxidants, phenolic compounds, and/or polysaccharides.

74.

$GABA_A$ RECEPTOR ACTIVITY OF OXYGEN-BRIDGED NEUROSTEROID ANALOGS

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Progesterone's metabolites like allopregnanolone (Allo) and its 5ß isomer (Preg), are produced in the nervous system and modulated the activity of the $GABA_A$ receptor. The rapid biotransformation of these steroids could be a disadvantage for therapeutic treatments. The development of synthetic analogs more stables with comparable or better activity may resolve this problem. The aim of this work was to elucidate the interaction of steroids having similar spatial conformation like Allo (SB1: 1-19 oxo; SB2: 4-19 oxo) or Preg (Ns6: 1-11 oxo Δ4) with the $GABA_A$ receptor. ^{3}H-muscimol (MUS 10nM) and ^{3}H-flunitrazepam (FLU 1nM) binding pattern were determined in cortex and cerebellum rat's synaptosomes. Incubations were made at 4ºC by 60-90 min with a range of 25 to 1000 nM of Allo, Preg, SB1, SB2 or Ns6. GABA (10µM) or Diazepam (1µM) were used for the non specific binding respectively. Allo, Ns6 and Preg stimulate the binding of both ligands (EC50 MUS= 22; 44; 118 nM ; FLU= 180;125;104 nM) meanwhile the others two steroids only stimulate FLU (EC50= SB1: 38nM SB2: 250nM) but not MUS binding. Further evaluations in live tissues or whole animals are necessary to validate these steroids as therapeutic drugs.
(UBACYT-M012 - PICT-727).

75.

3α-HYDROXY-6-19-OXIDOPREGN-4-ENE-20-ONE (Ns1) EFFECTS ON ASTROGLIOSIS INDUCED BY HYPOXIA IN ORGANOTYPIC CULTURES OF CEREBRAL CORTEX

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Some progesterone's metabolites produced in the nervous system are able to modulate the action of neurotransmitters on ion channels. One of these steroids Allopregnanolone (Allo) has showed a neuroprotective effect on organotypic cultures from cerebral cortex submitted to hypoxia. On the other hand preliminary studies have indicated that the synthetic steroid 6-19 oxo-pregnene (Ns1) shows a similar binding pattern to $GABA_A$ receptor like Allo. The aim of this study was to evaluate the possible protective action of Ns1 in an '*in vitro*' tissue culture system by determining the astroglial reaction (GFAP) during hypoxia. Tissue cultures were treated with similar concentration of Allo or Ns1 ($5x10^{-6}$M) or vehicle, 24h before and during hypoxia (1h). Then steroids were removed and 24 hours later, tissues were homogenized to determine the expression of GFAP by Western blot. Cultures subjected to hypoxia without steroid treatment showed a significant increase in the expression of GFAP (27% $p<0.05$). Pretreatment with Allo or Ns1 prevented this effect. Therefore Ns1 that shows like Allo similar ability to prevent astrogliosis induced by hypoxia is a possible candidate for future therapeutic applications.
(UBACYT M012 and PICT727).

76.

ANTIVIRAL ACTIVITY EVALUATION OF *Baccharis crispa Sreng*

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Baccharis crispa (carqueja) native specie of Cordoba, is used in infusions, for its antiseptic, antirheumatic, colagoga, diuretic and hepatic properties. Our aim was to evaluate the antiviral activity of 5 extracts of *B.crispa*: n-hexane (H), chloroform (Cl) methanol (M), cold and hot water extracts (AF and AC), on Simplex Herpes Type I (HSV-I), Venezuelan Equine Encephalitis (VEEV) and Saint Louis Encephalitis virus (SLEV). *In vitro* cytotoxicity: Several concentrations of the extracts were added in MEM and incubated in VERO cells. The cell viability was observed at 48 hs by neutral red assay (NR). Evaluation of antiviral activity: Subtoxic concentrations of extracts were inoculated on cell infected cultures and incubated at 37 ºC for 3 days for VEEV, HSV-I and 7 days for SLEV. Viruses, cell culture and different concentrations used from each extract were included as controls. The viral inhibition (%I) (estimated by RN assay) were: for Cl on VEEV (50-70%I) and on HSV-I (50-100%I); AC on HSV-I (50-60%I); AF disabled VEEV (40%I) and to HSV-I (50%I). H and M did not shown considerable antiviral activity. These results allow us to conclude that AC, AF and Cl inhibit VEEV and HSV-I. None analyzed extract inhibited SLEV. Further studies will be carried out in order to get a better understanding regarding antiviral properties of *B. crispa*.

77.

STUDIES OF THE BIODETERIORATION CAUSED BY INSECTS IN A VALUABLE HISTORIC COLONIAL SITE IN LA RIOJA PROVINCE

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Biodeterioration studies in a colonial valuable historic and turistic site in La Rioja were carried out. Analyses were made in detached material from the walls. The studied site is located at the South near to the alluvial fan of Capayán River in Famatina locality. Based on the presence of two blast furnaces, a small water reservoir and abundant rocks of vitrified waste material spread all over the ground, this site could have been a huge metal factory. Landscape setting was characterized by the presence of large adobe walls attaining up to 3.5 m height. From this group of walls two of them were much striking because of their severe deteriorated aspect. Deterioration was standing out by a vast number of ovoid cavities upholstering all over the Northern surfaces of the walls. Affected surfaces were eroded up to 15 cm inside the walls. Larvae and adults conserved within the cells aided to identify the biodeterioration causative agent as *Centris muralis*. It is a bee-like insect well known in this region as "abejorro blanco". Ovoid cavities consisted of the bee cells constructed to lay eggs and offspring development. Biodeteriorated walls exhibited an advanced degree of deterioration along with a high vulnerability to erosion. SEM micrographies exhibited important differences among biodeteriorated and nonbiodeteriorated walls.

78.

SELECTION OF BOAR SPERM CRYOPRESERVED WITH OR WITHOUT α-TOCOPHEROL

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Addition of α-tocopherol in the freezing extender prevented oxidative damage produced by cryopreservation, improving boar sperm functionality. Sephadex filtration improves sperm quality by removing dead and morphologically abnormal spermatozoa. Boar samples separated by Sephadex, previously cryopreserved with (T) or without (C) α-tocopherol were evaluated by comparing sperm quality parameters and the response to capacitation (40mM bicarbonate) and acrosomal reaction (30% follicular fluid) inducers. After selection, sperm motility, assessed by optical microscopy, and viability, evaluated by the eosin-nigrosin technique, were higher ($p<0.05$), without significant differences between T and C samples. The percentage of live sperm with intact acrosome, evaluated by trypan blue and DIC, increased in both samples post-selection ($p<0.05$). Cryocapacitation, assessed by CTC, was lower in T respect to C samples ($p<0.05$) pre- and post-selection. Percentages of bicarbonate-induced capacitation and follicular fluid-induced acrosome reaction increased after selection in both samples, with higher values in T samples ($p<0.05$). The presence of α-tcopherol and/or the removing of non viable spermatozoa would diminish ROS production improving sperm quality parameters. Samples cryopreserved with α-tocopherol and selected by Sephadex, would retain the ability to provide energy necessary to sperm motility and capacitation.

79.

DIRECT EFFECT OF GnRH AGONIST IN A CORPUS LUTEUM CULTURE FROM OVARIAN HYPERSTIMULATION SYNDROME (OHSS) MODEL DEVELOPED IN RODENTS

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Ovarian hyperstimulation syndrome (OHSS) is an iatrogenic complication caused by the induction of ovulation in fertility treatments. These factors contribute to increased vascular permeability and the formation of ovarian cysts. The objective was to analyze the direct effect *in vitro* in corpus luteum (CLs) from a OHSS model developed in rat on the levels of progesterone (P4), proliferation and apoptosis luteal. Prepubertal female rats were used and injected with high doses of PMSG (50 IU / day) for 4 days and after 24 hours were injected with hCG (25 IU). The rats were sacrificed 48 h after hCG injection. The CLs were isolated under the microscope by ovarian microdissection and incubated with and without LA (GnRH-a, 100ng/ml) for 3 hours at 37ºC. The results show that the addition of LA to the incubation medium significantly decreased P4 levels in CLs from the OHSS group compared to the untreated group. PCNA (proliferation marker) levels were significantly lower in CLs incubated with LA compared to untreated group. The levels of p17 active fragment of caspase-3 were significantly higher in the CLs from OHSS group incubated with LA compared to untreated group. In conclusion, it suggests that LA would act as an intraovarian factor on luteal cells from the syndrome, decreasing levels of P4 and cell proliferation, and increasing luteal apoptosis.

80.

THE MUSCLE SPINDLE: A CLUE FOR EARTH COLONIZATION BY VERTEBRATES. A PRIME OF PALEOBIOLOGY

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Introduction: Many structural and functional adaptations were necessary before first vertebrates were able to move from water to earth. Since Amphibians are considered to be the earliest living vertebrates settled on land, we chose the common toad *Bufo arenarum,* as a suitable model for studying some physiological and behavioral changes linked to the transit from water to land. Objectives: The objective was to investigate some of those adaptations associated with the motor control essential for migrating from the original aquatic environment to terrestrial life. Methodology: 1-open motor behaviors: postural and up-righting reflexes, walking clasping and escape, were thoroughly described in normal animals; 2-stretch responses of the forelimbs (sexual clasp) were tested in the following groups of male animals: a-normal controls; b-animals with a chronic lesion of the mesencephalic reticular formation or its reversible blocking by a microinjection of 1MKCl (0.5 µl) into the midbrain *tegmentum*; 3-finally the existence of monosynaptic spinal reflexes was tested. Results: 1-the existence of muscular proprioceptors was evident; 2-a permanent or transient unreleasable clasping, appeared after lesion or temporary exclusion of the reticular formation, respectively; 3-monosynaptic spinal responses were also shown. Discussion: amphibians exhibit antigravitatory and reproductive behaviors highly differenciated, without which earth colonization would has not been possible. These mechanisms are not present in fishes.

81.
STUDY OF THE PARTICIPATION OF GALECTIN-1 IN SPERM-EGG INTERACTION

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Galectin-1 (Gal-1) is a homodimeric animal lectin that plays a role in inmunomodulation and cellular adhesion mainly by binding to carbohydrates present in the cellular surface or the extracellular matrix. Given the important role of carbohydrates in different steps of fertilization, the aim of this study was to investigate the participation of Gal-1 in the sperm-egg interaction process. As a first approach, we evaluated by Western blot (Wb), the presence of Gal-1 in protein extracts from mouse epididymal sperm and epididymis using the testes as positive control. Results revealed the presence of Gal-1 in the epididymis but its absence in sperm. However, incubation of fresh and capacitated sperm with biotinylated Gal-1 *in vitro* and subsequent analysis of the cells by epifluorescence microscopy showed the ability of the protein to bind to the head of motile sperm. This binding was blocked when the assays were carried out in the presence of lactose, a specific ligand of this lectin. Finally, Gal-1 was detected by Wb in cumulus-oocyte complexes (COCs) obtained from superovulated Gal-1 wild type females but not in COCs from Gal-1 knock out mice. Together, the ability of Gal-1 to bind to sperm and its presence in COCs support the potential participation of this protein in gamete interaction.

82.
ACUTE TOXICITY OF AFICIDA® ON AQUATIC ORGANISMS

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Pirimicarb is a carbamate pesticide used mostly for aphid control in agriculture. So far, few data are available about its acute effects on aquatic organisms. In this study the acute toxicity of Aficida® (50% pirimicarb) was evaluated in two aquatic organisms, *Cnesterodon decemmaculatus* (Pisces: Poeciliidae) and *Rhinella arenarum* (Anura: Bufonidae). Individuals were exposed under laboratory conditions to crescents concentrations of Aficida® (80-400 mg/L). The median lethal concentration (LC_{50}) (mg/L) was the acute toxicity end-point employed and it was calculated by means of the probit analysis software (version 1.5). For both species, results demonstrated that mortality increased in a negative concentration-dependent manner. LC_{50} on *R. arenarum* reached values between 239-182 mg/L at 24 and 96 h of exposure, respectively. On *C. decemmaculatus*, those values raised up to 344-224 mg/L for 24 and 96 h, respectively. These results indicate that *R. arenarum* is a more sensitive species than *C. decemmaculatus* to the aficide exposure.

83.
ASSOCIATION OF HUMAN CRISP3 WITH SPERM AND ITS BEHAVIOUR DURING CAPACITATION

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Evidence from our group indicates that epididymal CRISP1 and testicular CRISP2, both members of the CRISP (Cysteine Rich Secretory Proteins) family, would be involved in fertilization in rodents and human. To investigate whether human CRISP3 present in seminal plasma also plays a role in this process, we analyzed the association of the protein with human sperm as well as its behaviour during capacitation. For this purpose, human sperm were subjected to different treatments and the permanence of CRISP3 in sperm was evaluated by Western blot and inmunofluorescence (IIF). Results showed the presence of two bands of 31kDa and 29kDa corresponding to glycosylated and deglycosylated CRISP3 in protein extracts from fresh, untreated sperm. While the protein corresponding to the 31kDa band was removed by washing with PBS, the one of 29kDa remained in sperm after their exposure to 0,6M NaCl, and was completely removed by 1% Tritón X-100. The 29kDa band was also present in protein extracts from both capacitated and ionophore-induced acrosome reacted sperm. Subsequent analysis of these sperm by IIF revealed the presence of fluorescent labelling in the acrosome and tail of capacitated sperm and in the equatorial segment of the acrosome reacted cells. The strong association of CRISP3 with human sperm and its permanence after capacitation and acrosome reaction supports the potential participation of this protein in the fertilization process.

84.
***Lagostomus maximus* NATURAL OVULATION**

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In the early 70s, Weir established the vizcacha's ovulation rate based on her own observations of 11 females, estimating it between 400 and 800 oocytes per reproductive cycle. Weir washed one of the two uterine horns of 6 females, the oviducts of 2 of them and both horns of 1 animal. The remaining uteri were fixed and analyzed for oocytes within them. Within the scope of female germline dynamics in *L.m,* a rodent with massive ovulation, the aim of this study was to verify ovulation rate. Animals were captured at ECAS during the 2 annual ovulatory periods (February/March, N=16 and September/October, N=10). Eggs and embryos were recovered by flushing of both horns and oviducts. Ovaries were screened for ovulation signs. Maximum ovulation for February/March was 326 oocytes with a mean value of 140, range 1-326. In September/October max value reached 205, with a mean of 116, range 29-205. These results confirm the existence of massive ovulation in *L.m* although the levels found in this study are lower than those reported by Weir.

85.

CARBOHYDRATE EXPRESSION IN UTERINE HORNS OF BALB/C MICE INFECTED WITH DIFFERENT STRAINS OF *Tritrichomonas fetus*

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The bovine tritricomonosis is a venereal disease caused by the protozoan Tritrichomonas foetus that produces embryonic death and abortion. The protozoan strains isolated from different herds in the country differ in their pathogenicity in Balb/c mice. In the pathogenesis of the disease, cell adhesion mediated by the protozoan surface glycoconjugates and host cells is very important. In a previous work, we reported that the labelling intensity of SBA and PNA lectins increased in luminal and glandular epithelia of the uterus of mice infected with *T. fetus*. The aim of this work was to confirm whether these changes are dependent on the strain of the inoculated protozoa. We used preputial smegma samples of bulls from Berker, Laprida, San Cayetano, Bolivar and Tres Arroyos, which were inoculated intravaginally in Balb/c mice. Five weeks after inoculation mice were sacrificed. Samples of uterine horns were processed for lectinhistochemistry and incubated with SBA and PNA lectins. In animals infected with the isolation from Bolivar the affinity for both lectins in luminal and glandular epithelia of the endometrium clearly increased. There is a partial relationship between the lectin binding pattern and the previous data obtained on the pathogenicity of the different strains.

86.

IDENTIFICATION OF MATRIX METALLOPROTEASES (MMPS) IN THE LAMA GLAMA OVIDUCT

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The MMPs are enzymes that hydrolyze components of the extracellular matrix. These proteins are involved in normal physiological processes and pathological events. Furthermore, MMPs are involved in reproductive function, regulating the structural changes in the uterus and ovary during the estrous cycle and pregnancy. Although,the MMPs have been associated with oocyte maturation and fertilization its function in the oviduct is still unknown. The study of the oviductal environment would be beneficial for the development of reproductive biotechnologies, especially on South American Camelids. The aim of this work was to identify the nucleotide sequences of some MMPs that are expressed in oviduct of lama. mRNA known sequence of MMP1, MMP2 and MMP9 mRNA from other mammals (cow, pig, horse, rat and human) were used to design the.primers. The texpected size products amplified, by oviductal RNA, were amplified, cloned and sequenced. The amplicon sequences showed a high identity percentage with their counterparts in other species, confirming that they corresponded to MMP1, MMP2 and MMP9. For the first time, we described and characterized MMP1, MMP2 and MMP9 mRNA fragments in llama. MMPs presence in the oviduct suggests that they might be involved in reproductive events that take place in this organ.

87.

RELATIVE HUMIDITY REGULATION DURING SOLID STATE FERMENTATION: EFFECT ON FUNGAL EXOENZYME PRODUCTION

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Introduction: Ligninolytic fungi produce exoenzymes (EE) of high industrial value. A better EE production is usually achieved culturing these fungi on Solid State Fermentation (SSF) than on submerged fermentation. SSF provides the fungus with a natural medium that not requires enzyme inducers addition. Relative humidity (RH) is a limiting factor when scaling up these systems. Objective: Regulate the quantity of water available for the microorganism with hydrogel in the medium and register its effects on EE production. Methodology: *Coriolus versicolor* var. *antarticus*, a white rot basidiomycete was cultivated on wheat bran as substrate and hydrogel (0.5%) with 75 (control without gel), 80, 83 and 85% RH; and EE production along 6 weeks was evaluated. Results: In comparison with the control, Laccase production increased 1.5 folds at day 28 of incubation with 85% RH. Manganese Peroxidase activity achieved a peak at day 14 with 80% RH and highest endoglucanase, endoxylanase and β-glucosidase enzymatic activities were registered after 3 weeks with 83% RH. β-xylosidase attained its maximum after 6 weeks with 85% RH. In conclusion: The employ of hydrogel in the medium to regulate the available water either increased and/or anticipated EE production.