BIOCELL
2009, 33(2): A191-A223

ISSN 0327 - 9545
PRINTED IN ARGENTINA

ARGENTINE SOCIETY OF BIOLOGY

(Sociedad Argentina de Biología)

Abstracts from the

Tenth Multidisciplinary Workshop

(Décima Jornada Multidisciplinaria)

December, 2008

Buenos Aires, Argentina

The abstracts were evaluated prior to publication by a scientific committee

C1.
MEMORY: FROM INVETEBRATES TU HUMANS
Maldonado H.
Laboratorio de Neurobiología de la Memoria. Instituto de Fisiología, Biología Molecular y Neurociencias-CONICET.

Recently, coinciding results of themes central in the field of neurobiology have been obtained using phylogenetically very distant animal species, from crabs to humans.
The possible universality of certain principles of organization is suggested, as well as the mechanisms and components sub-serving them in the field of neuroscience, and, more specifically, in the field of cognitive capacities, such as animal memory
In this presentation, the diverse arguments put forward in the actual debate over this supposed "mnesic universality" are analyzed. The historical antecedents of this discussion are revised, taking into account that they already appear in Darwin's first affirmations on this subject; Romanes works will be remembered as well as the critiques it evoked.
Finally, the author's own opinion on this debate is reviewed through examples of experimental results obtained in invertebrates, rodents and humans.

C2.
B-CELL ACTIVATION FACTOR FROM THE TUMOR NECROSIS FACTOR FAMILY (BAFF) DIFFERENTIALLY AFFECTS B CELL SUBSETS SURVIVAL AND CONSEQUENTLY AUTOANTIBODY PRODUCTION
Gruppi A.
CIBICI (CONICET) - Facultad de Ciencias Químicas. Universidad Nacional de Córdoba.

B cells face death throughout their lives, and the balance between their survival and death underlies the control of B cell expansion in response to pathogens, tolerance to self, and homeostasis. The control of B cell survival involves the regulation of several anti- and pro-apoptotic proteins including secreted factors, cytokines, intracellular proteins and death receptors. A fine-tuned balance is essential to preserve normal immune functions. B-cell activation factor from the tumor necrosis factor family (BAFF) is a key survival factor during B-cell maturation, a delicate immune checkpoint for B cells. Excessive BAFF production at this stage corrupts B-cell tolerance and leads to autoimmunity.
Microorganisms with pathogen associated molecular patterns (PAMP) activate B cells directly by binding to TLR and also indirectly by inducing myeloid cells to release BAFF. We found that BAFF can affect differentially B cell survival depending on the B cell subset and the PAMP that triggered B cell activation. Indeed, murine B cells activated concomitantly with a TLR-4 ligand and BAFF are protected from spontaneous apoptosis, but unexpectedly are more susceptible to Fas/CD95-mediated cell death. On the other side, we observed that BAFF repress in peritoneal B1 cells the expression of FcgammaRIIB (a receptor involved in controlling plasma cell survival and whose absence drives to autoimmunity) triggered by a TLR-9 ligand. The mechanisms underlying these processes and their consequences will be discussed.

C3.
SPERM ASSOCIATION "ROSETTES" IN RODENT EPIDIDYMIS
Monclus MA, Cabrillana ME, Vincenti AE, Fornés MW.
IHEM - CONICET - Facultad de Cs. Médicas-U.N. de Cuyo. E-mail: mmonclus@fcm.uncu.edu.ar

In rat and mouse, sperm associate forming Rosettes only in epididymal cauda. In mouse we described Rosettes for first time by optic and electronic microscopy. In rat, we isolated proteins from epididymal caudal fluid and tested theses fractions to re-associate isolated sperm *in vitro,* resembling the phenomenon observed *in vivo*. MALDI-TOF MS analyses identified the proteins present in the fraction with the highest sperm re-associating activity. Among them we found α-1-Antitrypsin and a new protein also with a "α-1-Antitrypsin like" domain, members of *serpins* (serine proteases inhibitors) family. The role of these proteins and the possible relations with epididymal sperm maturation remain unclear.

C4.
INVOLVEMENT OF THE PLASMINOGEN ACTIVATION SYSTEM IN MAMMALIAN OVIDUCT
Roldán-Olarte M.
Asociación de Biología de Tucumán. Fac. de Bioq., Qca. y Fcia, UNT – Dep. de Biol. del Desarrollo, INSIBIO. Chacabuco 461- S. M. de Tucumán. E-mail: emroldanolarte@fbqf.unt.edu.ar

The Plasminogen Activation System participates in different physiologic and pathologic processes that require a focalized and controlled proteolytic activity. It is known that this system is involved in several reproduction stages, as ovulation and embryo implantation. We determined the proteolytic activity of plasminogen activators (PAs) in porcine oviduct, showing that both (tissue type, t-PA, and urokinase type, u-PA) are present in the oviductal fluid. Semi-quantitative RT-PCR assays were performed to study t-PA and u-PA gene expression in porcine oviduct during the estrous cycle. We found that u-PA transcripts levels are higher in metestrus, while t-PA doesn't vary during the sexual cycle. The gene expression analysis in primary cell cultures of porcine oviductal epithelial cells demonstrated that both PAs are synthesized in the epithelium. An increased u-PA transcript level was observed under progesterone stimulation. The immuno-detection of u-PA receptor (u-PAR) in oviductal epithelium and porcine oocytes suggest that u-PA/ u-PAR complex could participate in the proteolytic plasminogen activation near to the oocyte and the apical epithelium. We propose that u-PA would initiate intracellular signals via u-PAR, important to the synthesis and secretion of molecules involved in the reproduction process that take place in the oviductal lumen.

S1.
GLYPHOSATE: THE HERBICIDE MOST COMMONLY USED IN SOYBEAN CROPS AND THEIR EFFECTS ON FRESHWATER ECOSYSTEMS

Pizarro H.
Depto. Ecología, Genética y Evolución, FCEyN, UBA. CONICET.

The introduction of genetically modified crops resistant to glyphosate has generated a significant increase in the use of the herbicide. Nowadays, Argentina is the second largest world-producer of soybeans resistant to glyphosate and this production has been accompanied by an increase in the use of glyphosate (over 17 million liters per year), applied at a rate of 10 liters per hectare. Despite its widespread use and assuming that it has low toxicity, there are very few studies on its impact on the environment. There is very little information on the effect of glyphosate on natural communities, and this situation is exacerbated in freshwater ecosystems where many pesticides accumulate and concentrate. We experimentally studied the effects of Roundup® (glyphosate formulation) on freshwater quality and on phytoplankton and periphyton communities using mesocosms. The total phosphorus concentration increased significantly with the addition of the herbicide, thus contributing to the phenomena of eutrophication. In the phytoplankton, the herbicide reduced the nano and microplankton densities and produced a significant increase of picocianobacterias. In periphyton, the density of algae was reduced with an increase in the proportion of cianobacteria, affecting both the community in a mature stage and its colonization and succession. Our results showed that Roundup® affects water quality and the overall functioning of aquatic systems, considering that both phytoplankton and periphyton are the basis of aquatic food webs.

S2.
GENETIC DIVERSITY IN SOUTH AMERINDIAN POPULATIONS

Carnese FR[1], Parolin ML[1], Goicoechea AS[1,2], Avena SA[1,2], Dejean CB[1].
[1]Sección Antropología Biológica, Instituto de Ciencias Antropológicas, FFyL-UBA, Argentina; [2]CONICET.

In the present study genetic diversity in South American Aboriginal groups was analysed. Several sets of markers and populations were considered in this work. Diversity at 11 protein loci and in mitochondrial DNA haplogroups were compared in 20 populations (N= 24.228): Chrirguanos, Pilagá, Chorote, Mocoví, Toba, Wichi, Mapuche(Arg.), Mapuche(Ch.), Tehuelche, Aymará, Lengua, Ayoreo, Guaraní, Kaingang, Macushi, Wapishana, Xavante, Yanomama, Makiritare and Ticuna. Other comparation was made, in relation to 11 protein loci, with class II HLA system, Y chromosome and autosomals STRs, which were compared among 12, 10 and 7 South Amerindian groups, respectively. Genetic diversity lowest value was observed at protein level (Ht= 0.308) and the highest in HLA-DRB1 locus (Ht= 0.902). The intrapopulation genetic variability (Hs) explained most of the diversity, with a variation range (Hs/Ht) from 0.753 at mtDNA to 0.937 at protein level. The lowest interpopulational genetic variability was observed at protein level (Gst'= 0.066), and the highest at mtDNA (Gst'= 0.257) and Y chromosome haplotype (Gst'= 0.226). Autosomal STRs (Gst'= 0.086) and HLA-DRB1 locus (Gst'= 0.126) presented intermediate values. Genetic variability calculated is discussed taking into acount the evolutionary, migratory, historic and demographic processes that South Amerindian populations went through.
Grant Sponsor: CONICET y UBACyT.

S3.
LIMNOLOGICAL BASE LINE STUDY IN THE URUGUAY RIVER PRIOR TO THE FUNCTIONING OF BOTNIA PULP MILL, AND PRESENT MONITORING PLAN

Izaguirre I, O´Farrell I, Pizarro H, Marinone C, Vinocur.
Fac. Ciencias Exactas y Nat. UBA. E-mail: iri@ege.fcen.uba.ar

A limnological base line study was carried out in the Uruguay River between Kms. 73.3 y 111.9, prior to the functioning of Botnia pulp mill. Phytoplankton and zooplankton communities and the main physico-chemical variables were analysed. Three sampling periods were compared, from September 2006 to April 2007. Limnological features and plankton communities were regulated by two main forces: a) the influence of the water discharge, which in turns affected suspended solids, transparency and nutrients; b) temperature variations along the year. Two high water level periods were analysed, one during a "sudestada", and another due to an increase in the water discharge upstream. Physico-chemical variables and plankton communities fluctuated in relation to seasonality and the hydrological variations. In late spring, the higher temperatures, together with a more stable water column, were the main factors regulating the plankton structure and the abiotic features. Higher temperatures favored higher plankton abundances. The biodiversity was relatively high for both phytoplankton and zooplankton. The density of potentially toxic algae was generally below the "alert level", although *Microcystis aeruginosa* and some *Anabaena* species frequently reached values that exceed the "vigilance level". To evaluate the potential impact of the pulp mill on the river, a bimonthly monitoring is carrying out from May 2008, analysing the planktonic communities and physical and chemical variables.

S4.
THE TREE OF LIFE OF ALL "FISHES:" SIGNIFICANCE, CHALLENGES, AND AN UPDATE ON THE STATUS OF THIS MEGA-PROJECT

Ortí G.

Introduction: Fishes are the dominant group of organisms in aquatic environments in terms of biomass and diversity. Current estimates set the number of living fish species at 32,500. Phylogenetic relationships among the major groups, however, remain unknown even after more than a century of study.
Objective: this presentation emphasizes the importance of a phylogenetic perspective for the study of biodiversity, stressing current challenges in both practical and theoretical arenas to undertake large-scale comparative analyses among fishes.
Methods: in the genomic era, design of an efficient approach to compare representative segments of the genome among a diversity of organisms remains an important challenge. Conceptual and practical tools to collect and analyze such information are being developed.
Results: bioinformatic analysis of genomic data bases produced a set of molecular markers designed to standardize large-scale comparisons among fishes. Explicit criteria should be used to define these markers to maximize their phylogenetic utility.
Discussion: analysis of complex multi-gene data sets to establish organismal phylogenies require a new paradigm. What is the best methodological approach to solve such a large-scale analytical challenge?

S5.
ENVIRONMENTAL IMPACT OF INTENSIVE BOVINE PRODUCTION SYSTEMS

Pérez Carrera A[1,2], Volpedo A[1], Yoshida N[1], Moscuzza H[1], Fernández Cirelli A[1,2].
[1]Centro de Estudios Transdisciplinarios del Agua, FVET, UBA. [2]CONICET. E-mail: alpc@fvet.uba.ar

The extension of the agriculture area in Argentina, as a consequence of climatic and economic factors, has produced the displacement of cattle breeding towards low aptitude lands (Northwest region) or the intensification of breeding systems (feedlots) in the Pampa plain. The impact of the intensive systems on the environment is based in the contribution of a great quantity of organic matter, nutrients (N and P) and pharmaceuticals. Runoff or infiltration processes transport the pollutants to superficial water bodies or to groundwater affecting their quality. Superficial water bodies suffer eutrophication and groundwater achieves high levels of nitrates. Other environmental effect of animal manure is the contribution of elements that increase the water turbidity producing a propitious medium for the development of pathogenic microorganisms, fact that can put on risk human health. The products more frequently used in feedlots are antibiotics, antiparasitic agents and growth promoters, which final fate is related to the chemical characteristics of the compound and to the environmental conditions. On the other hand, the climatic global change is producing different environmental effects that enhance the impact of the agricultural activities and also influence them by the reduction of grazing areas, the modification in the natural pastures and in the water quality.

S6.
HISTORICAL BIOGEOGRAPHY: THE GEOGRAPHICAL DIMENSION OF EVOLUTION

Crisci JV.
Museo de La Plata, La Plata, Argentina.

To listen to the voice of historical biogeography is a return to the birth of the evolutionary theory, as Darwin himself stated in the opening paragraph of "The Origin of the Species" (1859): "*When on board H.M.S. 'Beagle', as naturalist, I was much struck with certain facts in the distribution of the inhabitants of South America, and in the geological relations of the present to the past inhabitants of that continent. These facts seemed to me to throw some light on the origin of species...*"
Today, as in Darwin's time, the distribution of the living beings is an inexhaustible source of light on the evolution of life on Earth. Actually, there are few facets of evolutionary biology that cannot be illuminated by the study of the history of these distributions, the so-called historical biogeography. Furthermore, historical biogeography is passing through an extraordinary revolution that includes its fundamental principles, basic concepts, methods and relationships with other disciplines of comparative biology.
The specific objectives of this presentation are:
1- To discuss the overall situation in which historical biogeography occurs;
2- To outline the contemporary methodologies;
3- To present a list of critical issues that needs to be tackled.

S7.
CULTURAL HERITAGE AND DEVELOPMENT: THE IMPACT OF PRIVATE AND PUBLIC WORKS

Ratto N.
UBA, FFyL, MET.

Every productive activity involves several actors. What is curious or paradoxical is that *progress* for some can be *annihilation* for others, be it social products from actors of the present or the past. This dichotomy in values and situations can only be foreseen and calibrated through environmental impact studies in the framework of sustainable development. The essence lies in individuals, organizations and entire nations adopting this concept as a starting point to rethink the ways of interaction with the physical and socio-cultural environment. This problematic is in direct relation with the idea of *duality*, given that on the one hand there are written texts that specify why and how actions should be taken to preserve/conserve our cultural heritage, and on the other hand there are the results obtained, which are often the antithesis of the original aim to avoid or mitigate impact. This dualism is materialized in a *virtual reality* due to the existence of a declarative discourse that is not backed with executive actions. It is even stronger in the case of public works, especially provincial ones, which ignore the legislation existent their own jurisdictions and avoid social and environmental impact studies previous to any roadwork, awarding of land for agricultural tax deferment, tourism entrepreneurship, among others. Mainly, the case studies presented will focus on examples of private and public works and/or entrepreneurships in the province of Catamarca. The incorporation of heritage issues in the State policy agenda is advocated for, since *only what is valued is protected*.

S8.
WHAT IS (AND WHAT IS NOT) THE EVOLUTION

Rossi MS.
IFIBYNE-CONICET, LFBM, Facultad de Cs Exactas y Naturales, UBA. E-mail: srossi@fbmc.fcen.uba.ar

The theory of evolution for natural selection was so successful from the explanatory point of view, that natural selection is considered the almost exclusive mechanism of the evolution. We will discuss the enormous importance of hazardous processes in evolution. Also there will be revised the influence of the patterns of development, as source of evolutionary innovations. Another mistaken idea is that natural selection directs the change towards predetermined and perfect adaptations. Finally, the evolutionary biologists we will have to analyze the consequences of the epigenetic phenomena in the evolutionary process.

1.

HALOPHILE ARCHAEABACTERIA AS A MODEL FOR ASTROBIOLOGICAL STUDIES

Abrevaya XC[1], Cortón E[2], Mauas PJD[1].
[1]IAFE - CONICET - UBA; [2]QB - FCEN - UBA.
E-mail: abrevaya@iafe.uba.ar

Introduction: Halophile archaeabacteria inhabits in environments with high salt concentrations (3.4-5.1 M NaCl). They are relevant to astrobiological studies because are known inhabitants of halites and ancient evaporites in Earth, structures who were detected in Martian meteorites, so halophiles are proposed as plausible inhabitants of Mars-like planets or other extrasolar planets. Terrestrial planets around dM stars can be suitable places for the emergence and evolution of life and many of these stars emit large amount of UV radiation. ***Objectives:*** we examine UV-C effects on *Natrialba magadii* an haloalkalophile archaea to evaluate how these UV events can influence life development. ***Materials and Methods*:** *N. magadii* cultures were grown at mid-exponential phase (37ºC, rich media). Culture was diluted to reach OD_{600nm}= 0.05 and drops placed in Petri dishes. Samples were divided in groups: Non-irradiated, irradiated for 5, 10, 20, and 30 minutes. ***Results:*** OD values for each sample were obtained at different times after irradiation and plotted versus post-irradiation time. Our results show that there is a dose dependent delay in the growth of the different groups. ***Discussion:*** absence of growth for more than 30 hours, are probably attributable to t-RNA or DNA UV-related damage. Even after UV damage, the surviving cells were able to resume growth with nearly normal kinetics.

2.

ANTIVIRAL ACTIVITY OF EXTRACTS FROM *Aspidosperma quebracho blanco*

Aguilar J[1], Konigheim B[1], Núñez Montoya S[2], Contigiani M[1]
[1]Inst. Virología, Fac. Cs. Médicas. [2]Fcognosia, Fac. Cs. Qcas, IMBIV-CONICET, UNC, Cdad. Universit., Córdoba. Argentina.

Aspidosperma quebracho blanco (Apocynáceas), native plant of the argentinian Chaco, has alkaloids and flavonoids with several biological activities, which corroborate some of its numerous popular uses. Nevertheless, there are no precedents of antiviral effects for this species. Therefore, our objective was to evaluate the antiviral activity of extracts with different polarity (hexane, Cl_3CH, MetOH and aqueous), obtained from aerial parts of *A. quebracho blanco* againts the Herpex Simple Virus Type I (HSV-I) and Venezuelan Equine Encephalitis Virus (VEEV).
Decreasing dilutions of the extracts in MEM with DMSO (co-solvent) were used to evaluate the acute cytotoxicity *in vitro* in VERO cells, by means of the neutral red assay (NR). Subtoxic concentrations of each extract were inoculated in infected cellular cultures with the virus and they were incubated during 72 hs. at 37ºC. Viruses, cells and the different concentrations used from each extract were included as controls. The viral inhibition percentage (%I) was estimated by means of NR assay.
Inside of the 80-90% of cellular viability, the HSV-I was inhibited by all the extracts at different percentage; whereas the VEEV was only inhibited by the Cl_3CH and aqueous extracts. The Cl_3CH extract showed greater antiviral activity for both viruses; therefore it will be submitted to chemical studies in order to determine the active principles that are responsible for the observed bioactivity.

3.

GLYCOLYSIS REGULATION ON PORCINE OOTYTE *IN VITRO* MATURATION

Alvarez GM, Dalvit GC, Cetica PD.
INITRA, Biochemistry , School of Veterinary Sciences, University of Buenos Aires. E-mail: galvarez@fvet.uba.ar

We have previously demonstrated that glycolysis in cumulus-oocyte complexes (COCs) is stimulated by gonadotropins. The aim of the present study was to determine the effect of positive (AMP) and negative (ATP and sodium fluoride –NaF-) regulators on glycolytic pathway and meiotic maturation of oocytes. COCs were aspirated from ovarian antral follicles (3-8mm) of slaughtered gilts and selected under stereomicroscope, only oocytes surrounded by a complete and dark cumulus were used. Oocyte maturation was performed for 48 h in 199 medium supplemented with FSH and LH (control), added with different concentrations of the regulators. Glucose and lactate concentration in the culture medium was measured by spectrophotometry. Meiotic maturation was evaluated by presence of metaphase II. There was no effect of AMP neither on glucose consumption and lactate production nor nuclear maturation at any concentration used. There was a dose dependent inhibition of glucose consumption, lactate production and meiotic maturation with both ATP and NaF ($p<0.05$). AMP does not affect glycolytic activity and nuclear maturation. Inhibition of the glycolytic pathway diminish porcine oocyte *in vitro* maturation capability.

4.

BLOOD ANTIOXIDANT RESPONSES IN THE ANTARCTIC SKUAS *Catharacta maccormicki* AND *Catharacta antarctica lonnbergi*

Ansaldo M, Repetto MG, Inzillo LN, Wider EA, Montalti D.
E-mail: tincho@qb.fcen.uba.ar

*C. maccormicki (*South Polar skua) forages almost exclusively at sea (mainly fish and krill), while *C. antarctica lonnbergi* (Brown skua) monopolize the terrestrial food source (penguin eggs and chicks). Antarctic birds are exposed to severe environmental conditions, extremely low temperature and high UV radiation levels, which can increase oxidative stress.
Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities, as well as total glutathione (2GSH+GSSG), lipid peroxidation (TBARS) and protein oxidation (PO) indexes, were measured from blood samples. Results showed a response related with the type of food ingested and the age of the studied birds. South Polar skua chicks had higher blood SOD and GPx activities, as well as lower glutathione levels, than adults. On the other hand, Brown skua chicks did not show differences in the enzyme activities compared to adults, although differences in the glutathione levels were observed. GSH decreases with age; the increase observed in total glutathione could be due to an increase in the GSSG content and it is higher in adult Brown skuas. TBARS were significantly different between species but not comparing the ontogenesis. This study suggests that the type of diet plays a role in controlling the activity of the glutathione related antioxidant system.

5.

BLOOD ANTIOXIDANT STATUS OF THE ELEPHANT SEAL (*Mirounga leonina*) FEMALES AND PUPS

Ansaldo M, Inzillo LN, Carlini A, Wider EA.
E-mail: tincho@qb.fcen.uba.ar

Every September, the elephant seals (*Mirounga leonina*) arrive to the coast of Cove Potter (25 de Mayo island, Antarctica) to give birth. The period of lactation is of approximately 22 days. During lactation, mothers neither feed nor drink and lose approximately the third part of their weight, whereas pups treble their weight.
Antarctica possesses natural characteristics which potentially generate oxidative stress. In fact, Antarctic organisms should be protected against the above mentioned stress. The type of diet plays a fundamental role that can modulate the antioxidant defenses at blood level.
In the present work we measured the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferasa (GST), as well as lipid peroxidation (TBARS) and protein oxidation (OP), in blood samples of pups and females of *Mirounga leonina*.
The analyzed parameters did not show any significant differences ($p > 0.05$) between females and their pups.
Our results could be explained by a highly efficient transference of fats, proteins and water, through maternal milk which seems to be satisfactory maintaining the pup´s blood antioxidant defenses.

6.

INTRACRANIAL DURAL VENOUS SINUSES OF THE LLAMA (Lama galma)

Arzone C, Genoud P, Sánchez G, Valdés V, Vidal R.

As the blood that flows through the dural venous sinuses comes from the viscerocranium, the description of these structures in the llama is important due to the fact that infections of the facial region may potentialy spread towards the intracranial space. Moreover, dural venous sinuses contribute to hyipothalamic cooling. The study was performed on seven adult llamas. Coloured gelatin was injected as a repletive substance into tributary veins of the dural sinuses. Dissections were performed with rutinary technique and instruments. There are two systems of dural venous sinuses in the llama: dorsal and basilar. They have particular connections with caudal deep temporal vein, and the postglenoideus plexus -only presnt in camelids. Although the general structural pattern of the dural sinuses is similar in llamas, horses and dogs, the llama presents distinctive characteristics in the emisary veins.

7.

SUCRASE AND MALTASE ACTIVITIES IN HEPATOPANCREAS OF *NEOHELICE GRANULATA*: POST-INGESTA RESPONSE

Asaro A[1], López Mañanes AA[1,2], del Valle JC.
[1]Dpto Biología, FCEyN, UNMDP, Mar del Plata. [2]CONICET. E-mail: antonela_asaro@hotmail.com

Hepatopancreas has a central role in crustacean digestive physiology. Studies on key digestives enzymes in euryhaline crabs are lacking. The aim of this work was to study biochemical characteristics and post-ingesta response of sucrase (Suc) and maltase (Mal) activities in hepatopancreas of *N. granulata*. Adult males were acclimated 14 days in 35‰ salinity. The supernatant (10000xg 15min) from an hepatopancreas homogenate (0,1M Tris-HCl, pH 7,4) (4 ml buffer x g of tissue^{-1}) was used. Suc and Mal were assayed by hydrolysis of the corresponding substrate in 0.1 M maleate/OHNa 30ºC (pH curve: 3.5-8.3, S=28mM; substrate curve: S=0.56-42 mM, pH=6.4; ingesta: S=28 mM, pH=6.4). To study post-ingesta response, crabs were starved for 5 days (t0). Suc and Mal activities were assayed 2 and 4 h post-ingesta. The activity was expressed as µg glucosa x mg prot^{-1} x min^{-1}. Suc and Mal activities were similar at pH range 3.5-6.8, being 27-32% lower at pH 8 and exhibited Michaelis-Menten kinetics (Km(mM)=2.5 and 5.1). Suc and Mal did not vary after ingesta (Suc:t0=77.3±22.7, t2=67.4±17.2, t4=55.9±9) (Mal: t0=539.3±116.1, t2=406.0±102.5, t4=319±54.9) (ANOVA, $p>0.05$). At t0 Mal activity was Suc activity-dependent ($p<0.05$) whereas at t2 and t4 post-ingesta it was Suc-independent ($p>0.05$). The results show that *N. granulata* hepatopancreas exhibits Suc and Mal activities and suggest a cualitative modulation post-ingesta of maltase activity.

8.

CLEOME ACULEATA L. VAR CORDOBENSIS, A KIND OF PERENNIAL CYCLE. II. STUDY ANATOMICAL

Ateca N, Furlan Z, Gómez M.

Bibliographic background mention the species *Cleome aculeata* L. *var. Cordobensis* (Eichler & Griseb) Kuntze as grass of annual cycle , whereas the exomorphological study made by the authors determine the presence of a basal crown with yolks that make the species to be perennial. The objective of the present work is to confirm the perennial life cycle of the *C. aculeata var. cordobensis* through the anatomical study of the vegetative organs. They were made cross-sectional cuts on prepared series, and sequential cuts on root, main stem, and spaces between wood nuts of the axis recrudescence, nomophyls and petiole with safranin. The units were collected in the commune of San Jose del Morro (San Luis). The microphotographies were obtained with a Nikon binocular microscope and a Copix camera. *C. aculeata* var. *cordobensis* displays a main root of reserving nature, with secondary growth. The primary root is tetrarch with a main metaxylem vessel. The underground stem presents wood of diffuse porosity, pores simple and multiple and uni, bi and triseriate radios. The bark of both organs is characterized by the presence of loners and grouped sclereids. The epidermis of the annual aerial bud presents different types of glandular pluricellular tricommas. The stem is one eustele with collateral open sheaves, and in the basal spaces between wood knots there is activity of interfascicular cambium.

9.

CLEOME ACULEATA L. VAR CORDOBENSIS, A SPECIES OF PERENNIAL CYCLE. I. EXOMORPHOLOGICAL STUDY

Ateca N, Gómez M, Furlan Z.

Bibliographical backgrounds quote to *Cleome aculeata* L. var. *cordobensis* (Eichler & Griseb.) Kuntze as endemic shrub of annual cycle, upright habit and with ornamental value. The objective of the present work is to identify and describe the vegetative structures that justify the perennial cycle of the mentioned species. The exomorphological analysis was made on vegetative organs and the multiplication of portions of root with ligneous stem, in flowerpots. The plants were selected a vegetable community characterized by grasses and mounts, at 978 mts., of Argentina, county of San Luis, town of San José del Morro. The pictures were obtained with stereoscopic microscope and digital camera of 6 megapixeles and 12 X precision. The exomorphological analysis determined that Cleome aculeata var. cordobensis presents annual buds that are developed from yolks located in a crown form in the neck of the root and in the basal region of shafts, both ligneous and reserving organs. The air bud dies after a period of growth and from the same base are developed yolks that produce air buds in the following year. The vegetative structures and the observed in the transplant of Cleome aculeata var. cordobensis determine the cycle of perennial life.

10.

BIOLOGICAL CHARACTERIZATION OF A COMPONENT (gp39) OF THE OOCYTE VITELLINE ENVELOPE IN *Bufo arenarum*

Barrera D, García EV, Miceli DC.
INSIBIO (CONICET-UNT). Chacabuco 461. Tucumán (4000). E-mail: abarrera@fbqf.unt.edu.ar

In amphibians, the first portion of the oviduct or pars recta (PR) synthesizes and secretes a serine protease called oviductin. This enzyme produces a limited proteolysis of glycoproteins of the oocyte vitelline envelope (VE) during their transit through the PR. In consequence, the oocytes can be fertilizable by homologous sperm apparently by the exposure of sperm-binding sites on VE. Previous studies showed that oviductin action increases the relative amount of a VE glycoprotein of 39 kDa (gp39) and sequence analysis revealed that gp39 could be member of the ZPC proteins family. The aim of this work was to analyze if *B. arenarum* gp39 is involved in gamete interaction. For this purpose, spermatozoa were incubated with biotin conjugated VE glycoproteins. Also, we tested the sperm binding capacity to gp39 using antibodies generated against this gp in inmunocytochemical and inhibition of fertilization assays. The results showed that *in vitro* the sperm bind labeled gp39. Furthermore, the inhibitory effect of antibodies on fertilization indicated that gp39 might participate in sperm binding. Thus, considering the results obtained, it seems probable that *B. arenarum* gp39 is involved in the sperm adhesion mechanism to the oocyte envelope during fertilization.

11.

ANATOMY OF VEGETATIVE ORGANS OF *PANICUM MAXIMUM* CV. *GATTON PANIC* (PANICEAE)

Beltramini V, Reyna ME, Ateca NS.

Panicum maximum Jacq. cv. *Gatton panic* is vigorous specie adapted a 760 a 1000 mm precipitation. The cultivar has been demonstrated good recourse for live stock breeding in Córdoba province (Argentina). Anatomy of vegetative organs such as root, stem and leaves was studied in this work. Temporary slides of cross sections have been made following standard techniques and they were stained with safranin and safranin-astral blue. Photographs were taken under Nikon phase contrast 2, SONY camera of colour video and Aver TX GO 007 Plus Program, Slow View. The exodermis cells have suberin lamella covered by a cellulose walls most voluminous on the external tangential and radial walls. The cortex is sclerified beneath the exodermis. Endodermis has two layers and the thickening is most voluminous on the inner tangential walls. The vascular cylinder comprises the pericycle that is composed of two layers of sclerenchyma cells and vascular tissues. Phloem strands alternate with protoxylem and they are scattered through the metaxylem vessels too. The stem is a typical atactostele with collateral vascular bundles. Fiber strands occur between the small bundles and epidermis. Strands of chlorenchyma alternate with the fiber strands. This consisted in cells appeared to radiate from to the Kranz sheath where chloroplasts are peripheral placed. Leaves present PCK subtype of Kranz anatomy with mestomatic sheath between vessels.

12.

SEASONAL CHANGES ON RAM SEMINAL PLASMA LIPIDS

Bernardini A, Hozbor F, Alberio R, Cesari A.

Sheep have seasonal sexual activity in temperate regions. Males show seasonal variations in sperm production and quality, being the best semen the one from fall. The seminal plasma (SP) also presents seasonal fluctuations and changes in SP proteins have been determined. However the SP also contains a large amount and variety of lipid components whose annual changes and role in sperm function have not been fully elucidated. The aim of this study was to analyze the lipidic profile in ram SP obtained at different seasons. Seminal lipids were extracted with (2:1) chloroform:methanol from SP collected during the fall, winter, spring and summer and separated by thin layer chromatography (TLC). Chloroform: methanol (98:2) was used as mobile phase to separate cholesterol and an alkaline solvent sistem was used to separate phospholipids. The cholesterol concentration in SP from the different seasons was also measured by an enzimatic method. Phosphatidylcholine and phosphatidylserine were the major phospholipids in ram SP. The amount of these phospholipids, as well as the amount of phosphatidylethanolamine, fosfatidilinositol and cholesterol was the lowest in SP obtained after the reproductive season. The opposite was for fosfatidilinositolbisphosphate. There are seasonal variations in SP lipids that might be related to the variation of the semen quality already known.

13.

DEVELOPMENT AND INCIDENCE OF POLIPLOIDIES ON BOVINE PARTHENOGENETIC EMBRYOS ACTIVATED WITH DEHYDROLEUCODINE

Bevacqua R, Canel N, Salamone DF.

The search of activating agents for assisting somatic cell nuclear transfer (SCNT) is of big interest, as available drugs are still inefficient. Objective: to evaluate the potential of Dehydroleucodine (DhL) as bovine oocyte activator according to: 1) embryo development capability; 2) ploidy of produced embryos. Methods: cumulus oocyte complexes were collected from slaughtered ovaries and *in vitro* matured. After being denuded, oocytes were exposed to ionomycin 5 µM for 4 min and randomly allocated into the treatments: a) incubation with DhL 5 µM for 3 h; b) incubation with DhL 5 µM and cytochalasin B 5 µg mL^{-1} for 3 h (Io-DhL/CB); c) incubation with 6-Dimethylaminopurine 2 mM for 3 h (Io-DMAP) and d) no treatment. A control group was *in vitro* fertilized, following Brackett and Olliphant protocol. All treated oocytes were *in vitro* cultured during 9 days. Giemsa staining was used to determine the ploidy of 4-8 cells embryos of the activation treatments which produced blastocysts. Results: although the percentage of blastocysts produced by Io-DhL/CB (4,44) is statistically lower ($p<0.05$) than the one generated by Io-DMAP (20,93), the percentage of poliploid embryos was lower for the first group (18,75 vs. 80%). If Io-DhL/CB was employed to assist SCNT, it could be possible to reduce the rates of poliploidy and consequently, to increase the produced clones viability.

14.

IMPLICATIONS OF DIFFERENTIAL EXPRESSION OF $GABA_B$ RECEPTORS IN INFANTILE RAT MALE AND FEMALE PITUITARIES

Bianchi M[1], Calvo V[1], Libertun C[1,2], Lux-Lantos V[1].
[1]IBYME-CONICET, Fac de Medicina - [2]UBA.

Previously, we reported sexual differences in pituitary $GABA_B$ receptor ($GABA_BR$) expression at early stages of postnatal development, which are androgen dependent, and that disappeared towards the adulthood (*Neuropharmacology* 2001, 40:185-192; *Neuroendocrinology* 2004, 80:129-142). These differences may be associated to the pituitary sexual differentiation process through differential regulation of gene expression. We evaluated whether the differences in the number of pituitary $GABA_BR$ is associated to differences in intracellular pathways involved in the activation of these receptors. We performed primary cell cultures from male and female, 6-7 day-old rat adenohypophyses. $ERK_{1/2}$ activation (determined by Western blot) induced by Baclofen, a $GABA_BR$ agonist, (Bac: 10^{-4}M) was lower in male cultures, without sex differences in the response to GnRH (10^{-7}M) response. Bac 10^{-4}M decreased IP_3 levels (determined by ionic exchange cromatography) only in female cultures, which also showed higher IP_3 basal levels. Bac 10^{-4}M decreased intracelular AMPc basal levels (determined by RIA) in female primary cultures but not in males.
In conlusion, the sexual difference in pituitary $GABA_BR$ expression is accompanied by differences in the intracellular pathways involved post receptor activation.
CONICET, UBA, ANPCYT.

15.

IN VITRO* STEROID SYNTHESIS IN THE BRAIN OF PEJERREY, *Odontesthes bonariensis

Blasco M, Pérez MR, Somoza GM.
IIB-INTECH. Chascomús. Buenos Aires. Argentina. E-mail: mblasco@intech.gov.ar

Estrogens are key steroids in the development and function of the CNS in numerous vertebrate species. The enzyme P450 aromatase is responsible for the aromatization process. The studies on this enzyme have been generally performed by the demonstration of its activity, without showing the produced steroids profile. In teleosts fish, two aromatase variants has been characterized: *CYP19A1* (gonadal) and *CYP19A2* (brain). In pejerrey, it is known that the *CYP19A2* activity is greater in male brain than in female. The main objective of this work was to analyze the *in vitro* steroid production from tritium labeled androgens in pejerrey. Then, brain fragments from the forebrain and midbrain periventricular regions were incubated in L15 medium at 18°C for 6 h., using tritium labeled androstenedione (A4) or testosterone (T) as precursors. The steroids were extracted and resolved by thin layer chromatography and reverse phase liquid chromatography. For brain fragments incubated with A4, the main estrogens produced were estrone (E_1) and estradiol (E_2) whereas T incubation results in E_2. This data show that both estrogens are synthetized by adult brain of male pejerrey.

16.

DEVELOPMENT OF LC-MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF ANDROGEN IN FISH SERUM

Blasco M, Carriquiriborde P, Marino D, Ronco AE, Somoza GM.
[1]Laboratorio de Ictiofisiología y Acuicultura. IIB-INTECH. Chascomús. [2]CIMA, Cs. Exactas, UNLP, La Plata. Buenos Aires.

The 11 oxygenated androgens are known to be the biologically active androgens in fish. The RIA or ELISA methods generally used for their quantification relied on specific antibodies and usually not commercially available tracers. Furthermore, the volume of serum for multiple steroids quantifications is difficult, when not impossible, to be obtained. In this context, this work was focused on the development of a method for serum steroids determinations in goldfish (*Carassius auratus*) as a model, using a mass spectrometer coupled liquid chromatography (LC-MS) system. An Agilent 1100 LC system equipped with a reverse phase (C-18) column was used. The isocratic mobile phase consisted of 55% acetonitrile in 0.1% aqueous formic acid. The steroids: T, 11-KT y 11βOHA4 were extracted as usual, then dried and resuspended in mobile phase. Under these conditions, the three steroids were clearly resolved and quantified. The detection limit was < 0.25 ng/mL and the quantification limit < 0.5 ng/mL. The equivalent to 20 µL of sera was used for each determination. The goldfish serum T, 11KT and 11βOHA4 levels obtained for defined gonadal stages, were in agreement with the previously reported values obtained elsewhere by using RIA/ELISA. Thus, the LC-MS could be considered a valid alternative for fish physiology studies involving at least the androgens assayed here.

17.
INTRACELLULAR MECHANISMS INVOLVED IN PORCINE SPERM CAPACITATION

Breininger E, Satorre MM, Beconi MT.
Área de Química Biológica, INITRA. Facultad de Ciencias Veterinarias, Universidad de Buenos Aires.
E-mail: ebreininger@fvet.uba.ar

This study evaluates the participation of different capacitation inducers in intracellular mechanisms involved on the capacitation of cryopreserved porcine spermatozoa. Bicarbonate, adenosine and caffeine were used as capacitation inducers. These compounds produce cAMP by activating adenylate cyclases through different pathways, except caffeine that increases cAMP by the inhibition of phosphodiesterase. Capacitation was evaluated by epifluorescence chlortetracycline technique, protein tyrosine phosphorylation and the ability of spermatozoa to undergo the acrosome reaction. The participation of protein kinases were evaluated indirectly with specific inhibitors. Significative differences were observed in the level of acrosome reaction, in decreasing order with bicarbonate, caffeine or adenosine. A MW 32 kDa tyrosine-phosphorylated protein was detected with all the capacitation inducers, the highest intensity was detected with bicarbonate. Additionaly, a MW 14 kDa protein was only observed with bicarbonate. Protein kinase A, protein kinase C and tyrosine kinase were differentially activated depending on the inducer evaluated. The differences observed in the acrosome reaction could be due to the different intracellular responses triggered by the inducers.

18.
BETA-ADRENERGIC RECEPTORS INHIBIT PROLIFERATION AND STIMULATE ADHESION VIA DIFFERENT SIGNALLING PATHWAYS IN MCF-10A HUMAN BREAST CELLS

Bruzzone A, Lüthy IA, Paris H.
Instituto de Biología y Medicina Experimental - CONICET, Buenos Aires, Argentina and INSERM U858, Institut de Médecine Moléculaire de Rangueil, Toulouse, France.

Beta-adrenergic stimulation is linked to enhanced cyclic AMP (cAMP), which activates protein kinase A (PKA). However, another pathway involving the exchange protein directly activated by cAMP (Epac) has been described. Treatment of different human breast cancer cells with beta-adrenergic agonists is associated with diminished cell proliferation. The objective of the present work was to study the effect of a beta-agonist on the proliferation and adhesion of non-tumoral human breast cells, MCF-10A, and to investigate the signalling pathways involved in these actions.
MCF-10A cells incubated for 48 hs in the presence of 0.2 µM isoproterenol (ISO) exhibited a significant diminution of cell proliferation with respect to control ($37 \pm 3\%$, $p<0.001$), associated with attenuated phosphorylation of Erk 1/2. This latter effect was blocked by the PKA inhibitor H-89 and mimicked by forskolin, 8-Br-cAMP and 6-Bnz-cAMP (BNZ) but not by the Epac stimulator 8-CPT-2'-O-Me-cAMP (CTP), indicating involvement of PKA.
The exposure of MCF-10A cells to 0.2 µM ISO for 4 hs caused a significant enhancement of cell adhesion ($74 \pm 2\%$ *vs* $1.2 \pm 0.1\%$ of adherent cells after trypsin-EDTA treatment under mechanical agitation, $p<0.0001$). ISO-induced cell adhesion was not affected by H-89. On the other hand cells treated with CTP, but not BNZ, exhibited enhanced adhesion. As assessed by immunocytology with specific antibodies the effect of ISO was related with a redistribution of beta1-integrin and with reinforced cell-cell contacts.
As a conclusion, beta-adrenergic stimulation of MCF-10A cells causes diminished proliferation via the PKA-Erk 1/2 pathway and enhanced cell adhesion via Epac signalling.

19.
MORPHOLOGICAL ALTERATIONS IN THE SPERMATOPHORE OF *CHERAX QUADRICARINAUS* (PARASTACIDAE, DECAPODA)

Bugnot AB, López Greco L.
Departamento de Biodiversidad y Biología Experimental, FCEN, Universidad de Buenos Aires.

Cherax quadricarinatus is a crayfish native of Australia and Papua Nueva Guinea. It is cultured in many countries, including Argentina, and the researches concerning its biology are focused on the knowledge of the optimal conditions for its culture, and they intend developing specific techniques for reproduction and growth in captivity. In this context, the objective of this study was to evaluate the effect of the annual cycle on the macro y microscopic structure of the vas deferens of *C. quadricarinatus*. Macroscopic and histological analysis of the vas deferens have been done in animals sacrificed in summer, autumn, winter and spring. The reproductive system of males from spring and summer did not show any morphological abnormality. By the way, males sacrificed in winter and autumn presented abnormalities in the vas deferens evidenced as a brownish macroscopic coloration, and an advanced alteration in the microscopic structure of the spermatophore and the epithelium of the vas deferens. These alterations remind the deterioration and melanization of the vas deferens reported for penaeid and caridean shrimps in captivity. This work is the first to report this kind of abnormality in crayfishes.
UBACYT X458 y PICT 2004-953.

20.
SPERM PRODUCTION IN *CHERAX QUADRICARINATUS* (PARASTACIDAE, DECAPODA)

Bugnot AB, López Greco L.
Departamento de Biodiversidad y Biología Experimental, FCEN, Universidad de Buenos Aires.

Cherax quadricarinatus is an Australian freshwater crayfish that is cultured in Argentina at present. Until now, no studies have been performed on male reproduction in this species, although it has been demonstrated that sperm production affects reproduction of caridean and penaeid shrimps in captivity. Hence, the objective of this study is to evaluate the effect of the size of the male, temperature and annual cycle on the sperm production of *C. quadricarinatus*. The sperm count and mortality have been estimated, the reproductive system was weighted, and a macro and microscopic analysis of it was conducted. The size of male presents a significant regression with the sperm count and the reproductive system weight, but not with sperm mortality. The spermatophore structure has macro and microscopic differences between sizes. These results show that there is a maximum in sperm production at the usual sizes used for reproductive stock in culture. The sperm production is higher in summer, which demonstrates that males present a reproductive cycle through the year. There is a strong tendency to increase sperm production at 27ºC, so this temperature would be optimal for male reproduction.
UBACYT X458 y PICT 2004-953.

21.

PARTICIPATION OF THE NPC2 LYSOSOMAL CHOLESTEROL TRANSPORT PROTEIN IN OVARIAN PHYSIOLOGY

Busso D, Oñate-Alvarado MJ, Balboa E, Zanlungo S, Moreno RD.
Pontificia Universidad Católica de Chile.

The lysosomal NPC2 protein transports cholesterol, the substrate for the synthesis of steroids. Our previous studies showed that female NPC2 deficient-mice (NPC2-/-) were infertile, with atrophied, non-ovulating ovaries. In this work we compared different parameters of the ovarian physiology in adult wild type (+/+) and NPC2-/- females. The progression of folliculogenesis (by histology), as well as the proliferation and apoptosis of follicular cells (immunodetection of BrdU and active caspase-3) were similar. In female NPC2-/- mice, a defect in plasma estradiol concentration was observed, together with a lower expression of aromatase (RT-PCR) and an accumulation of cholesterol (Folch: *9±0,7 vs. 6±1,0 mg/g in +/+) in the ovary. The administration of exogenous gonadotropins induced a subnormal ovulation in the NPC2-/- females, with *5±2 vs. 22±2 oocytes in +/+ mice, and anomalous concentrations of steroids (Estradiol *52±6 vs. 19±1 pg/ml in +/+; Progesterone *14±2 vs. 21±2 ng/ml in +/+). These results show that a deficiency in NPC2 affects steroidogenesis, the luteinization of follicular cells and the process of ovulation, and suggest that the availability of lysosomal cholesterol could be necessary for ovarian physiology. *p<0,05.

Funding: FONDECYT 3080022 to DB, 1070622 to SZ and 1070360 to RDM.

22.

INTRAUTERINE DEVELOPMENTAL DEFECTS IN HDL RECEPTOR SR-BI KNOCKOUT MICE

Busso D, Leiva A, Moreno RD, Rigotti A.
Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile.

The cell surface receptor SR-BI mediates cholesterol uptake from HDL lipoproteins into cells. During the generation of SR-BI knockout mice, heterozygous crosses produced less SR-BI -/- pups than those expected by the mendelian distribution. In this study we evaluated if the SR-BI deficiency affected preimplantation and/or postimplantation embryo development. Embryos obtained from SR-BI +/- matings were compared to those obtained from wild-type matings. No quantitative or qualitative differences were observed in 2-cell embryos, nor in their development *in vitro* up to the blastocyst stage. At day 12 of pregnancy, the number of postimplantational embryos was similar between both types of matings. However, normal embryonic morphology was significantly lower in the SR-BI +/- than in SR-BI +/+ females (5,2±1,5 vs. 9,5±2,5; p<0,05). Genotyping of postimplantational embryos showed that 89% of those with SR-BI -/- genotype were abnormal: 62% were amorphous and the remainder were smaller than the normal size. These results show that the expression of SR-BI in the fetus or in extraembryonic membranes of fetal origin is critical for postimplantational development, supporting the importance of this lipoprotein receptor for the acquisition of maternal cholesterol.

Financed by FONDECYT 1070634 (AR), 1070360 (RM).

23.

INVOLVEMENT OF EPITHELIAL CADHERIN IN FORMATION OF THE BOVINE OVIDUCTAL SPERM RESERVOIR

Caballero JN[1], Pérez-Martínez S[2], Gervasi MG[2], Cetica PD[3], Vazquez-Levin MH[1]
[1]IBYME (CONICET-UBA), [2]CEFYBO (CONICET-UBA), [3]INITRA (School of Veterinary Sciences, UBA).

Introduction: After mating, spermatozoa with high fertilizing ability are selected by cell-cell interaction with the Oviductal Epithelial Cells (CEO) until ovulation occurs. Epithelial cadherin (E-cad) is a homophillic calcium-dependent cell-cell adhesion molecule, anchored to the actin cytoskeleton through β-catenin (β-cat). **Objectivs**: 1) To characterize E-cad and β-cat localization in both interacting cells and in spermatozoa released from the interaction, 2) To evaluate the participation of E-cad in the sperm-CEO interaction. **Methodology:** Localization studies were carried out by immunocytochemistry, and the role of E-cad in the interaction was evaluated by competition assays in the presence of specific anti E-cad antibodies. **Results**: E-cad and β-cat were immunodetected in CEO and adhering spermatozoa. The presence of DECMA-1 anti E-cad antibody caused a significant (p<0.001) decrease in the number of sperm bound to CEO cultures. In spermatozoa released from CEO, the signal for E-cad and β-cat was lost in the acrosomal cap. Same results were obtained after sperm incubation under capacitating conditions in a defined medium. **Conclusions:** E-cad would participate in the formation of the oviductal sperm reservoir by interaction of the acrosomal cap with the CEO. The release may involve the loss of E-cad in the acrosomal cap after capacitation in the presence of heparin.

24.

CADMIUM IN DRINKING WATER MODIFIES THE CELLULAR ACTIVITY OF PITUITARY GLAND ASSOCIATED WITH CHANGES IN MRNA OF PROLACTIN AND PHOSPHOLIPASE D

Calderoni AM, Biaggio V, Acosta M, Filippa V, Oliveros L, Gimenez MS, Mohamed F.
Dpto. Bqca. y Cs. Biológicas. Univ. Nac. de San Luis. IMIBIO-SL CONICET. 5700 San Luis. E-mail: amcal@unsl.edu.ar

Cadmium (Cd) is widely used in industrial applications and is an important contaminant of agricultural products. As an endocrine disruptor, Cd modifies the pituitary hormone release. We have shown that serum prolactin and phospholipase D (PLD) activity in the rat pituitary gland are decreased after Cd treatment. Now, our objective was to determine the morphological changes provoked by the 15 ppm Cd in drinking water during two months, in the pituitary gland, in adult Wistar male rat At the end of the treatment, the glands were dissected and fixed by stand methods and submitted to immuhystochemical and morphometric analysis. Cd induced a decreases in the number of lactotroph (PRL-ir) and an increases in the number of folliculo-stellate (S-100-ir) and apoptotic cells (Hoechst reactive). The RNA of pituitary gland was extracted (using TRIZOL) to determine the gene expression of prolactin and PLD by RT-PCR. The mRNA levels of prolactin and PLD were decreased by Cd treatment in relation to the controls. These agree with the serum prolactin and PLD activity previously found in our laboratory. Cd modifies the cellular activity of pituitary gland.

25.

INSULIN TRANSPORT TO MITOCHONDRIA

Camberos MC, Passicot GA, Cao G, Cresto JC.
CEDIE, Endocrinología, Htal. de Niños "R. Gutierrez"; Anatomía Patológica, Htal. de Niños "P. Elizalde", Bs. Aires, Argentina.

Mitochondria respond to hormonal changes. Otherwise we showed that insulin-degrading enzyme (IDE) is present in mitochondrial matrix.
Objectives-The study was directed to know if IDE transport insulin and if it increases its incorporation to mitochondria. **Methods**-IDE was obtained from muscle rats from successive chromatogramphics steps. Hepatic mitochondria were isolated with Parson´ procedure, recovered and incubated at 25ºC with 100% oxygen, insulin 1ng/tube and ^{125}I-insulin (10^5 c/m). Depend on the experiment, IDE, substrates and inhibitors were added. In some experiments, isolated mitochondria were studied with immu-nofluorescence and confocal microscopy. **Results**-Electro micros-copy showed normal shape mitochondria. There was not insulin degradation by IDE at 25ºC. IDE increased and bacitracin, dinitrophenol and apyrase decreased insulin incorporation to mitochondria. Cromatographic profiles showed a complex formed by insulin and mitochondrial transporters, and after dissociation free insulin was recovered. Confocal studies showed that insulin is transported to mitochondrial matrix. **Conclussions**-1) IDE transport insulin using its own transporters (TOM and TIM). 2) Insulin is transported into mitochondria. 3) The insulin transport is active.

26.

MITOCHONDRIAL FUNCTION AND CREATINE KINASE SHUTTLE ARE INVOLVED IN TYROSINE KINASE ACTIVITY ON THE BOVINE SPERMATOZOA CAPACITATION

Casas E, Córdoba M
Instituto de Investigación y Tecnología en Reproducción Animal (INITRA), Química Biológica, Fac de Ciencias Veterinarias. UBA.

The aim of this work was to determine the relation of phosphorylation by tyrosine kinase with oxygen uptake and creatine kinase B (CK-B) activity, as factors that participate in the metabolism to obtain the sperm energy. Genistein was used as specific inhibitor of tyrosine kinase. CK-B activity was registered spectrophotometrically at 340 nm. Oxygen uptake was measured polarographically. Capacitation was evaluated by chlortetracycline and the viability by trypan blue stain. Data were analizad by ANOVA and Tukey test ($P<0.05$). Capacitation percentages, with and without genistein, were different vs control, but not significant differences were observed in sperm viability ($p<0.05$). In spermatozoa capacitated in the presence of genistein, CK-B activity (26,8±7.6 $x10^{-2}$/ 10^8esp, oxygen uptake (7.39±1.73µL O_2/h/) and capacitation percentage (12.3±2.4%) decreased vs their controls ($p<0.05$). Tyrosine phosphorylation is required to maintain mitochondrial function and CK-B activity that are necessary for capacitation induction.

27.

THE EXPRESION OF PROLACTIN RECEPTOR mARN IS NOT REGULATED BY ADRENERGIC COMPOUDS IN THE T47D HUMAN BREAST CANCER CELL LINE.

Castillo LF, Sarappa G, Pérez Piñero C, Bruzzone A, Lüthy IA.
Instituto de Biología y Medicina Experimental – CONICET, Buenos Aires, Argentina.

Catecholamine secretion increases during stress, and breast cancer diagnosis generates distress to most patients. Another hormone that increases during stress is Prolactin (PRL), which exerts a mitogenic effect acting through the prolactin receptor (PRLR). PRL is also synthesized by normal and tumor breast tissue, giving place to an autocrin/paracrine loop. Previously, using the Nb2 bioassay we have shown that T47D cells increase PRL synthesis and release in the presence of α_2-adrenergic agonists. Then, part of the mitogenic effect generated by adrenergics compounds could eventually be due to increased PRL synthesis by the cells. We have previously demonstrated α_2-adrenoceptors (α_2-RA) expression on T47D human breast cancer cells, associated with a mitogenic effect. Rauwolscine (RAU), an α_2-adrenergic antagonist, behaved like an inverse agonist. The objective of the present work was to elucidate if different adrenergic compounds are able to regulate PRLR mRNA expression at the transcriptional level. RT-PCR assays of the long isoform of PRLR were performed. The cells were incubated with different adrenergic compounds: 1 or 10 nM Epinefrine (EPI) or Dexmedetomidine (DEX) in the presence or absence of 1 nM RAU. After treatment for 24 or 48 hs we extracted total RNA with TRIZOL and then RT-PCR assays were carried out. Western blotting and immunocitochemistry for the PRLR were performed. The expression of the long, intermediate and delta-S1PRLR isoforms of the PRLR was verified in the T47D cell line by immunocytochemistry and Western blotting. The receptors were more concentrated near the plasmatic membrane. The intermediate isoform was preferentially expressed by approximately 50% as compared to the rest of PRLR isoforms. Expression of mRNA for the long isoform of the receptor was observed in all treatments and times tested, the increase being approximately 50% after 24 h with Epi 1 nM. The stimulation of T47D cell proliferation mediated by the α_2-RA could be partly mediated by an increase in PRL synthesis and release by these cells but not by the regulation of the RNAm PRLR at the transcriptional level.

28.

EFFECTS OF EARLY EXPERIENCE ON A CONSUMMATORY SUCCESSIVE NEGATIVE CONTRAST PROCEDURE IN SHEEP

Catanese F, Cuello MI, Freidin E, Distel R.
Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), CONICET, Bahía Blanca, Argentina.

Studies with sheep have shown that diet selection is established during particular sensitive periods, and that dietary experiences early in life could lead to a development of preferences for that food in adulthood. In the present experiment we explore the persistent effect of early experience with a high or a low quality basal diet in sheep. We assessed preference and incentive valuation in adulthood using a Consummatory Successive Negative Contrast procedure (cSNC). Early-experience phase lasted for 6 months, lambs of one month old were divided into two groups: one group received a basal high quality food (HQ), while the other group received a low quality basal food instead (LQ). Both groups received also an energy-protein supplement. When subjects were 10 months old the cSNC procedure began. 12 animals from each group were assigned to a contrast or a control condition. In preshift sessions, contrast-HQ (n=6) and contrast-LQ (n=6) received a high quality reward, while control-HQ (n=6) and control-LQ (n=6) received a less-preferred, low quality food (LQ). In the postshift phase all subjects received LQ. RESULTS: In the preshift phase the contrast-LQ group ate more of the high quality food than subjects in the contrast-HQ treatment. After the shift, contrast-LQ ate significantly less of LQ than controls-LQ (i.e., a negative contrast effect). Present data indicate that animals in contrast-LQ group might have perceived a sharper contrast between high and low quality food received during pre and postshift phases, which implies that early experience with a food type left persistent consequences in adult consummatory behavior. The mere exposure to a food early in life does not necessarily increase its value for the subject in adulthood, but also comparisons between foods in the early alimentary environment could affect what sheep learn about foods, and how they value foods later in life.

29.

O2.- RADICALS AND H2O2 HAVE DISTINCT ROLES IN THE REGULATION OF ROOT GROWTH IN SALIX SEEDLINGS

Causin HF[1], Láinez VR[1], Maroder H[2].
[1]Lab. Anatomía y Embriología Vegetal, D.B.B.E., F.C.E.N., UBA. Ciudad Universitaria, 1428 C.A.B.A.; [2]Dto. Cs. Básicas, UNLu, Ruta 7 y 5, Luján, Pcia. de Bs. As.

When salix seeds are exposed to light and ambient temperature they exhibit a marked accumulation of reactive oxygen species (ROS), which correlates to a decrease in normal germination percent and increased loss of seed viability. While exogenous supply of H2O2 scavengers increases normal germination percent, the presence of O2.- scavengers (particularly Mn2+) inhibits root growth, suggesting that O2.- formation is required for normal growth of this organ. In the present work we show that, after 24 h germination and in the absence of external Mn2+, seedling roots of non aged seeds exhibit an intense O2.- production in the sub-apical meristem and the protodermal layer as indicated by NBT staining. As root growth proceeds, O2.- formation is actively maintained in the same regions. When increasing Ca2+ concentrations are exogenously supplied together with Mn2+, root growth as well as NBT staining are completely restored, suggesting that O2.- formation is Ca2+ dependent and that Mn2+ might be competing with calcium in the signaling process. On the other hand, H2O2 scavengers only partially delay root growth rate, but markedly stimulate the formation of root hairs. The opposite effect is observed when H2O2 is induced to accumulate, indicating that factors affecting the homeostasis of different ROS in salix seedlings can alter root growth and/or morphology in a specific manner.

30.

IMMUNOHISTOCHEMICAL LOCALIZATION OF ANDROGEN RECEPTORS IN PROSTATE OF VISCACHA (*Lagostomus maximus maximus*)

Chaves M, Filippa V, Mohamed F, Aguilera-Merlo C, Domínguez S. Proy. 22/Q603. Cát. de Histología y Embriología. UNSL. E-mail: emchaves@unsl.edu.ar

The prostate is an annexes gland of the reproductive masculine system that morphofunctionally depends of the levels of circulating testosterone. In the viscacha, the gland has 2 lobes that not surround in its totality to the urethra. Histologically two zones are described: central (CZ) and peripheral (PZ). The objective of the present work is to study the distribution of androgen receptors (AR) in the different prostate zones. Prostates of adult male viscachas were processed by conventional technologies of optical microscopy and the AR was immunohistochemically identified using the antibody AR (N-20): SC-816. The obtained results demonstrated that in the PZ, the prostate adenomeros showed nuclear immunostaining in numerous epithelial cells and muscular fibers. In the CZ scarce immunoreactive epithelial cells were observed. They have a heterogeneous pattern of cytoplasmatic inmunolabeling. Some adenomeros without labeling were observed. On the other hand, the blood irrigation in CZ was higher than in PZ. In conclusion, these results demonstrate variations in the localization of the AR suggesting different sensitivity to androgens in the prostate CZ and PZ zones, probably due to the blood irrigation.

31.

SPECIALIZATION OF SCOPAL HAIRS OF TWO NATIVE BEE SPECIES OF THE GENUS *MELISSODES* LATREILLE (HYMENOPTERA, APIDAE, EUCERINI)

Cilla G, Roig-Alsina A.

Most females of nonparasitic bees transport pollen on specialized brushes of hairs called scopae. Such scopae often exhibit modifications in relation to the type of pollen carried. Females of *Melissodes* Latreille collect pollen with the forelegs, which is transferred to the scopae on the hind tibiae and basitarsi. In this contribution we study specilization of the scopal hairs of the native species *Melissodes tintinnans* Holmberg (n=3) and *M. rufithorax* Brèthes (n=3). One hind leg from each female was removed and examined under scanning electronic microscopy. Both species transport dry small pollen grains (<40 µm) from disparate tribes in the Asteraceae and other plant families. The analysis showed similar structures for the two species. The scopae have densely compacted long hairs with a simple shaft and a low proportion of scattered hairs with: few long slender branches or short spurs in an helicoidal arrangement. The grains are placed between the long selender branches, kept by the densely packed simple hairs. The specialized hairs, electrostatic charges on the surface of the bees, as well as pollenkitt on the surface of some types of pollen, contributed to accumulation and transport of dry pollen on the scopae of the two species of *Melissodes*.

32.

DIFFERENTIAL ACCUMULATION OF THYLAKOID PROTEINS IN A BARLEY PLASTID TRANSLATION MUTANT

Colombo N, Prina A.
Instituto de Genética, CICVyA, INTA, Castelar.

CL2 barley is a mutant of the chloroplast gene *infA* which presents a delay in plastid protein synthesis, associated with a delay –and sometimes a failure- in chloroplast early development and plastid ribosomes formation.
The goal of this research is to characterize the accumulation of chloroplast-encoded and nucleus-encoded thylakoid proteins along a development gradient of the first leaf. Western blots were made using thylakoid proteins extracts and antibodies against components of the photosynthetic complexes with chemiluminiscence detection. A differential accumulation of chloroplast thylakoid proteins compared with the control genotype was observed in coincidence with data of chloroplast ribosomes accumulation previously determined. In addition, differences in the accumulation of nuclear proteins of the different thylakoid photosynthetic complexes were observed. These results corroborate that CL2 presents a less efficient early plastid translation. Besides, the differential accumulation of nucleus-encoded proteins reveals the existence of a retrograde signal from the chloroplast to the nucleus.

33.

ACUTE TOXICITY EVALUATION *IN VIVO* OF THE NATURAL ANTHRAQUINONES

Comini L[1], Fernandez I[2], Núñez S[1], Cabrera J[1], Rivarola V[2].
[1]Fcognosia, FCQ, UNC, IMBIV-CONICET. C.U. Cba., Arg. [2]Dpto. Biol. Mol. Fac. Cs. Exs. FQca y Nat. UNRC. Río IV, Argentina.

Rubiadin (**1**) and soranjidiol (**2**), are anthraquinones (AQs) isolated from the phototoxic plant: *Heterophyllaea pustulata*, which exhibits photosensitizers properties, with the ability to produce both singlet oxygen and/or superoxide anion under radiation condition. An important research line studies photosensitizers compounds and their potential uses in photodynamic therapy, oriented mainly in antitumoral therapy. Considering these antecedents we evaluated the photodynamic activity of these AQs *in vitro* against a human breast carcinoma cell line, obtaining satisfactory results. Therefore, the next step would be to evaluate their photodynamic activity *in vivo*. In this work, the objective was to study the acute toxicity of **1** and **2** on different organs, in order to establish the non toxic dose, which is necessary for future phototherapeutic studies *in vivo* that evaluate the tumor rejection when AQs are administered under irradiation conditions. Acute toxicity was evaluated by histopathology test of kidney, liver, spleen and skin tissues, which were obtained from the Balb/c female mice at 1, 2 and 7 days after the intraperitoneal injection of three concentrations of each AQ and from the control animals. The obtained results demonstrated that **1** and **2** to proven doses produce no permanent alterations in the tissues analyzed in comparison with the tissues of control animals. Therefore, these AQs could be used in phototherapeutic studies.

34.

SERUM EFFECT ON MITOCHONDRIAL ACTIVE AREA OF 1 TO 4 DAYS IVP BOVINE EMBRYOS

Crocco M[1,2,3]; Lauría de Cidre L[3,4]; Alberio R[2]; Mariano MI[1,3]
[1]Inst Nac de Parasitología "Dr. MF Chabén" - ANLIS MALBRAN; [2]Biotecnología de la Reproducción- EEA-INTA Balcarce; [3]CONICET; [4]Histología Animal - Mastocitos y Biología Tumoral - FCEyN -UBA.

Serum action on different stages of embryo development shows diverse belongings. The mitochondrial dynamics taking place in embryos cultured during the first four development days are here described, analising the proper time when serum should be added. A total of 35 embryos were analysed (5 per stadium and treatment): first to fourth day grown in free serum medium, three days, the last 24 hours serum added, and four days cultivated the latest 24 hs and 48hs with serum. TEM micrographs were used to quantify the area size occupied by the different mitochondria morphological type. The results were statistically treated with Kruskal-Wallis and Dunn tests. Total mitochondrial activity was assessed using Mitotracker deep red probe. All embryos between days 1 to 4 show hooded mitochondria as the most representative type. The second type is the swollen, which active surface presents a notorious reduction. On state of fusion and orthodox types where present too. Mitochondrial activity is revealed in all stages studied. Dynamics for hooded type appears similar to swollen type, a fact that may be ascribed as a proof that the same developmental changes govern both dynamics, and perhaps this behavior shows that swollen mitochondria aren't completely inactive. Estrous cows' serum increased mitochondrial activity toward day 4th. The best result during the 4th day is found when serum was added at the 3rd day.

35.

LATENT INHIBITION IN A SPATIAL LEARNING TASK IN AMPHIBIANS

Daneri MF[1,2], Casanave EB[2], Muzio RN[1].
[1]Laboratory of Behavioral Biology, IBYME, & Faculty of Psychology, UBA; [2]Laboratory of Animal Physiology, UNS.

Latent inhibition, retarded conditioning to a stimulus that has been previously repeatedly presented without reinforcement, was examined in the toad *Bufo arenarum*. Partially dehydrated animals were daily trained in a spatial learning task using a visual cue signaling the presence of a pool with water in an open field. Toads of the Pre-exposed group experienced the unreinforced visual cue for 5 sessions before starting training. Subjects in the Control group (with no pre-exposure to the visual cue) needed only 10 sessions to learn the task, while animals in the Pre-exposed group needed 16 sessions (ANOVA, $F_{1,10}$=80,52, p<.001). Once the learning criterium was achieved, an extinction phase was conduced: 11 sessions were necessary to extinguish the response for the Control group, while only 8 sessions for the Pre-exposed group (ANOVA, $F_{1,10}$=25,6, p<.001). These results reveal the presence of the latent inhibition phenomenon; the pre-exposure to the visual stimulus retarded the association with the reinforcer and accelerated the extinction. This has been extensively observed in mammals and birds, but is the first time reported in amphibians, showing that it is a mechanism evolutionarily preserved.

36.

ANALYSIS OF SALIVARY AND SERUM FATTY ACIDS AS MARKERS OF LIPIDIC INTAKE IN HUMANS

Defagó MD, Repossi G, Perovic N, Valentich MA, Actis A.

Introduction: the lipidic intake is reflected in biological fluids. For instance, the serum cholesterol is frequently used as a marker of its dietary intake. No evidences about the correlation between dietary, salivary and serum fatty acids (FA) have been found. **Purpose:** to analyze the correlation between dietary, salivary and serum FA. **Methodology:** nine healthy subjects of both sexes who attended two hospitals of Córdoba, Argentina, in 2007 participated in the study. A validated quantitative-qualitative food frequency questionnaire was applied. The *Interfood v.2* software gave information on FA of habitual intake (mg/day). Saliva samples were collected by the subjects according to the international procedure and those of blood by the lab personnel. The lipids were extracted by the Folch method and the FA methylesters were analyzed by gas chromatography (mg/ml saliva or serum). The Spearman correlation test was applied to study the association between dietary, salivary and serum FA. **Results:** a positive correlation (r>0 <1) between diet-saliva-serum for 4:0 and 14:0 FA was observed. A positive correlation was also found for 16:0, 18:0, 18:1 n-9 and 18:2 n-6 between diet-serum and for 20:4 n-6 between diet-saliva**. Discussion:** these preliminary results show a differential correlation between dietary-salivary and dietary-serum FA.

37.

GnRH AND GONADOTROPIN ONTOGENY IN MICE LACKING THE EXPRESSION OF A FUNCTIONAL $GABA_B$ RECEPTOR ($GABA_{B1}$KO). GnRH PULSATILITY IN ADULT HYPOTHALAMI IN BOTH GENOTYPES

Di Giorgio N, Catalano P, Bonaventura M, Libertun C, Lux-Lantos V.
IBYME-CONICET.

Previously, we have shown that GnRH contents in hypothalami (HT) increased with age in $GABA_{B1}$KO and wild-type (WT) mice; $GABA_{B1}$KO mice showed altered GnRH contents in HT at 4 postnatal days (PNDs) and in adults. Here we studied GnRH contents (RIA) in olfactory bulbs (OB), embriologically related to HT, and in frontoparietal cortex (FC), as a control area, at 4, 12 and 20 PNDs and in adults, in both sexes and genotypes. We determined the pituitary content and serum LH and FSH levels (RIA) at these ages. *In vitro* GnRH pulsatility in adult hypothalamic explants was also evaluated in both sexes and genotypes. GnRH contents increased with age in OB, being levels in adult $GABA_{B1}$KO female mice lower than in WT controls, $p<0.05$, similar to what was observed in hypothalamus. On the contrary, GnRH contents in FC decreased with age, showing no differences between genotypes. LH pituitary contents were similar between genotypes during development in each sex, except for PND 4 in which they were increased in $GABA_{B1}$KO mice compared to WT mice, $p<0.05$. FSH pituitary contents showed no differences between genotypes during development. We observed an increase in serum LH levels and a decrease in serum FSH levels in $GABA_{B1}$KO mice at PND 4 with respect to WTs, $p<0.05$. An increased frequency of GnRH pulsatility ($p<0.05$), with no changes in pulse amplitude, was observed in HT from adult $GABA_{B1}$KO females. These results demonstrate that the absence of functional $GABA_B$ receptors alters gonadotropic axis ontogeny and physiology, especially in females.
CONICET, UBA, ANPCYT.

38.

STUDY ON OIL AND PROTEIN BODIES IN COTYLEDONARY TISSUES IN SEEDS OF DIFFERENT SIZES OF *CUPHEA GLUTINOSA*

Di Santo ME, Cardinali FJ, Thevenon MA.
Lab. de Botánica. Dpto de Biología. FCEyN. UNMdP. Funes 3250. (7600) Mar del Plata. Argentina.E-mail: mariedisanto@gmail.com

Cuphea glutinosa stores its lipidic and proteic reserves in oil (OB) and protein bodies (PB) in the cotyledons. Studies on this species showed changes in the distribution and size of these organelles. The degree of variation in seeds of different sizes is unknown.
The objective of this work was to determine the number and size of OB and PB in big and small seeds. The weight of 1000 big seeds was 1.011 ± 0,003586 g. and of 1000 small seeds was 0,557 ± 0,002698 g. The measurements of OB and PB were conducted on photographs from the SEM. Measurements of the number and size of OB and PB in the palisade and spongy parenchyma were conducted with the software ImageJ. The results were statistically analysed. The size of OB and PB by area unit were similar in both tissues, both for big and small seeds. The number of PB was higher for big seeds. The number of OB was remarkably higher for small seeds, for both tissues, being this parameter the adjustment variable for the accumulation of reserves.

39.

TOXICITY & REDOX CYCLE MECHANISM OF NOR LAPACHOL DERIVATIVES TRYPANOCIDAL NAPHTHOQUINONES

Elingold I[1], Taboas M[1], Casanova MB[1], Da Silva E[2], Ferreira V[2], Pinto AV[3], Dubin M[1]
[1]CEFYBO-UBA-CONICET, Facultad de Medicina, UBA, Buenos Aires, Argentina; [2]Universidade Federal Fluminense, Brasil. [3]Nucleo de Pesquisas em Produtos Naturais, Universidade Federal do Río de Janeiro, Brasil.

Chagas disease, affect about 20 million people in Latin America. Some reports indicate that may in the future, it can extent by the increase of global temperature. In this work, we assay some toxicity and redox cycle mechanism on rat liver microsomes and mitochondria, of new naphthofuranquinones(ENSJ5; ENSJ13 and ENSJ14) synthesized from nor lapachol, with activity against *T. cruzi*. All compounds inhibited NADPH and *tert*-butyl hydroperoxide dependent microsomal lipid peroxidation when NADPH was present, but did not inhibit without the presence of this cofactor. In rat liver mitochondria, the drugs, whit malate-glutamate as respiratory substrate, increased the O_2 consumption in state 4. Only ENSJ5 could inhibit the respiratory state 3 (10 and 50μM). As consequence, the respiratory control index (RCI) was diminished and the oxidative phosphorylation was uncoupled. Whit succinate, all compounds increased state 4 and diminished ICR at 50μM. These results support the hypothesis that, in our experimental conditions, these naphthoquinones inhibit microsomal lipid peroxidation by diverting reducing equivalents from NADPH. Further, these compounds produce toxic effects in mitochondria caused principally by acting as oxidative phosphorylation uncoupled agents.

40.

NEONATAL EXPOSURE TO BISPHENOL A ALTERS THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS IN FEMALE RATS

Fernandez M, Bourguignon N, Lux-Lantos V, Libertun C.
IByME-CONICET.

Previously we demonstrated that animals neonatally exposed to Bisphenol A (BPA) showed precocious puberty and altered ciclicity and pituitary response to GnRH during adulthood. Objectives: To study the effects of the neonatal exposure to BPA on: 1) pulsatile GnRH release on postnatal day (PND) 13, 2) serum hormone levels and ovarian weight (OW) in adults. Methods: Sprague-Dawley females were injected sc from PND1-10 with BPA [500μg/50μl ("H"), 50μg/50μl, ("L") in oil], or vehicle (C). We determined: 1) In PND13, pulsatile release of GnRH: hypothalamic explants were incubated for 6 h and the medium renewed at 9-min intervals. GnRH was measured (RIA) and the results analyzed using the Cluster8 algorithm. 2) In adults in estrus, OW, and serum E2, P4 and PRL (RIA). Results: 1) BPA-exposed groups exhibited significantly higher pulse frequency than C, evidenced by an increase in the peaks/hour and a reduction in the interpulse interval ($p<0.05$). 2) Adults neonatally exposed to BPA showed lower OW and higher serum E2 than C ($p<0.05$). Besides, H showed higher serum PRL and lower P4 than C ($p<0.05$). Discussion: Neonatal exposure to BPA induced an increase in GnRH pulse frequency in infantile females (precocious hypothalamic maturation), inducing precocious puberty. Adults neonatally exposed to BPA showed ovarian alterations and H also showed hiperprolactinemia; this could contribute to the alterations in ciclicity. The results presented show that exposure to BPA during the neonatal period irreversibly alters reproductive parameters in female rats.
CONICET-UBA-ANPCYT.

41.

SHEEP USE OF NATURAL AND ARTIFICIAL GRASSLAND IN FUEGIAN ECOTONE

Fernández Pepi MG, Stampacchio M, Rauber R, Collantes M, Arriaga M.

The herbivorous exert great influence in the dynamics of the terrestrial biomas through pasturing. The knowledge of the botanical composition of herbivorous diet is necessary for the election of handling alternatives of use of land. Our aim was to compare the sheep diet in two areas of the steppe in Tierra del Fuego: a natural pasture (Ea. Maria Behety: **MB**) and an artificially improved pasture (Ea. Cullen: **C**). The diet was analyzed by means of the microscopical identification of present botanical remains in the defecations. The relative frequencies of species were obtained and analyzed based on their forms of life, in each considered site, and considering also spring / summer defecations. The analysis was realized by Principal Component Analysis and one-way ANOVA. In addition the Sörensen Index (**SI**) were used to compare the similarity of diets. Additionlly, in **C,** diets between highlands and lowlands pastures were also compared. The values obtained with **SI** (all SI > 0,75) indicate that according to plant species ingested all sheep diet are similar. At the same time we find significant differences in the frequency of ingestion of soft grasses, native herbaceous dicotyledons, exotic herbaceous dicotyledons, invader herbaceous dicotyledons and sedges and rushes, being the two first consumed ones most frequently in all the cases. Differences found among diets not only reflect food offert but also may be associated to environmental conditions, location within the land and phenologic condition of the plant species, all which may show the habitat use made by sheep.

42.

STRESS AS THE TRIGGER FOR TEMPERATURE MALE SEX DETERMINATION IN PEJERREY. EFFECTS OF CORTISOL ON THE EXPRESSION OF *AMH* AND *CYP19A1*

Fernandino JI[1], Hattori RS[2], Strüssmann CA[2], Somoza GM[1].
[1]IIB-INTECH. Chascomús. Argentina. [2]Tokyo University of Marine Science and Technology. Tokyo, Japan.

Recent studies in pejerrey provided evidences on the involvement of cortisol in the masculinization at high temperatures (29ºC, MPT) where 100% males were obtained. Furthermore the administration of cortisol to pejerrey larvae raised at "sexually neutral" temperature (24ºC; ♀ 50%: 50% ♂; MixPT) induced a male-biased sex ratio. Then, in order to access if the increase of cortisol may have an effect on the testicular differentiation cascade, *amh* and *cyp19a1* (gonadal aromatase) expression was quantified using Real Time PCR on total RNA extracted from larvae grown at MixPT fed with food supplemented with cortisol (50mg/kg food). The treatment produced changes in the *amh* and *cyp19a1* expression similar to those found in males exposed to MPT. These changes suggested that cortisol may have a key role in the testicular differentiation process triggered by high temperatures in this species.

43.

CADMIUM INTOXICATION AND OXIDATIVE STRESS IN RAT SERUM

Ferramola M, Giménez MS.
Department of Biochemistry and Biological Sciences, National University of San Luis. E-mail: ferramolamariana@gmail.com

Cadmium intoxication increases oxidative stress parameters in several tissues. The aim of this study was to assess the effect of Cd intoxication on the antioxidant system and lipids content in serum, and the effect of a diet based on soy protein. Male Wistar rats were separated in six groups: 1, 3 and 5 were fed with a diet based on casein; 2, 4 and 6 were fed with a diet based on soy. Controls (1 and 2) received tap water; groups 3 and 4 received tap water with 15 ppm Cd^{2+}; groups 5 and 6, tap water with 100 ppm Cd^{2+}. Food and water was administered *ad libitum,* during 2 months. Animals were decapitated under light ether anesthesia. We determined: TBARS (Jentzsch y col., 1969), Proteins (Layne y col., 1957), Paraoxonase-1 (PON-1) activity (Beltowsky y col., 2002), total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides (Wiener Lab). TBARS values were increased in group 5, compared to controls ($p<0.05$). PON-1 paraoxonase activity was diminished, also in group 5 ($p<0.05$). PON-1 arilesterase activity increased in group 5 compared to group 1 ($p<0.05$), and diminished in groups 4 and 6, compared to group 2 ($p<0.05$). No differences were found in total cholesterol, HDL, or LDL cholesterol among the groups. Triglycerides augmented in group 2, compared to the others ($p<0.05$). Rats' exposure to oral cadmium intoxication modifies oxidative stress parameters in serum, and there seems to be a protective effect exerted by soy administration. Serum lipids content weren't modified in this experimental model.

44.

IMMUNOHISTOCHEMICAL ANALYSIS OF RAT MUC1 EPITHELIAL MUCIN DURING DEVELOPMENT: TEMPORAL EXPRESSION AND TISSUE SPECIFIC LOCALIZATION

Ferretti V[1], Lacunza E[1], Barbeito C[2], Segal-Eiras A[1], Croce MV[1].
[1]Center of Basic and Applied Inmunological Research, Faculty of Medical Sciences, UNLP, Argentina. [2]Laboratory of Histology and Embryology, School of Veterinary Sciences, UNLP, La Plata, Argentina.

Introduction: The MUC1/Muc1 mucin (MUC1 in humans and Muc1 in others species) is a high molecular weight transmembrane glycoprotein expressed at the apical surface of most epithelia. It has been established that MUC1 expression coincides with the onset of epithelial sheet and glandular formation during embryonic development. **Objective**: To analyze Muc1 expression and tissue specific localization in different embryonic stages of the rat. **Methodology**: a total of 8 embryos of each gestational stage (13 to 20 days of gestation), 8 neonates and 8 adults were included. By Immunohistochemistry, employing the anti-MUC1 CT33 antibody, the following tissues were analyzed: esophagus, stomach, small intestine, salivary gland, liver, pancreas, trachea, lung and kidney.. **Results**: Muc1 expression was observed in the stomach, lung and kidney at the gestational age of 13 days (D13). In embryonic pancreas, Muc1 expression appeared at D14 stage. In small intestine, a strong reaction was observed from D15 while esophagus and trachea showed expression from D18. In all cases, the reaction was restricted to epithelial cells with a predominantly apical pattern. In the liver, a positive reaction was detected from D16, being the only organ analyzed which showed a cytoplasmic pattern. The ducts of the salivary glands showed reaction from D18 with an apical pattern; at D20, mucous and serous acini were distinguished and Muc1 expression was detected. **Discussion**: In several of the organs analysed, rat Muc1 mucin was expressed in coincidence with epithelial differentiation which may suggest a possible role of this mucin during epithelia formation.

45.

ST. LOUIS ENCEPHALITIS VIRUS TRANSMISSION BY *Culex quinquefasciatus* MOSQUITOES COLLECTED IN CORDOBA CITY

Flores FS[1], Diaz LA[1], Almirón WR[2], Contigiani MS[1].
[1]Inst. Virología FCM UNC; [2]Centro de Investigaciones Entomológicas de Córdoba, UNC. E-mail: virolog@cmefcm.uncor.edu

St. Louis encephalitis virus (SLEV) is maintenance by transmission cylces including mosquitoes and birds. Culex quinquefasciatus mosquitoes was incriminated as a potential vector for SLEV transmission in Córdoba city. The aim of this project was to evaluate vectorial transmission of SLEV by *Cx. quinquefasciatus* mosquitoes collected in Cordoba.
Mosquitoes females were fed on 3-4-day-old viremic chicks, containing 3.5 log pfu/ml, previously inoculated with SLEV 78V-6507 strain isolated from *Cx. quinquefasciatus* in Santa Fe in 1978. Those mosquitoes were incubated at room temperature during 15 days, after that were re-feeding over non viremic chicks. Virus detection was carried out by plaque forming unit/ml (pfu/ml) whit monolayer cultures of Vero cells under agarosa.
Overall, 90.9% (80/88) mosquitoes became infected showing their susceptibility to the infection by SLEV. In a preliminary essay the non-infected chicks on which mosquitoes were re-feeding developed viremias between 3 and 5 log pfu/ml. Those results indicate the ability of *Cx. quinquefasciatus* mosquitoes to transmit SLEV and contribute to the knowledge of this specie as a competent vector for SLEV in the center region of Argentina.

46.

CHARACTERIZATION OF DYSADHERIN IN HUMAN SPERMATOZOA AND MALE REPRODUCTIVE TRACT ORGANS

Gabrielli NM[1], Matos ML[1], Chemes H[2], Quintana S[2], Vazquez-Levin MH[1].
[1]IBYME, [2]CEDIE, CONICET, Buenos Aires, Argentina. E-mail: gabrielli@dna.uba.ar

Introduction: Dysadherin (Dys) is a membrane glycoprotein described in tumoral tissues and in some normal cells. A regulatory role upon epithelial cadherin (Ecad) function has been reported for Dys. Our group has previously reported the presence of Ecad in human spermatozoa and provided evidence on its participation in gamete adhesion. **Objetives**: To evaluate the expression of Dys in the male reproductive tract, as well as its presence in spermatozoa and colocalization with Ecad. **Methods:** RT-PCR for Dys mRNA detection, Western inmunoblotting and immunocytochemistry with anti Dys (NCCM53) for protein analysis. **Results:** RT-PCR studies revealed the presence of Dys mRNA in human testis, epididymis and spermatozoa. Western immunoblotting of testicular and sperm extracts showed a 91 kDa form, whereas in the epididymis a 50 kDa protein was detected; differences in Mr would be attributed to changes in O-glycosilation. Dys was immunolocalized in the acrosome of spermatids and in the acrosomal cap and flagelum of ejaculated spermatozoa. Dys colocalized with Ecad in the acrosomal region. **Conclusion:** Dys is present in the human testis, epididymis and spermatozoa; its colocalization with Ecad may anticipate a modulatory role upon Ecad adhesive function.

47.

LIPOPOLYSACHARIDE EFFECTS ON BRONCHIOLAR CLARA CELLS

García LN, Roth FD, Maldonado CA.

Bronchiolar Clara cells (CCL) are clue in lung homeostasis. They secrete CC16, its main secretory protein, with anti-inflammatory and immunomodulatory activity, and surfactant D with antibiotic properties. We previously demonstrated in an asthma model that Th2 inflammation induces mucous transdifferentiation of CCL, and CC16 diminution. However, their response to Th1 inflammation and the effectiveness to turn on innate immunity has not been defined yet. We here evaluated the effects of lipopolisacaride (LPS), gram negative endotoxin, on Clara cells biology. Female Balb/c mice were instillated via intratraqueal with 100 µl of LPS (30 ug/ml). 24 hours after exposition, lungs were dissected and processed for morphologic analysis, CC16 expression by immunohistochemistry followed by morphometry, and analysis of inflammatory cells in brocheolar lavage (BAL). Morphologic analysis, by high resolution fotonic microscopy and electron microscopy, evidenced that LPS treatment induced Clara cells atrophy, apical cupole lost, and nuclear alterations. We also registered a significant diminution of CC16 epithelial content CC16 ($p<0,01$) with lost of free cytoplasmic label though still remaining scarce secretory granules under the plasma membrane. In correlation, BAL exhibited a 28% of neutrophils. These results evidenced that LPS induces severe damage on Clara cells in the conditions here applied, precluding the analysis of the early cell response to inflammation.

48.

DOPAMINE AND ITS D2 RECEPTORS: ACTIONS IN THE GHRH-GH AXIS

García-Tornadú I, Perez-Millán MI, Risso G, Cataldi N, Libertun C, Rubinstein M, Becu-Villalobos D.
IByME-INGEBI-CONICET.

Disruption of D2 receptors (D2R) alters the GHRH-GH-IGF-I axis, and impairs body growth in the adult male mice. D2R knockout mice (KO) have lower body weight with lower serum GH levels during the first weeks of life. We also demonstrated that *in vitro* dopamine does not play a role *per se* at the pituitary level in GH secretion. The aim of this work was to characterize the D2R knockout dwarf mouse (KO). We determined the GH pulsatility in adult KO mice measuring the expression of Major Urinary Protein (MUPs), an indicator of GH receptors binding time in the liver, and therefore of the GH pulsatility. Wildtypes showed higher levels compared to KO animals, maintaining the sexual difference for these proteins. We also determined the RNAm level of expression of GHRH and Somatostatin (STT) using real time PCR. A decrease in GHRH levels and an increase in STT levels were observed in KO animals with respect to wild types. We observed that a treatment with recombinant GH, twice a day, from postnatal day 7 thereafter for 30 days, was able to reverse the decreased body growth phenotype of KO animals. These results demonstrate that the absence of dopaminergic action through D2R modifies the GH axis at central level, determining a phenotype of delayed body growth reversible with recombinant GH treatment.

49.

GENE IMMUNIZATION TO PROACROSIN BLOCKS MALE FERTILITY IN MICE

García L[1], Veaute C[1], Lustig L[2], Vazquez-Levin MH[3].
[1]Fac. Bioqca y Cs. Biológicas, UNL, Santa Fe. [2]Centro de Investigaciones en Reproducción, Fac. Med., UBA. [3]IBYME-CONICET, Buenos Aires. Argentina.

Introduction: The sperm serine protease **Acrosin** plays several key roles on sperm function during fertilization. Our group has demonstrated the ability of proacrosin cDNA injection to induce an immune response in female mice. This study assessed the humoral response of male mice to gene immunization with proacrosin and its effect upon fertility. **Methods**: Male Balb/c mice were inoculated (i.m.; 5 times; 3 weeks apart) with an eukaryotic expression vector, containing proacrosin cDNA under CMV transcription control (pSF2-Acro, 40µg/dose; control:plasmid without insert). **Results**: Immunized mice exhibited high titers of antibodies towards proacrosin (ELISA). After mating with fertile female mice, all mice with anti-proacrosin antibodies levels higher than 4 times the preimmune value were infertile. Antibody levels were not associated to histological changes in the testis, or alterations in sperm # motility or A23187 Ca^{++} ionophore-induced acrosome reaction (AR). Immune sera inhibited induced AR in control spermatozoa (% AR: 18±2 vs 75±2; $p<0.05$). **Conclusion**: Gene immunization of male mice towards proacrosin induced a humoral response that was associated to a reduced fertility. This could be attributed, at least in part, to inhibition of AR.

50.

ANALYSIS OF PROGESTERONE (PROG) PLASMA LEVELS IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS (ALS)

Gargiulo Monachelli GM[1,2,3], Rodríguez GE[1,3], Meyer M[2], Sica REP[3], De Nicola A[2,3], González Deniselle M[2,3].
[1]Ramos Mejia Hospital. Motoneuron Disease Section. [2]IByME-CONICET. [3]Faculty of Medicine, UBA.

Introduction: ALS is a fatal neurodegenerative disease. Poor prognostic factors described are: 1) older age, 2) shorter time from onset to diagnosis and 3) bulbar onset patients. PROG demonstrated antioxidant and neuroprotective properties in animal models of ALS. **Objectives:** The aim of this study was to assess plasma levels of PROG in ALS patients and healthy subjects, and to correlate these levels with prognostic factors described in this disease. **Methodology:** We selected 13 patients with definite or probable ALS and 8 healthy subjects and determined PROG plasma levels by radioimmunoassay (RIA). We analyzed results among these groups and in relation with prognostic factors, genre and time since diagnosis (evolution of the disease) with the Two-way ANOVA. **Results:** We studied 7 women and 6 men in the ALS group and 2 / 6 in the control group. All women were postmenopausal. No difference was observed in age: ALS 51.62 ± 3.07 years and controls 52.38 ± 4.33. Plasma levels of PROG were: ALS 0.43 ± 0.08 ng/ml and controls 0.35 ± 0.03 ng/ml ($p = 0.42$). No difference was either obtained when comparing genre, time onset to diagnosis or time since diagnosis. Nevertheless, we found significantly higher levels in patients < 55 years, with less than 24 months of evolution, without variation among controls (Two-way ANOVA $p = 0.031$). We also found higher levels in spinal rather than bulbar onset patients (borderline significance). **Conclusion:** No differences were found in PROG plasma levels between overall ALS patients and controls. We found higher PROG levels according to age (patients < 55 years) and to site of onset (spinal), both considered better prognostic factors. We suggest increased PROG levels in subgroups of improved prognosis could be affording neuroprotection.

51.

MORPHOLOGY OF THE POPLITEAL AND THE SUB ILIAC LYMPH NODES OF THE PIG (*Sus Scrofa Domesticus*)

Gauna Añasco L[2], Diaz M[1], Valverde I[1], Candotti G[1], Oliva G[1].
[1]Cátedra de Anatomía. [2]Cátedra de Histología y Embriología. Facultad de Cs. Veterinarias. UBA. E-mail: gauna@fvet.uba.ar

Pig lymphatic system shows morphological variations in the popliteal (pln) and sub iliac lymph nodes. The aim of this study was to analyze the morphological features of popliteal lymph node used in bromatological inspection and sub iliac lymph node. 8 specimens of different races were dissected, 4 Landrace pigs were injected with modified Gerot paste. Sub iliac and pln lymph nodes was routinely processed for optical microscopy and the sections stained with H/E. These lymph nodes were covered by adipose tissue. Of 8 dissected specimens, in accordance with other authors, deep pln were absent in 5 specimen of Landrace while in the rest of the pigs there was a number variation. Only superficial pln was always present, in contrast with the descriptions of other authors. With optical microscopy it shows an inverted and disorganized cortex, medulla is very small and is located in the cortical area. Cortex lymph follicles of pln had a reduced size, surrounded by abundant internodular lymphoid tissue.
The analysis of the results for the sub iliac lymph node allowed to verify the coincidences with the descriptions of other authors in reference of location and quantity of this lymph node, while the histological characteristics allowed to identify the inner compartmentalization of the organ that which would be interpret as various lymphatic units.

52.

LOCATION OF HIGH AFFINITY NEUROTROPHINS RECEPTORS IN RABBIT'S (*Oryctolagus cuniculus*) THYMUS

Gauna Añasco LG[1], Soñez MC[1], Di Matteo AM[1], Gazzaneo P[1], Lombardo DM[1], Vega Alvarez JA[2].
[1]Cátedra de Histología y Embriología. Fac. de Cs. Veterinarias. UBA. [2]Universidad de Oviedo España. E-mail: gauna@fvet.uba.ar

Neurotrophins are growth factors involved in the development of central and peripheral nervous system and lymphatic system of domestic and wild animals that we have studied. The objectives of this work were to determine the localization of high affinity receptors in rabbit's thymus and compare with others domestic and wild species (pigeon, chicken, pig, bovine, horse, llama, caiman, fish, armadillo and human). Samples from 12 young rabbit thymus were fixed in 10% buffered formaldehyde and processed by the routine histological technique, included in paraffin. On serial sections an indirect immunocytochemistry method (ABC) was applied employing anti-Trks A, B and C diluted 1:100. Negative controls consisted in the omission of the first incubation with the primary antibody. TrkA was expressed in reticulo-epithelial cells type III and IV similar to those that were localized in pigeon, human and horse, and cortex stroma. TrkB was expressed in monocyte-macrophagic cells of cortex and medullar area like fish, pigeon, mouse and llama species. TrkC was identified in dendritic cells around Hassall corpuscles in medullar area, similar results were observed in rat and pig. Trks proteins may play a paracrine function for an adequate environment for T lymphocytes maturation in rabbit's thymus.

53.
LIPID PEROXIDATION ASSAYS IN CANINE FRESH SEMEN

Gavazza M, Gutiérrez AM, Marmunti M, Palacios A.
Cát. Bioquímica, Fac. Cs. Veterinarias, Cát. Fisilogía Animal, Fac. C N y M, UNLP, La Plata, Buenos Aires.
E-mail: marianagavazza2002@yahoo.com.ar

The LP is a potencial cause of infertility in males of numerous species. The levels in which the spermatozoa loses mobility *in vitro* is correlate with the rate of LP that suffers. The objective of this work was to know the fatty acid composition and analyze the sensitivity to the LP (ascorbate-Fe^{++} dependent) in spermatozoa obtained from different samples of canine fresh semen. LP was evaluated using chemiluminescence (CL) (cpm/mg of protein) and fatty acid (FA) profile by means of gas chromatography. The saturated FA content found in the analysed spermatozoa was approximately 40%, whereas in the total unsaturated FA content was approximately 60%. When the control and ascorbate-Fe^{++} dependent samples were compared, it was observed a significant increase in the light emission = CL. Consequently, significant decrease in the percentage of the PUFA was obtained being more affected: C18: 2 n6, C20: 4 n6, C22: 4 n6, C22: 5 n6 and C22: 6 n3. The unsaturation index used to evaluated the alterations generated during the LP, was correlated with the data before mentioned. Our results indicate that semen contains great amounts of PUFA, which were vulnerable to the LP. The alteration in the PUFA composition will be the base common of different degenerative processes.

54.
VARIATION OF COMT AND MAO-A GENES IN 2 ARGENTINIAN PROVINCES

Glesmann LA, Martina PF, Vidal Rioja L, Catanesi CI.

Individual differences in the response to pain stimuli are caused in part by genetic polymorphisms which affect the pain perception. Cathecolamines are involved in pain modulation, and some of the genes coding for the enzymes that control their metabolism are polymorphic. In Argentine the allelic frequencies of such genetic variants are unknown. Our population has partially an European ancestry, with a considerable Native American component; moreover, the ethnic composition of each Argentinian province can vary, depending on the prevalent immigration. The aim of this work is to characterize the allelic variants of the genes COMT and MAO-A in individuals of the provinces of Buenos Aires (BA) and Misiones (MN), for comparing both populations. DNA obtained from blood or cotton swab (BA=100, MN=45), was used to analyze a VNTR in MAO-A and a SNP polymorphism in COMT by PCR and electrophoresis. The results fit to Hardy-Weinberg expectations, except for VNTR in MN, due to an excess of heterozygote individuals. COMT-G/C polymorphism showed high prevalence of allele G in both provinces (>60%), while MAO-A VNTR presented as modal, the allele with 4 repetitions (BA=53%, Mnes=45%). Private alleles were found for the VNTR, but differences were not significant between both samples. Although these results will be confirmed in a bigger sample, these polymorphisms did not allow to detect population differences in the genetic composition of the two analyzed provinces.

55.
PHYSIOGNOMIC CHARACTERIZATION OF THE VEGETATION IN TWO SITES OF A FOREST OF CALDENAL

Gómez M, Corral A, Bogino S, Furlan Z, Escudero S, Verzino G.

The district of caldén (***Prosopis caldenia* B.**) occupies a large area in the province of San Luis. Originally comprised about 900,000 ha. But recent studies show an annual loss of 1.4% of the surface of the forest, despite its importance as elements of Environment and stabilizer as components of forest systems with productive potential. The limited information on local production and ecological aspects of caldenal, is, among other factors, a strong impediment to the implementation of sustainable management practices. The aim of this study was to analyze the structure and floral composition of different situations caldenal serve as a starting point for drawing up proposals for sustainable management of the resource. We present the first results. The study area is located at a place 25 km from Villa Mercedes. We describe two situations: an open woodland, with dense and shrubland an agroforestry system of open woodland with winter grass. Distribution curves were constructed diameter of the trees and analyzed the richness and diversity, the latter through the index of Shannon. We identified two tree species genero of Prosopis and six shrub (Aloysia, Porlieria, Lycium, Celtis, and Schinus Geoffroea in shrub).

56.
SIZE AT MORPHOLOGICAL SEXUAL MATURITY OF THE "SPIDER CRAB" *LIBINIA SPINOSA* (BRACHYURA) IN PATAGONIAN GULFS

González-Pisani X[1,2,3], Barón PJ[1,2], López Greco LS[2,3]
[1]CENPAT, [2]CONICET; [3]DBBE, FCEyN, UBA, Argentina. E-mail: xgpisani@gmail.com

In the Brachyuran crabs, the size of the chelipeds is relevant for male mating success and the width of the pleon to maximize the egg carring capacity of the females during incubation. The detection of discontinuities in some morphometric relationships associated to these structures allows to establish the size of morphologic maturity, generally related to the puberty molt. With the aim of determining the size of morphological maturity in *Libinia spinosa*, 385 individuals (206 females and 179 males) of different sizes were captured in the northern Patagonian gulfs (41-43ºS) by trapping and SCUBA diving at depths between 5 and 40 m. Width of carapace (CW) and different dimensions of the chelipeds and pleon were measured. In females, discontinuities were detected in the relationship pleon width (PW) and the CW, both morphologically immature and mature individuals being present in the range of 41.3-50.9 mm CW. The smallest ovigerous female had a CW of 41.3mm. For males, a breakpoint was detected in the relationship of cheliped length (CL) and CW at 37 mm CW (size rangel of 32-43mm) AC. The size at morphologic maturity of females was similar to that found in *Leurocyclus tuberculosus* (40.33 mm CW), another Majoidea associated to the captures of *L. spinosa*, and the size obtained for the males was lower than the size registered for that species (55 mm CW).
PIP-CONICET 5835.

57.

HPV VIRAL ONCOGENE EXPRESION AND INTEGRATION STATUS IN CERVICAL LESIONS

Gonzalez Ledesma M, Girgulsky LC, Mauro JE, Stella IY, Eiguchi K.

Introduction: HPV16 y 18, and oncogenes E6/E7 over-expression, have been associated with cervical cancer (CC) development. Their expression regulation is altered by viral integration, being this process a possible malignant progression marker. Aim: Study E6/E7 oncogenes from HPV16 and 18 expression and viral genome status in cervical samples. Materials y Methods: n=18 CIN I -G1, n=24 CIN II/III -G2, n=32 CC -G3) derived from Hospital GA C.Durand GCBA were analyzed. HPV was detected and typificated, and Viral oncogene expression analyzed. Integration status was analyzed by PCR-APOT. Results: We observed viral genome frequencies of 0.78 G1, 0.86 G2 and 0.90 G3. HPV16/18 infection was greater in G3 (0.50 G1, 0.80 G2, 0.93 G3 $p<0.001$), some of which showed coinfection. Viral oncogene expression was greater in G2 and G3 (0.22 G1, 0.74 G2, 0.80 G3 $p<0.01$). Viral integration increased in high grade lesions (HPV16: G1 0.0, G2 0.20, G3 0.47 $p<0.01$; HPV18 G1 0.0, G2 1.0, G3 0.7 $p<0.01$); coexistence of episomal and integrated genome were only seen in HPV16 Conclusions: We observed an increase of HPV16 and HPV18 integration status related high grade and CC lesions. HPV18 was only detected in the integrated form independently of the analyzed group. We can conclude that viral integration could be considered a malignant progression marker for cervical lesions induced by HPV.

58.

HERBICIDE DICAMBA AND ITS COMMERCIAL FORMULATION BANVEL®

González NV, Soloneski S, Larramendy ML.
Cátedra de Citología, Fac. Cs. Naturales y Museo, U.N.L.P. Calle 64 nº 3. La Plata, Argentina. E-mail: nvgonzal@hotmail.com

The genotoxic effect of dicamba and its commercial formulation Banvel® (57% dicamba, Syngenta Agro S.A. Argentina) was evaluated by the analysis of the frequency of micronuclei (MN) in binucleated cells and the determination of the nuclear division index (NDI). CHO-K1 cells were cultivated at 37ºC, in the presence of cytochalasin B, during 24 h of continuous treatment with 0-500 µg/ml doses of either compound. The obtained results showed: 1) a highly significant increase of the MN frequency within the 300 - 500 µg/ml concentration-range dose of dicamba ($p< 0.001$); 2) highly significant increase of the MN frequency the two highest Banvel® concentrations ($p< 0.001$); 3) a trend to a diminution in the NDI for all the assessed concentrations of both compounds. These results confirm our previous investigations about the genotoxic potential of the pure herbicide as well as for the commercial formulation commonly used in Argentina.

59.

HPV INFECTION UN CERVICAL LESIONAS ANS ITS POSIBLE RELATION WITH CODON 72 TP53 POLYMORPHISM

Gori MS, Girgulsky L, Gonzalez Ledesma M, Mauro JE, Stella I, Eiguchi K.

Introduction: Cervical cancer (CC) has, in our country, 6.7% annual mortality rate, and HPV has been recognized as the etiological factor for its development. One of the mechanisms involved in malignant progression includes the interaction between E6 viral oncoprotein and p53 protein. It has been suggested that TP53 Arg variant confers more susceptibility to develop different types of cancer, even though its real significance in CC remains controversial. Aim: Study the relation between codon 72 TP53 polymorphism and CC susceptibility. Materials and Methods: We evaluated CIN I n=16 -G2, CIN II/III n=21- G3, CC n=32 -G3, healthy controls n=27 -G1 samples. HPV was detected and typificated, and TP53 polymorphism was evaluated by specific PCR. Results: We detected viral DNA in 15% G1, 68% G2, 76% G3 and 81% G4, 82% of which showed infection with either HPV16 or 18. TP53 poblational frequencies were AA 0.68, AP 0.23 and PP 0.09 ($p<0.01$). This tendency maintained between groups. No significant differences between group allelic frequencies were found. TP53 frequencies in HPV positive samples were AA: G1 0.00, G2 0.73, G3 0.76, G4 0.66; AP/PP: G1 0.05, G2 0.16, G3 0.21; G4 0.58. Discussion: Given the poblational frequencies obtained, we cannot infer that AA genotype background can confer a higher CC susceptibility.

60.

RELATIVE MASS OF DIFFERENT KINDS OF FEAHTERS IN THE BROWN SKUA

Graña Grilli M, Montalti D.
Dept. Biología, Aves, Instituto Antártico Argentino y UNLu.

In birds, the different kinds of feathers play different functions as insulation of temperature, water and radiation, flight and mimetism, among other. For that reason, the relative mass of each kind of feather could indicate its adaptable importance to a particular environment or ecological habit. Our objective was to determine the relative mass to the body mass of the different kinds of feathers in the brown skua Stercorarius antarctica. Different kinds of feathers (coverts, down, primaries, secondary and rectrices) have been extracted from specimens coming from Esperanza Bay and Potter Peninsula, Antarctica, these have been put to dry weight and weighed to estimate its relative mass to the body mass. The contribution of the whole of the feathers to the body mass was superior to 9%. Of all the kinds of feathers studied, no differences betwen sexes have been found. About 6% of the body mass correspond to coverts and 1% to down, this would be related to the importance of these feathers in the insulation to low temperatures. Approximately 2% of body mass correspond to feathers related to the flight; half of this mass is distributed among 20 secondary and 12 rectrices, and the rest belongs to the 20 primary ones which stand out its preponderance on flight. In this latter ones, there is an increase in mass and lenght towards the outermost, which gives the wing its pointed shape characteristic in those birds which, like this migratory species, cross great distances.

61.
DIFFERENTIAL LEAKAGE OF UROTHELIAL VESICLES INDUCED BY DIETARY FATTY ACIDS
Grasso EJ, Bojanich MA, Calderón RO.
Instituto de Biología Celular, FCM-UNC.

The subplasmalemal vesicles (SPV) of the urothelial upper cells (umbrella cells) undergo a dynamic process of internalization and reinsertion into the plasma membrane regulating the surface area during the micturition cycle. To study the effect of lipid membrane composition on the vesicle leakage, three groups of rats (Wistar) were fed with differential diets enriched either in oleic acid(18:1n-9) or linoleic acid (18:2n-6) and a commercial diet. The SPV were loaded with the fluorophore-quencher pair HPTS-DPX by induced endocytosis, and the increase of fluorescence by release of the HPTS was determined by the fluorescence "requenching" procedure. Basically, the HPTS released is titrated with externally added DPX. In this way the state of the contents of the vesicles, internal HPTS quenching (Qin) can be determinate. The values of the Qin from control and linoleic acid derived vesicles changed by the externally added DPX indicating that the release mechanism was the graded type; by contrary the SPV from oleic acid were not affected by the DPX added indicating an all-or-none release mechanism. The results showed differential diet effects on the vesicle membrane "permeability" whose physiological consequences, yet unknown, on the urinary bladder physiology may have pathological implicances.

62.
OXIDATIVE STRESS IN THE BRAIN. EFFECT OF CONJUGATED LINOLEIC ACID ORAL SUPPLEMENTATION
Guajardo M, Terrasa A, Palacios A.
Cátedra de Bioquímica. Fac. Cs. Veterinarias. U.N.L.P. E-mail: mguajardo@fcv.unlp.edu.ar

The brain is vulnerable to oxidative damage due to its high content of polyunsaturated fatty acids, (PUFAs), high oxygen consumption and relative lack of antioxidant enzymes. Oxidative damage has been implicated in various neurological disorders. Conjugated linoleic acid (CLA) has shown to exert anti-inflammatory and antiproliferative activities in different organs. This work evaluates the *in vitro* non-enzymatic lipid peroxidation of brain homogenate and mitochondria of two groups of Wistar rats: control and *c9, t11* CLA-supplemented (30 mg/day during 10 days). Lipid peroxidation was monitored by chemiluminescence and changes in the fatty acid composition by GLC. After incubation of brain homogenates and mitochondria in an ascorbate-Fe^{2+} system at 37°C during 180 min, it was observed that the total cpm originated from light emission (chemiluminescence), was lower in those preparations isolated from CLA-supplemented group than in the control group, as an example homogenate (1 mg of protein): 1534,411 ± 80,391 and 1767,279 ± 39,064 cpm respectively. The fatty acid composition was substantially modified during the lipid peroxidation with considerable decrease of PUFAs. In the control group the homogenate and mitochondria PUFAs contents diminished from 24.09 ± 1.35 to 10.76 ± 2.74 and 25.13 ± 4.34 to 12.25 ± 1.48%, respectively. No significant differences were observed when CLA group was compared to the control group. We conclude that in our experimental conditions, CLA did not protect the PUFAs from oxidative damage in the brain.

63.
TGFβ1, SEXUAL STEROIDS AND EXTRACELLULAR MATRIX IN EXPERIMENTAL PROLACTINOMAS
Guida MC, Recouvreux V, Becú-Villalobos D, Díaz-Torga G.
IBYME -CONICET.

Prolactinomas are the most frequent among pituitary tumors and they are usually benign. TGFβ1 is a cytokine locally synthesized by lactotropes that regulates cellular function, inhibits cell proliferation and prolactin synthesis. TGFβ1 remains inactive by interaction with LAP and other extracellular matrix (EM) components. EM remodeling can cause TGFβ1 activation principally by Thrombospondin-1 (TSP1). The aim of this study was to evaluate TGFβ1 expression and regulation in prolactinomas, using as a model adult female rats with chronic estrogenic or estrogen+progesterone treatment during 4 weeks. TGFβ1 expression, measured by Western blot, was reduced after estrogen (E2) treatment. However, progesterone (P4) co-treatment was able to counteract the estrogen effect. Similarly, TSP1 expression was inhibited by estrogen, effect which was also prevented by P4 co-treatment. Pituitary expression of the EM structural component Laminin, was also inhibited by estrogens, but.P4 treatment was unable to counteract the estrogenic effect, and it also inhibited laminin expression per se. Our results suggest a complex TGFβ1 regulation by E2 and P4, in which P4 opposes estrogenic effects normalizing the expression of TGFβ1 and its principal activator TSP1. This protective P4 effect could enhance TGFβ1 proliferative inhibitor activity, promoting tumor reversal.

64.
INFLUENCY OF THE DIET ON THE COMPOSITION OF FATTY ACIDS AND SENSITIVITY TO LIPID PEROXIDATION IN TESTIS AND HOMOGENATES OBTAINED FROM CHINCHILLA LANIGERA
Gutiérrez A, Gavazza M, Marmunti M, Palacios A.
Cát. Biochemistry, Face. Cs. Veterinary, Cát. Physiology Animal, Face. CNyM, UNLP, The Silver, Buenos Aires.

Many studies have demonstrated that lipid peroxidation increases in function of the degree of unsaturation of fatty acids (FA) present in biological membranes. The objective of this study was to evaluate the influence of the diet on the FA composition and sensitivity to the lipid peroxidation (LP) in brain (BR) and testis homogenates (T) of Chinchilla. The LP it was evaluated by means of two parameters: chemiluminescence (CL) and FA profile analyzed by means gas chromatography. In the diet the polyunsaturated FA content and the total of no-saturated were 2 and 4 times greater than the found in T and BR. The AG C20: 4 and C22: 6 were not found in the diet. The analysis of the CL demonstrated that the homogenates of BR were sensible to the LP process and those of T were not affected by this process. Our results indicate that 1) the composition of FA of the diet was not responsible for the content of bysallilic observed in BR and T homogenates. 2) The lack of relationship between the saturated FA and sensitivity to the LP observed in T suggest that others factor/es could be involved in the protection of this organ the oxidative damage.

65.

FATTY ACID COMPOSITION OF THE FEATHERS AND UROPYGIAL GLAND SECRETION OF ROCK DOVE *COLUMBA LIVIA*

Gutiérrez AM, Montalti D, Reboredo GR, Palacios A.
Cát. Fisiología Animal FCNyM, Cát. Bioquímica, FCV, UNLP. E-mail: anamaria_gutierrez@yahoo.com

The uropygial gland secretes a complex and variable mixture of lipids that the birds extend for the surface of the feathers (PL). Our objective was to compare the composition of the fatty acids (AG) of the total present lipids in the PL and the secretion of uropygial gland (SGU) of rock dove. The birds were anesthetized by ether, there took samples of the different types of PL (Tectrices (T), powder down (PP) and coat feathers (C)) and of the SGU. The lipids were extracted by Folch's method and the profile of AG was analyzed by means gas chromatography. The AG saturated of the homogenates of PL and of the SGU were the C16:0 and C18:0. The percentage of unsaturated was 30% in the PL and 58% in the SGU. The content of monounsaturated and of unsaturated of the SGU was 2 times higher than in the PL. T presented the highest content of not saturated than the PP and the C. Our results seem to be an indication that in the PL the saturated AG are the most predominant and they probably could contribute inflexibility to optimize his function..The high contained of unsaturated found in the SGU would explain one of the most important functions of the gland the oiling. This preserving in the PL the physical structure, elasticity and protecting them from the environment.

66.

CARACTERIZATION OF THE ANGIOREGULATORY DLL4-NOTCH SYSTEM IN CORPUS LUTEUM (CL) FROM PREGNANT RATS

Hernandez F, Irusta G, Peluffo M, Tesone M.
Instituto de Biología y Medicina Experimental (IBYME)-CONICET.

Introduction: Notch family, particularly Delta- like ligand (DLL4) and its receptors Notch1 and 4 were recently identified as novel factors involved in angiogenesis regulation. In healthy adult animals or human beings, physiological angiogenesis is mainly limited to the reproductive system. The ovarian vasculature, particularly that associated with the dominant structures (preovulatory follicle and CL) during the ovarian cycle, is one of the few sites where nonpathological development and regression of blood vessels occurs in the adult. **Objectives:** to evaluate the expression of Notch ligand DLL4 and Notch receptor 4 in the rat CL during different stages of pregnancy and postpartum. **Methodology:** CL´s were microdissected for western blot and ovaries were fixed for inmunohistochemistry analysis at specific stages of pregnancy (days 7, 17, 19, 21 of gestation) and postpartum (day 1 and 4). Luteal function was investigated by measuring intraluteal Progesterone (P) levels by RIA. **Results:** A significant drop in luteal P content was observed by day 21 of pregnancy when luteolysis occurs (day 7: 157.8±10.7 vs. day 21: 49.1±6.7 pg P/ug protein). DLL4 expression peaked on day 7 of pregnancy and significantly decreases on day 21. A significant decrease of Notch receptor 4 was observed in postpartum. Immunostaining of perycites was observed surrounding the CL´s using αSMA antibody. **Discusion:** These results suggest an important role for the Notch system during progression and luteolysis in pregnancy in the rat CL.

Supported by: ANPCYT and CONICET.

67.

RELATION BETWEEN DIET AND RELATIVE MASS OF THE SALT GLAND

Ibañez L, Montalti D, Acosta Hospitaleche C.
Museo de La Plata, CONICET, Instituto Antártico Argentino.

The salt gland is a cranial structure very developed in marine birds whose function is the extrarenal excretion of salts. It is known that the relationship mass of the salt gland /body mass differs in each taxon. To test if these variations respond to alimentary differences, we analyze the relationship between the salt gland mass and the body mass in 8 Antarctic species with different alimentary habits: *Pygoscelis antarctica,*(n= 3) *P. papua* (6)*, P. adeliae* (4)*, Oceanites oceanicus* (3)*, Stercorarius antarcticus* (13)*, S. maccormicki* (10)*, Larus dominicanus* (4) and *Chionis alba* (3)*.* The specimens were weighted, the gland was obtained by dissection and weighted with an analytical balance. Percentages of gland mass/body mass obtained were 0.142 (*O. Oceanicus*), 0.079 (*C. alba*)*,* 0.074 (*L. dominicanus*), 0.074 (*S. antarcticus*), 0.055 (*P. adeliae*), 0.054 (*S. maccormicki*), 0.043 (*P. antarctica*) and 0.032 (*P. papua*). According to these results, the gland in krill-eater species of Spheniscidae would be heavier than those of the piscivorous, due to the higher ingest of water and salts of the preys. Additionally, the high value obtained in *O. oceanicus*, would respond to the consume of crustaceans and their pelagic habits. Comparing to published data and our own observations we can claim that the gland is heavier in marine birds consumers of invertebrates than in fresh water birds from the same order.

68.

COOPERATIVE EFFECT OF THIOL REDUCING AGENT AND PROTAMINE ACCEPTOR DURING SPERM DECONDENSATION *IN VITRO*

Julianelli V, Romanato M, Calvo L, Calvo JC.

Sperm chromatin decondensation *in vivo* requires a thiol reducing agent, GSH, and a protamine acceptor, heparan sulfate. The aim of this study was to analyze the interplay between both agents during human sperm decondensation *in vitro*. Semen specimens were obtained from normozoospermic (WHO criteria) volunteers. Spermatozoa were washed and resuspended in HTF-BSA medium and incubated with heparin + GSH or DTT at 37°C, adding both reagents either simultaneously (60') or sequentially: thiol reducer (30') + wash + heparin (30') and viceversa. The % decondensed spermatozoa (%Des) was determined by phase contrast microscopy. %Des was similar for both thiol reducing agents (n=5, Student, NS), suggesting that %Des is characteristic of the sperm sample, independently of the thiol reducer used. %Des was significantly lower when heparin and GSH were used sequentially, regardless of which reagent was added first. DesR (%Des relative to %Des obtained with heparin + GSH 60') was 56±5% with heparin as first reagent and 44±14% when GSH was used first (n=4, ANOVA + Tukey, $p<0.05$). Sequential use of heparin and DTT significantly reduced %Des when heparin was added first (DesR 56±16%, n=3, ANOVA + Tukey, $p<0.05$) but remained the same when DTT was first. These results suggest the existence of a cooperative effect between protamine acceptor and thiol reducing agent, which varies according to the nature of the thiol reducer.

69.

ANTIVIRAL ACTIVITY SCREENING OF *Caulerpa Mexicana* (*Caulerpaceae, Chlorophyta*)

Konigheim B[1], Aguilar J[1], Colorado J[2], Contigiani M[1]
[1]Inst. de Virología, Fac. Cs. Médicas, UNC. Cba., Arg. [2]Grupo de Investigacion en Prod. Naturales Marinos, Universidad de Antoquia, Medellín, Colombia. E-mail: virolog@cmefcm.uncor.edu

Caulerpa Mexicana Sonder ex Kützing (*Caulerpaceae, Chlorophyta*), seaweed from the Colombian Caribbean, has recently shown having antibacterial effect and modulating activity of immunological system. Nevertheless, there are no reportes of antiviral effect from this especie. Our objective was to evaluate the antiviral activity of the ethanol extract (EtOH) obtained from *C. Mexicana* against the herpes simplex virus type I (HSV-I), Venezuelan Equine Encephalitis virus (VEEV), Saint Louis encephalitis virus (SLEV) and Junin virus (JV).

Decreasing dilutions of EtOH extract in MEM with DMSO (co-solvent) were used to evaluate the acute cytotoxicity *in vitro* in VERO cells, by neutral red assay (NR). Subtoxic concentrations of extract were inoculated on infected cellular cultures with the viruses and they were incubated during 72 hs (VEEV and HSV-1) and 7 days (SLEV and VJ). Viruses, cells and the different concentrations used from each extract were included as controls. The viral inhibition percentage (%I) was estimated by NR assay.

Inside of the 80-90% of cellular viability, EtOH extract inhibited to JV (50-80%I) and VEEV (60-100%I). No antiviral activity was observed against SLEV and HSV-I. These results encourage us to determine chemical compounds which are responsible for the observed bioactivity.

70.

EFFECTS OF BONE MORPHOGENETIC PROTEIN 4 AND NOGGIN, ON BOVINE *IN VITRO* PRODUCED EMBRYO DEVELOPMENT

La Rosa I, Fernandez y Martín R, Paz D, Salamone D.

Bone Morphogenetic Proteins (BMPs) are responsible of many events of differentiation during various developmental stages in all vertebrates. A powerful BMP4 inhibitor is Noggin. The aim of this study was to investigate the effects of BMP4 and Noggin supplementation on *in vitro* produced bovine embryo development. Oocytes were aspirated from abattoir ovaries, *in vitro* matured for 22hs, and then *in vitro* fertilized. Presumptive zygotes were randomly assigned one of the following treatments: CR2 (control), CR2 supplemented with 100ng/ml of BMP4, and CR2 plus 100ng/ml of Noggin. Cleavage rate and cells number of were evaluated at day 2. Blastocyst and hatching Blastocyts and their cell number were evaluated until day 10. Cleavage rate was significantly lower in Noggin than in Control groups. Embryos from the three groups contained similar number of cells at day 2 of culture. Development to Blastocyst stage was statistically lower in both BMP4 and Noggin groups compared to Control. Percentage of hatching embryos was considerably higher for Control. Blastocysts did not differ in the number of cells. The inhibitory effects of Noggin suggest that endogenous BMP4 has an important role in bovine IVP embryo development. Exogenous BMP4 caused a negative effect on Blastocyst formation and hatching indicating that is a right balance of BMP´s signals what is needed for a proper embryonic development in Bovine.

71.

PROSTATIC STROMAL CELL RESPONSE TO LPS EXPOSITION

Leimgruber C, Quintar AA, Petiti, JP, Maldonado CA.
Centro de Microscopía Electrónica. U.N.Córdoba.

We previously demonstrated that bacterial prostatitis produces changes in smooth muscle cells (SMC) phenotype. Considering the pivotal role of these cells in gland homeostasis maintenance through stromal/epithelial interactions, our objective was to analyze their response to lipopolysacharide (LPS). Primary cultures of stromal prostatic cells from Wistar rats were performed with cells being plated in selective media MCDB 131. First, the cells were pre-incubated with TGFβ1 (1ng/mL) from 7 to 9 days in order to evaluate the homogeneity of cell population. TGFβ1 treatment resulted in the increment of smooth muscle α-actin cells compared with control cells without treatment. Afterwards, TGFβ1-treated stromal cells, were incubated with LPS (1ng/mL) for 24 hs. Cells were processed for morphological analysis and ICQ at optical and electron microscopy level. We evaluated expression of cytoesqueleton proteins, innate immune molecules (TLR4, TNFα) and NFκB translocation. LPS induced changes in actin distribution which appeared clumped in "patches". Secretory-like granules of low electron density appeared dispersed in the cytoplasm. Moreover, TLR4 and TNFα expression increased in cytoplasm and NFκB translocated to the nucleus. These observations let us conclude that SMC respond to LPS, evidencing cellular activation to express innate immunity molecules and extracellular matrix molecules. Results stand out the importance of SML that demonstrated to take active part in prostate response to inflammatory stimuli.

72.

ANTIANGIOGENIC TREATMENT OF EXPERIMENTAL PROLACTINOMAS

Luque GM, Ornstein A, Cristina C, Becu-Villalobos D.
Instituto de Biología y Medicina Experimental. CONICET.

In a subset of human prolactinomas dopamine resistance is diagnosed, and tumors are usually aggressive and invasive. The dopaminergic D2R female knockout mouse (KO) represents a useful experimental model to study alternative therapies. In the present study we evaluated the effect of an anti-VEGF treatment on the development of pituitaries from D2R KO mice transplanted in silastic capsules into receptor mice. Treatment consisted of intratumor injection of 2ug/20ul of VEGF-TRAP every three days during four weeks. Control transplanted pituitaries received the vehicle. At four weeks mice were decapitated. Silastic transplants from vehicle treated mice were highly vascularized while VEGF-TRAP injected transplants were almost transparent. By immunohistochemistry we determined that in both transplants there was a majority of lactotrophs, but in vehicle treated pituitaries, prolactin containing tissue grew outside the silastic tube. Immunostaining with von Willebrand factor revealed that vessels were tortuous in vehicle treated transplants, and well formed in the VEGF-TRAP group. Total, as well as CD31 positive, small vessels were significantly decreased in the VEGF-TRAP group, in endocrine as well as in the surrounding tissue. Intrapituitary concentration of prolactin was 8.99 ± 0.55 and 4.84 ± 2.94 ng/mg tissue in vehicle and VEGF-TRAP treated group, respectively, and there was also a decrease in circulating prolactin levels at 4 weeks 35.0 ± 7.7 and 21.6 ± 4.0 ng/ml, respectively. These results indicate that VEGF-TRAP decreases tumor vascularization and prolactin output in experimental prolactinomas.

73.

EVIDENCE ON THE SECRETION OF GLUCOSE-REGULATED PROTEIN 78 (Grp78) BY THE HUMAN OVIDUCT AND ITS INVOLVEMENT IN GAMETE INTERACTION

Marín-Briggiler CI[1], González-Echeverría MF[2], Munuce MJ[3], Ghersevich S[3], Caille AM[3], Corrigall VM[4], Vazquez-Levin MH[1].
[1]IBYME, CONICET-UBA; [2]Fertilab, Bs. As.; [3]Lab. de Est. Reproductivos, Fac. Cs. Bioq. y Farm., Univ. Nac. de Rosario, Argentina; [4]King's College London, London, United Kingdom.

Introduction: Grp78 is a chaperone found in the cellular surface and in the extracellular space; however its presence and function in the female reproductive tract remain unknown. *Objectives:* To determine the expression and secretion of Grp78 by human oviduct epithelial cells, as well as its participation in gamete interaction. *Methods:* Grp78 expression and secretion by the human oviduct was analyzed by immunohistochemistry and Western immunoblotting, respectively. The effect of recombinant Grp78 (rec-Grp78) upon sperm-*zona pellucida* (ZP) interaction was determined using the hemizona assay (HZA). *Results and Discussion:* Grp78 was found in the surface of oviduct epithelial cells; soluble Grp78 was detected in oviductal fluids from women in the periovulatory period and in oviductal tissue conditioned medium, indicating its secretion both *in vivo* and *in vitro*. Presence of rec-Grp78 in the HZA led to a decrease in the number of spermatozoa bound to the ZP. When calcium ions from the incubation medium were replaced by strontium, sperm-ZP interaction was enhanced by rec-Grp78, suggesting that Grp78 modulates gamete interaction in a calcium-dependent manner.

74.

IMMUNOHISTOCHEMISTRY PERFORMANCE FOR BOVINE VIRAL DIARRHEA DIAGNOSIS IN FORMALIN-FIXED TISSUES OF ABORTED BOVINE FETUSES

Marini MR, Pezzone N, Canal AM.

Introduction: Bovine viral diarrhea virus (BVDV) exhibits a worldwide distribution. A high prevalence of fetus infection has been demonstrated in Argentina, although diagnostic laboratories can't arrive at an etiological diagnosis in 60% of the abortion cases.
Objective: To measure concordance between Viral Isolation (VI), Direct Immunofluorescence (DIF) and immunohistochemistry (IHC) results for identifying BVDV in samples of aborted fetus' tissues.
Methods: 50 aborted fetuses from milk producing region of Santa Fe were examinated by VI, DIF and IHC. Previous to the Streptavidin-Biotin-Peroxidase IHC in formalin-fixed, paraffin-embedded tissues, an antigen retrieval technique with proteinase K was performed. Cell line 157 monoclonal antibody (VMRD Inc.) was used, and the chromogen substrate was aminoethylcarbazol.
Results and Discussion: all test performed were positive in 12 fetuses (24%) and negative in 13 (26%). IHC failed to detect 4 fetuses that were positive with VI and DIF, probably due to incomplete antigen retrieval, whereas IHC detected 13 fetuses that were negative with the other techniques. This results showed that IHC is a powerful method for demonstrating BVDV antigens, even in autolitic fetuses.

75.

cAMP-EPAC AND PI3K SIGNALLING IN 3T3-L1 PRE-ADIPOCYTE DIFFERENTIATION

Martini CN, Vila MC.
Depto.Quim.Biol.FCEyN,UBA.

Adipogenesis is stimulated in 3T3-L1 fibroblast by a combination of insulin, dexamethasone, and methylisobutylxanthine (MIX). Mitotic clonal expansion (MCE) precedes differentiation of 3T3-L1 fibroblast to adipocytes. MIX increases cAMP content, which is the activator of protein kinase A (PKA). However, PKA-independent cAMP signaling has also been described. In this paper, it was found that H89, an inhibitor of PKA, was able to block MCE but not differentiation of 3T3-L1 fibroblast. Consistently, MCE (evaluated by cell-counting) did not occur in the absence of MIX in the differentiation mixture but was recovered by overexpression of a catalytic subunit of PKA. On the other hand, differentiation of 3T3-L1 fibroblast to adipocytes, which was analyzed by triglyceride staining or quantification, did not occur when MIX was not present in the differentiation mixture and it could not be recovered by overexpression of a catalytic subunit of PKA. Differentiation was restored by addition of either dibutyryl-cAMP (db-cAMP) or 8 CPT-2 Me-cAMP. The latter activates cAMP-EPAC but not PKA signaling. In addition, LY 294002, an inhibitor of PI3K, is known to inhibit both MCE and differentiation. To investigate the role of each inducer of the differentiation mixture on PI3K activation, we analyzed PKB phosphorylation and found that complete differentiation mixture or insulin, but not dexamethasone or MIX alone, were able to increase PKB phosphorylation. These results indicate that MIX signaling through cAMP-EPAC, which is independent of PI3K, is required for 3T3-L1 fibroblasts differentiation to adipocytes.

76.

CHARACTERIZATION OF A NOVEL TRANSCRIPT OF HUMAN EPITHELIAL CADHERIN

Matos ML[1], Lapyckyj L[1], Gabrielli NM[1], Reventos J[2], Abal M[2], Vazquez-Levin MH[1].
[1]IBYME, CONICET-UBA. Buenos Aires, Argentina. [2] Hospital de Vall d'Hebron, Barcelona, Spain. E-mail: laumatos@yahoo.com.ar

Introduction: Epithelial cadherin (Ecad) is a 120 KDa transmembrane glycoprotein involved in cell-cell adhesion. Changes in its expression have been related to tumor development. To the present time only Ecad variant forms have been identified from genomic mutations associated to cancer. We have previously reported the identification of a novel Ecad mRNA (Ecad-var). **Objetives**: To characterize the expression of Ecad-var in *in vitro* models and to assess its levels in normal/tumoral tissues. **Methods and Results**: 1) *In silico* analysis indicate that Ecad-var mRNA would result from alternative splicing 2) Translation of the Ecad-var transcript in transfected cells renders a 94 kDa secretory protein 3) Cells transfected with Ecad-var showed altered shape and adhesion, as well as lower growth and higher mortality rates 4) Ecad-var transcript levels are controlled by Nonsense Mediated Decay in MCF-7 cells, 5) Low levels of Ecad-var mRNA were detected (qRT-PCR) in all epithelial cells/tissues evaluated 6) An increased in the cadE-var/cadE-wt (qRT-PCR) ratio was found in ovarian and endometrial tumors compared to controls. **Conclusion**: Changes in the expression of Ecad-var may relate to some cell alterations associated to tumor development.

77.

TEMPORAL RESPONSE TO PROGESTERONE IN SPINAL CORD DEGENERATION OF THE MUTANT WOBBLER MOUSE (Wr)

Meyer M[1], Gonzalez Deniselle MC[1,2], Garay L[1,2], Gargiulo Monachelli G[1], Lima A[1], De Nicola AF[1,2]
[1]IBYME -CONICET. [2]Depto. de Bioquímica Humana, Fac. Medicina, UBA.

Introduction: In the Wr mouse, a mutation of gene Vsp 54 gene produces spinal motoneuron degeneration and astrogliosis. Aim: To evaluate temporal changes and the response to PROG, a group of Wobblers (identified by prior genotyping analysis for the Vsp 54 mutation) and controls received a PROG pellet (20 mg, 18 days before sacrifice) at different ages: 1-3 months (initial), 5-8 (symptomatic) and 12-13 months (late stage). **Methods**: The following parameters were analyzed: number of vacuolated neurons, Chat expression (choline acetyl transferase) and two astrocyte proteins: glial fibrilary acidic protein (GFAP, astrocyte specific marker) and glutamine synthetase (GS, an enzyme that catalyzes the reversible formation of glutamine from glutamate) with quantitative immunohistochemistry. **Results**: Vacuolated motoneurons, absent in controls ± PROG, were easily detected in Wr of 1-3 months , decreasing at 5-8 months ($p< 0.05$) and at 12-13 months ($p<0.01$). PROG reduced the vacuolated motoneurons at 1-3: 7.6 ± 1.3/area, 5-8: 3.5 ± 1.4/area and 12-13 months: 2.9 ± 1/area ($p<0.001$ vs. Wr of similar periods). Chat immunoreactivity was reduced in the three periods of untreated Wr mice, but increased after they received PROG ($p<0.01$). The GFAP+ astrogliosis measured (389 ± 56 astrocytes at 1-3 months/mm^2) and stayed elevated without temporal changes. PROG decreased significantly GFAP+ cells at all stages (1-3 months: 119 ± 1.7; 5-8 months: 117 ± 10; 12-13 months: 98 ± 12, $p<0.001$ or lower vs. Wr without treatment). In contrast, GS decreased in Wr without treatment at 1-3 months (336 ± 78 cell/mm^2), 5-8 months (418 ± 81/mm^2) and 12-13 months (191± 45/mm^2, all periods $p<0.01$ vs. control 536 ± 27/mm^2). PROG elevated GS at all periods in comparison with Wr without treatment (651 ± 72/mm^2, 617 ± 76/mm^2 y 535 ± 86/mm^2, $p<0.001$). **Conclusion**: Our results showed that PROG efficiently prevented motoneuron and glial cell alteration in the Wr mouse, suggesting therapeutic possibilities at all stages of the motoneuron disease.

78.

SYNTHESIS OF POLY(NEUTRAL RED) ON GRAPHITE ELECTRODES AS CONDUCTIVE POROUS SUPPORT FOR CHLOROPLASTS AND THEIR POTENTIAL USE AS PHOTOSYNTHETIC BIOSENSORS

Núñez Pölcher PN[1], Cortón E[1,2].
[1]Dpto. de Química Biológica, FCEN, UBA. [2]CONICET. E-mail: pnpolcher@qb.fcen.uba.ar

Introduction: Electroactive polymers have recently gained popularity with their widespread use in electrochemical sensors. This is due to their ease of synthesis and remarkable properties. The current work focuses on phenazine dye polymers, particularly on neutral red (NR) films and their potential use as support for photosynthetic structures for use in biosensors. Photosynthetic biosensors find most of their applications in environmental tech-nology. Widely used herbicides share a common mode of action by inhibiting PS II, thus making these sensors an excellent tool in environmental screening. **Objectives**: This work aims to assess the feasibility of use of poly-NR films in photosynthetic biosen-sors, and to devise a method that allows their construction by wiring chloroplasts to the film. **Methodology**: A film of PNR was synthesized on pencil leads by means of cyclic voltammetry (CV) and freshly isolated chloroplasts were deposited by means of dipping or by polymerization in a NR-chloroplast suspension. With the electrodes thus obtained, CV was carried out under different light intensities in two redox mediator solutions. **Results**: For 20mM ferricyanide + 50μM menadione bisulfite (pH 7.4), a difference in current could be appreciated between +300 and +500mV in electrodes with a PNR film polymerized in the pre-sence of chloroplasts. **Discussion**: The observed change may be due to reoxidation of ferrocyanide produced by reduction of ferri-cyanide by PS II.

79.

MORPHOLOGY OF PAROTID, MANDIBULAR LATERAL AND RETROPHARYNGEAL LYMPH NODES OF PIG (*Sus Scrofa Domesticus*)

Oliva G[1], Candotti G[1], Diaz M[1], Valverde I[1], Gauna Añasco[2]
[1]Cátedra de Anatomía. [2]Cátedra de Histología y Embriología. Facultad de Cs. Veterinarias. UBA. E-mail: gabiol@ciudad.com.ar

The exportation of pig meat requires exhaustive bromathologic inspections which include the lymphatic system. The objective of this study was to contribute to the interpretation of lymphatic drainage through the morphologic analysis of parotid, jaw and retropharyngeal lymph nodes (ln). 8 Landrace pigs were dissected, employing Gerota modified paste to identify the lymphatic system. 5 ln were fixed with formalin 10% for optic microscopy and 3 lymph nodes were fixed using glutaraldehyde 2.5% and processed for transmission electron microscopy. Parotid gland covered the three ln appointed. Parotid ln had a compact structure, localized ventral of jaw articulation, caudal of masseter muscle. The mandibular ln were located in relation to jaw angle, on the ventral edge of the linguofacial vein. Lateral retropharyngeal lymph nodes located caudal to caudal auricular vein. The last two ln presented heterogeneous colour. The H/E sections showed three zones separate by connective tissue that would be interpret each other as single lymph node recover by common capsule, as has been described by other authors. The electron microscopy did not reveal ultrastructural differences with other lymph nodes studied.

80.

LIPID PEROXIDATION OF MICROSOMES OBTAINED FROM RAT LIVER AND KIDNEY: EFFECT OF *CIS*-9, *TRANS*-11-CONJUGATED LINOLEIC ACID ISOMER

Palacios A, Leaden P, Piergiacomi V.
Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, UNLP, La Plata, Bs. As., Argentina. E-mail: apalacios@fcv.unlp.edu.ar

The polyunsaturated fatty acid composition, chemiluminescence and peroxidizability index of microsomes obtained from rat liver and kidney were studied after oral administration of *cis*-9, *trans*-11-conjugated linoleic acid isomer (*CLA*). After incubation of microsomes in an ascorbate Fe^{++} system (120 min at 37°C), was observed that the total counts per min/mg protein originated from light emission: chemiluminescence, was lower in liver and kidney microsomes in the *CLA* group than in the microsomes obtained from control group. The effect of *CLA* on the polyunsaturated fatty acid composition of native liver microsomes was evidenced by a statistically significant $p<0.007$ decrease of linoleic acid C18:2 n6. When peroxidized microsomes obtained from liver and kidney of both groups (control and *CLA*) were compared with respective natives, was observed that C18:2 n6, C20:4 n6 decreased in all membranes used in this work, whereas in microsomes obtained from liver *CLA* and control groups also decrease C22:6 n3. As a consequence, the peroxidizability index - a parameter based on the maximal rate of oxidation of fatty acids - showed significant changes in liver and kidney microsomes. These changes were less pronounced in membranes derived from rats receiving *CLA per os*. Our results would confirm and extend previous observations that indicated that *CLA* could act as an antioxidant, protecting membranes from deleterious effects.

81.
ERK1/2 AND p38 ACTIVATION IN NON-APOPTOTIC CELL DEATH INDUCED BY BROMOCRIPTINE

Palmeri C, Petiti J, Sosa L, Gutiérrez S, De Paul A, Mukdsi J, Torres A.
Centro de Microscopía Electrónica. UNCórdoba.

The mitogen-activated protein kinase (MAPK) signal transduction pathways are known to be involved in various processes of growth, differentiation and cell death. The purpose of the present study was to examine the roles of ERKs and p38 in cell death induced by Bromocriptine (Bc) in hyperplasic pituitaries.
Male Wistar strain rats were estrogenized with estradiol benzoate (15mg) for 30d (E), the last 5d Bc was administered (0,3mg/100g/d) (E+Bc). Control group without treatment (C). Cell death characterization was analyzed by Electron Microscopy. Phosphorylated ERK1/2 (P-ERK) and p38 (P-p38) were detected in cytoplasmic and nuclear fractions by WB. Subcellular localization of these kinases was studied by ICQ at Electron Microscopy level. Statistics: ANOVA-Tukey.
The predominant cell death type induced by Bc was a non-apoptotic mechanism characterized by nuclear and cytoplasmic shrinkage, striking vacuolization of organelles, compatible with paraptosis. Bc significant increased ($p<0,05$ vs C and E) P-ERK and P-p38 expression in nuclear fraction. Bromocriptine induced an intense immunogold labelling for P-p38 and P-ERK1/2 at nuclear level in pituitary cells.

82.
INSULIN DEGRADATION IN MITOCHONDRIA

Passicot GA, Camberos MC, Cao Gabriel, Cresto JC.
CEDIE, Endocrinología, Htal. de Niños "R. Gutierrez"; Anatomía Patológica, Htal. de Niños "P. Elizalde", Bs. Aires, Argentina.

Insulin-degrading enzyme (IDE) was found in mitochondrial matrix and it was capable to degrade insulin. **Objective**-Our aim was to demonstrate that mitochondria can incorporate and degrade insulin. **Methods**-IDE was obtained from muscle rats from successive chromatographics steps. Hepatic mitochondria were isolated with Parson´ procedure, recovered and incubated at 30ºC with 100% oxygen, 1ng/tube insulin and ^{125}I-insulin (10^5 c/m). Experiments with or without IDE in variable conditions were performed at the same temperature. Insulin degradation was determined by chromatography in Sephadex G50 superfine and eluted with 1 M acetic acid. Insulin was assessed by its chromatographic position and with insulin antibodies. **Results**-At 30ºC insulin degradation was higher than at 25ºC. Insulin increments in mitoplasts were similar in controls and IDE. At 300 sec. with IDE there was not insulin in mitoplasts but persisted in controls. In mitocondrial supernatants at time "0" there was not insulin in both: control and IDE. There was a radioactive peak in position of labeled tyrosine (insulin totally degraded). Only the control showed a small peak of insulin at 30 sec. That is, all insulin was taken and degraded by mitochondria at time "0". Dose/response studies with increased doses of insulin showed saturation of transport and degradation system. Inhibitors of IDE decreased insulin degradation. **Conclussions**-1) With physiologi-cal insulin concentrations all insulin was incorporated and degra-ded at "0" time. 2) It was apparent that mitochondrial transporters accumulate and regulate insulin previous to its degradation.

83.
A NEW MODEL FOR THE STUDY OF PROLACTINOMAS: MICE WITH TISSUE-SELECTIVE DELETION OF THE D2R

Pérez Millán MI, Luque G, Ornstein AM, Becú-Villalobos D, Rubinstein M.
INGEBI IBYME. CONICET.

For the study of dopamine agonist resistant prolactinomas we have developed a conditional knockout mouse with the Cre/loxP technology. Previously, we generated transgenic mice that express Cre recombinase within the anterior pituitary gland under the transcriptional control of the prolactin promoter (Prl+Cre) (Medicina 67: suppl III: 155 2007). We have obtained five lines of these transgenic mice, and using real time pcr we evaluated the expression of Cre in the pituitary, and its absence in different tissues (hypothalamus, kidney, liver). We then crossed two of these lines, number 6 and 8, with low and high expression of Cre respectively, with mice that have exon 2 of the D2R flanked by *LoxP* sequences. In order to verify the deletion of the gene of the D2R in lactotropes, we evaluated serum prolactin levels, basally and after a stimulus with haloperidol (3mg/kg) in both lines. Line 6, and not line 8, showed a basal prolactin level greater than paired wildtypes, and failed to evoke an increase in prolactin levels after the stimulus with haloperidol, like in wildtype mice. This indicated that the D2R in the pituitary in this line is not functional. This new transgenic mouse provides a valuable tool for the study of prolactinomas.

84.
ANNUAL SCREENING OF GASTROINTESTINAL NEMATODES IN A DAIRY FARM IN BUENOS AIRES PROVINCE: CORRELATION WITH MILK PRODUCTION

Perri AF, Lázaro L, Licoff N, Formía N, Fernandez G, Miglierina M, Fernandez MB, Mejía ME, Becu-Villalobos D, Lacau-Mengido IM.
Lab. Reg. Hipof. IBYME-CONICET y Esc. Inchausti, UNLP.

Gastrointestinal parasitism is a major constraint in grazing livestock production systems. An intensive parasitologic screening was conducted to evaluate the parasitic degree of infestation of the dairy farm of Inchausti (UNLP, 25 de Mayo. Bs.As) and to estimate its effect on milk production. During one year (Mar 2007 - Feb 2008) grass forage samples were taken every fortnight from every paddock where each herd grazed (10 herds, 24 samples) to obtain, identify and count infesting larvae. Individual fecal samples were taken monthly from every animal in the farm, to quantify nematode eggs per gram of feces (EPG). Individual daily milk production was registered. Larvae belonged to *Cooperia, Ostertagia, Haemonchus and Trichostrongylus* geni in this order of prevalence. EPG was higher in the calves from the second and third rearing categories. In the adult cows, EPG was ten fold lower than in calves. EPG did not correlate with milk production but lactating curves from animals with positive EPG counting in the first postpartum sample had a shorter period of maximal production than cows with EPG=0. We conclude that in this dairy farm there is a high offer of nematode parasites with infestation in all the rearing and producing categories of animals, and that this event affects milk production.

85.

FATTY ACID COMPOSITION OF MITOCHONDRIA AND MICROSOMES OBTAINED FROM DIFFERENT RAT TISSUES SUPPLEMENTED WITH cis-9, trans-11-CONJUGATED LINOLEIC ACID

Piergiacomi V, Leaden P, Palacios A.
Cátedra de Bioquímica, Facultad de Ciencias Veterinarias, UNLP, La Plata, Bs.As., Argentina. E-mail: vpier@fcv.unlp.edu.ar

The conjugated linoleic acid is produced in the rumen of cattle and other ruminants during the microbial biohydrogenation of linoleic and linolenic acids. CLA is an *in vitro* antioxidant, and in the cells it protects membranes from oxidative attack. In this work was analysed the effect of the cis9-trans11 conjugated linoleic acid isomer (c9-t11 CLA) on the fatty acids composition of mitochondria and microsomes obtained from liver, kidney, lung and heart of rat. The isomer c9-t11CLA was administrated *per os* (30 mg daily during 10 days), used a group of animals as control. The lípids were obtained from mitochondria and microsomes using Folch method's. Fatty acids were transmethylated and were analyzed by GLC. The composition of fatty acids shown alterations in mitochondria: was observed decrease of the percentage of some fatty acids in the organelles obtained from the animals that received CLA than in the control group. Mitochondria of liver showed changes in the araquidonic acid C20:4 n6 and the docosahexaenoic acid C22:6 n3, in kidney was affected only the araquidonic acid and in lung only the docosahexaenoic acid. Whereas in all the tissues decreased the linoleic acid C18:2 n6, except in mitochondria of heart that no show any change in the profile of fatty acids. When was analysed the porcentual composition of the fatty acids of microsomes obtained from both groups, control and CLA, observed only in liver the decrease of the linoleic acid C18:2 n6 ($p<0.007$). The results obtained are in agree with previous studies, that suggest that the conjugated linoleic acid diminished the quantity of polynosaturated fatty acids in the membranes.

86.

RESPONSE TO REDUCED SALINITY AND EMERSION OF PROTEOLYTIC ACTIVITY IN HEPATOPANCREAS OF *Neohelice granulata*

Pinoni SA, López Mañanes AA.
Dpto. Biología, FCEyN, UNMDP, Mar del Plata. CONICET. E-mail: sapinoni@mdp.edu.ar

We previously demonstrated the response of enzyme activities in muscle of the euryhaline crab N. granulata from Mar Chiquita coastal lagoon (Pcia. Bs.As.) to environmental factors. The aim of this work was to study the post-ingesta effect of salinity and emersion on proteolytic activity in hepatopancreas. To study the effect post-ingesta of salinity, male crabs were acclimated 14 days in 35 (osmoconformation) or 10‰ (hiperregulation) salinity (S) and starved for 5 days before experiments (t0). Proteolytic activity was determined at to and 2 and 4 hs post-ingesta. To study the effect of emersion, crabs acclimated to 35 S were submerged for 24hs (t0) and then exposed to air during 2 and 4hs. The supernatant of 10000xg 15 min from an hepatopancreas homogenate (0.1MTris-HCl pH 7.4) (4 ml buffer x g of tissue-1) was used. Proteolytic activity was assayed by measuring azocasein hydrolysis (1 % p/v) in 0.1M Tris-HCl, pH 7.5. The activity was expressed as enzyme units x h-1xmgprot-1(UE). 5 independent experiments were done and ANOVA was used for statistical analysis ($p<0.05$). In 35S no variations in activity occurred after ingesta (to=5,3UE) whereas in 10S the activity was lower than that the activity at to (t0=4,9UE, t2hs=2,5UE,t4hs=1,9UE). The activity increased upon emersion of crabs for 4h (t0=5,8; t4hs=11,9UE). The results suggest a role of hepatopancreas and proteases in mechanisms of adjustment secondary to hyperregulation and emersion.

87.

PRE- AND POST- GAMMA IRRADIATION FETAL BOVINE SERUM GENOTOXIC EVALUATION

Pilili JP, Molinari G, González NV, Soloneski S, Reigosa ML, Larramendy ML.
Cátedra de Citología, F.C.N.y M., U.N.L.P. La Plata, Argentina. E-mail: jpilili@yahoo.com.ar

Fetal bovine serum (FBS) is an essential component to propagate cells *in vitro*. Different methods are used by the industry to eliminate xenobiotic agents before their use, among them the ionizing radiations application. The aim of the present work was to analyze the existence of xenobiotic agents in 18 FBS lots manufactured by Internegocios S.A., Mercedes, Pcia. Bs. As., assessed pre- and post-irradiation. The analysis of the frequency of sister chromatid exchanges (SCEs) and micronuclei (MNs) in CHO K_1 cells were used as genotoxicity endpoints. The results showed SCEs increase in 44% of the lots (71% of non-irradiated sera and 29% of irradiated sera) and in MNs only in a sample non-irradiated (6.25%). These results would render evident the presence of agents with genotoxic capacity as much in lots of FBS irradiated as in non-irradiated. Thus, the irradiation not only grants sterility to FBS but eliminates or inhibits those xenobiotics with deleterious capacity present in them before irradiation. Finally, variations in the conditions during irradiation (temperature, time of exposure) could generate compound with clastogenic and/or aneugenic capacity that could affect the correct propagation of cells in culture.

88.

HISTOLOGY OF THE GILLS AND KIDNEY OF *Corydoras paleatus* JENYNS, 1842 (SILURIFORMES, CALLICHTHYIDAE). PRELIMINARY OBSERVATIONS

Plaul SE[1], Andrés Laube PF[1], Díaz AO[2], Barbeito CG[1].
[1]Histología. F. Cs. Veterinarias, UNLP; [2]Dpto. de Biología. F. Cs. Ex. Nat. UNdMP. E-mail: splaul@museo.fcnym.unlp.edu.ar / pfandres@fcv.unlp.edu.ar

The freshwater animals are hyperosmotic in relation to the environment. The osmoregulatory capacity depends on the characteristics of tissue containing differentiated cells as the epithelium of kidney and gills. The teleost *Corydoras paleatus* is an ornamental benthonic fish resident in environment with low oxygen pressure. The aim of this work was to describe the histological structure of the gills and kidney of this species. Kidney and gills samples were fixed in 10% buffered formalin, processed for paraffin inclusion and subsequently stained with H&E technique. The kidneys have two different regions. In the cranial region the haemolymphopoietic tissue is abundant; while in the caudal region the aspect is indicative of an excretory and osmotic regulation function. The epithelium of the filaments and lamellae of the gills have many cells similar to goblet cells. The histological characteristics of this fish are similar to the ones found in other freshwater teleost. New studies are necessary to determinate the existence of adaptations of this species to the hypoxic environment.

89.

EFFECTS OF *GRIFOLA SORDULENTA* ON GENOTOXICITY INDUCED BY DMBA IN *DROSOPHILA MELANOGASTER*

Postemsky P[1], Palermo AM[2], Curvetto N[1].
[1]CERZOS-CTT-BB- CONICET, UNSur, Bahía Blanca. [2]CITEFA, Buenos Aires, Argentina. E-mail: apalermo@citefa.gov.ar

Certain edible mushrooms are attributed to have antigenotoxic activity, thus their consumption could help humans to prevent cancer. *G. sordulenta,* a native edible polypore mushroom of Argentina, was evaluated for this putative antigenotoxicity using the eye-SMART test in *D. melanogaster* and 7-12-dimethylbenz(α)anthracene (DMBA; 25µmol/ vial) as mutagenic agent. Heterozygote larvae (*white/white*$^{+}$) were grown in media with 500mg of colonized wheat flour, or 500mg of wheat flour and either DMBA solution, its solvent or water as controls. Toxicity (% of surviving larvae) and genotoxicity were evaluated (white spots per 100 red eyes in eclosed adults). Results: Survival in controls was high (89% to 92%), but when DMBA was added to the growing media, it decreased (62-67%) significantly. The number of spots per 100 eyes was as follows: water = 21, solvent = 21, DMBA = 88, *G. sordulenta* colonized media plus DMBA = 65. The corresponding values for eyes with spots were 18, 18, 57 and 35% respectively. Conclusions: a) DMBA (25µmol/vial) significantly decreased about 30-35% the larvae survival relative to control media; b) DMBA is highly mutagenic, causing more than one mutant clone/eye; c) the addition of *G. sordulenta* to DMBA growing media lowered in 30% its mutagenic effect (χ^2 Test, $p<0.05$); d) the protective activity could be assigned to the antioxidant properties attributed to mushrooms of *Grifola* genus.

90.

DIFFERENTIAL GENE EXPRESSION IN PREFRONTAL CORTEX IN AN OPERANT CONDITIONING TASK

Rapanelli M, Frick LR, Zanutto S.
Instituto de Biología y Medicina Experimental, Vuelta de Obligado 2490, Buenos Aires, Argentina. E-mail: rapanelli@dna.uba.ar

The molecular mechanisms underlying learning of complex rules are not completely understood. Thus, we studied the expression of immediate early and plasticity-related genes in an operant conditioning learning task, which consists on a food reward after pressing a lever. To study the temporal course of these events, adult rats Incompletely Trained (IT, 55-65% of responses), or Trained (Tr 100% of responses and a latency time below 5 s). We evaluated mRNA expression levels of cAMP Response Element Binding Protein (CREB) Brain-Derived Neurotrophic Factor (BDNF), Synapsin-I, Calcium/Calmodulin-dependent protein Kinase II (CaMKII), c-fos and c-jun, in the prefrontal cortex (PFC) of IT, Tr and Controls (C) using Real-Time RT-PCR. BDNF, CREB, CAMKII, Synapsin-I, c-fos and c-jun expression levels were increased in the PFC of IT animals compared to C. On other hand, BDNF, CREB and c-jun were restored after Tr vs. C and IT, whereas CaMKII and c-fos remained high in Tr vs. C. Instead, Synapsin-I levels were partially restored. Our results suggest that in first stages of learning, animals need neural activation and synaptic plasticity in PFC. But once the animal learns the rule synaptic plasticity decrease and neural activation persists in PFC.

91.

EFFECTS OF EXOGENOUS FATTY ACIDS ON PROLIFERATION IN TWO CELL CANCER LINES

Repossi G, Eynard AR.
Instituto de Biología Celular, UNC.

Introduction: The type and content of fatty acids (FA) supplementation have deep influences on the growth and behavior in human cancer cells. Effects of dietary polyunsatturated fatty acids (PUFA) on cancer have reported in our laboratory. **Aim:** analyse effects of PUFAs eicosapentaenoic (EPA) 20:5n3 and arachidonic (AA) 20:4n6, also FA oleic (OA) 18:1 and palmitic (PA) 16:0 on proliferation in MCF7 and T98G cancer lines. **Methodology:** The cells lines MCF7 (human breast cancer) and T98G (human neuroblastoma) were cultured in three 96-well plates (1.5-2 x 10^4 cells per well) in DMEM medium supplemented with each free FA in 50 or 100 µM concentration and were 24, 48 and 72 hours incubated at 37ºC. The cell proliferation was evaluated by MTT method. Data obtained were statistically analysed by ANOVA ($p<0.05$) and "post hoc" LSD Fisher Test. **Results:** T98G: showed a decline of cell survival with rise AA concentrations, whereas in contrast PA stimulate cell growth, both with maximal effect at 24 hs of incubation. EPA and OA showed no significant differences with control. MCF7: Data for 72 hs incubation show that addition of 100 µM EPA suppresses cell proliferation, in contrast OA in 50 and 100 µM concentration increase population sizes, whereas AA and PA showed no significant effects. **Discussion:** this results show a differential modulation by FA on cell proliferation in differents cell cancer lines. In MCF7 cells the effects of EPA and OA on growth rate have been associated with ERK kinase activation and eicosanoids metabolism (Chamras *et al.*, 2002, Soto-Guzman *et al.*, 2008). T98G inhibition may be due a rise of endocannabinoids, metabolites AA derived, capables of restrict growth in cancer cells (Bifulco & Di Marzo, 2002).

92.

EVALUATION OF PANICUM MAXIMUN CV. GATTON PANIC (PANICEAE) SEEDLINGS

Reyna ML, Beltramini V, Ateca NS.

Although the International Rules for Seed Testing of ISTA definite normal and abnormal seedlings in considerable detail for Panicum maximum Jacq, not additional help provide for Gatton panic cultivar, with its detailed instructions and many illustrations, is vital if the principles of seedling evaluation are to be applied uniformly. The objective of this study was explained with the use of diagrams and colour photographs of P. maximum cv. Gatton panic normal and abnormal seedlings. This work is a most valuable guide for seed analysts. Germination test used 8 x 0,250 grs replicates of intact spikelets over moisture germination paper placed in square plastic boxes. The study was carried out at the incubators of Facultad de Ciencias Agropecuarias Seed Testing Laboratory. The normal seedlings are composed by persistent coleorhiza and first adventitious root. Later adventitious roots arise near mesocotyl. The coleoptile with an opening near its apex may show antocianic pigments and whitish mesocotyl elongates first and/or second leaf. Abnormal seedlings: primary root stunted, missing, broken, split from de tip, spindly, trapped in the seed pericap, with negative geotropim and less than two adventitiuos roots. Shoot (mesocotyl and coleoptile) short and thick, split right through, missing, constricted, twisted, glassy or positive geotropism. Terminal bud and leaves damage or missing. Seedlings decayed as a result of primary infection in some part of the two systems are considered abnormality.

93.

EFFECT OF THE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) INHIBITION ON ENDOMETRIOTIC LESIONS (EL) DEVELOPMENT, IN A MURINE MODEL

Ricci A[1], Olivares C[1], Bilotas M[1], Meresman G[1], Barañao RI[1].
[1]IByME.

One of the main factors involved in the neovascularization of the ectopic endometrial tissue in endometriosis (EDT) is VEGF, which is produced by the endometrial tissue as well as by the peritoneal macrophages. In addition, it is known that VEGF levels in the peritoneal fluid (PF) and in lesions from patients with EDT are increased compared to controls and eutopic endometrium respectively. The aim of this work was to evaluate the effect of two VEGF inhibitors: Bevacizumab, an anti-VEGF humanized antibody; and a polyclonal anti-murine VEGF antibody, on EL development and VEGF levels in the PF *in vivo*, in a murine model of EDT. After fifteen days of having induced EDT, treatments began according to the following scheme: Bevacizumab group: intraperitoneal (i.p.) injection every three days of 5mg/kg of Bevacizumab (in physiologic solution); anti-murine VEGF group: one i.p. injection per week of 5mg/kg polyclonal antibody (in PBS); and control groups, received injections of physiologic solution or PBS the same days. By the second week of treatment, animals were sacrificed, the EL volume was determined and the PF was collected to assess VEGF levels by ELISA. Treatment with Bevacizumab significantly reduced the mean volume of the EL developed per animal ($p<0.05$ vs Control), as well as the peritoneal VEGF levels ($p<0.05$ vs Control). However, there were no differences in any of these parameters after the anti-murine VEGF treatment. On the other hand, there was no significant difference in the number of lesions developed per animal with any treatment. Our results suggest that Bevacizumab reduces the size rather than the number of developed lesions. Furthermore, it has been shown that the antibody reduced peritoneal VEGF levels in the EDT murine model.

94.

SUPEROXIDE ANION AND NITRIC OXIDE PRODUCTION DURING HEPARIN-INDUCED CAPACITATION IN CRYOPRESERVED BOVINE SPERMATOZOA

Rodriguez PC, Valdez LB, Zaobornyj T, Boveris A, Beconi MT.
E-mail: prodriguez@fvet.uba.ar

Previous results in bovine spermatozoa showed that heparin increases oxygen uptake and induces capacitation, a process that involves the participation of reactive oxygen and nitrogen species. The aim of this work was to study the production of superoxide anion (O_2^-) and nitric oxide (NO), during heparin-induced capacitation in cryopreserved bovine spermatozoa. Capacitation, oxygen uptake and production of NO and O_2^- were determined: a) in the presence of 10 IU/ml heparin, b) in the presence of 10 IU/ml heparin + 500µM L-NAME and c) without heparin (control). Oxygen uptake at 30 min, was doubled, respect to the control, with the addition of heparin (6±2.1 vs 13±2.9 nmol/min/10^8esp). Spermatozoa capacitated with heparin produced NO (1.6±0.2 nmol/min/10^7 cells, at 30 min) reaching a plateau between 15 and 45 min of incubation. L-NAME significantly diminished NO production (43%) and capacitation (85%). A similar hyperbolic increase was observed in oxygen uptake, NO production and capacitation. O_2^- production increased during the first 15 min of capacitation, reaching values of 1.2±0.3 nmol/min/10^7 cells and was prevented by 2 µM DPI. Progressive motility and sperm viability were not affected by the treatments. These results indicate that O_2^- and NO are produced during heparin-induced capacitation in cryopreserved bovine spermatozoa.

95.

DIFFERENCES IN INITIAL SYMPTOMS BETWEEN AMYOTROPHIC LATERAL SCLEROSIS (ALS) AND PRIMARY LATERAL SCLEROSIS (PLS) IN A REFERRAL CENTRE

Rodríguez GE[1,3], Gargiulo Monachelli GM[1,2,3], González Deniselle MC[2,3], De Nicola AF[2,3], Sica REP[3].
[1]Ramos Mejia Hospital. Motoneuron Disease Section. [2]IByME-CONICET. [3]Faculty of Medicine, UBA.

INTRODUCTION: ALS and PLS are neurodegenerative diseases with motoneuron compromise. In the former, both upper and lower motoneurons are involved and in the latter, only the upper motoneuron degenerates. OBJECTIVES: The purpose of this study was to determine epidemiologic differences between ALS and PLS. METHODOLOGY: We reviewed the Motoneuron Disease Section database in the Ramos Mejia Hospital for patients with ALS according to the El Escorial criteria and for patients with PLS according to the Pringles criteria. We gathered information on when did the disease start, which was the first symptom and when was the diagnosis made. Statistical analysis for parametric variables was conducted using the Student´s T-test, and for non parametric the Chi-square (X^2). RESULTS: We found 166 ALS patients and 14 PLS patients. In the ALS group 65 were women (39.2%) and 101 were men (60.8%). In the PLS group 10 were women (71.4%) and 4 were men (28.6%) (X^2 $p = 0.018$). The mean age in ALS was 55.49 years and in PLS 55.64. We observed a four-fold increase in the mean time from onset to diagnosis in PLS patients compared to ALS (80.64 months vs 20 months $p < 0.0001$). The most frequent initial symptom for both diseases was weakness in one extremity (ALS 50.9% and PLS 28.6%). We also registered weakness in 2 extremities and instability more frequently in PLS than ALS patients (14.3% vs 5.5% and 14.3% vs 0.6% respectively). CONCLUSION: We found more women in PLS patients (71.4%) than in ALS patients (39.2%). Time onset to diagnosis was longer in PLS patients probably due to its more benign course and lower incidence. Weakness in 2 extremities and instability were more frequently observed as initial symptoms in PLS patients.

96.

GLYCOPROTEIN P EXPRESSION IN *FASCIOLA HEPATICA*

Scarcella S, Felipe A, Alzola R, Solana H.
Dpto. Cs. Biologicas FCV-UNCPBA, Campus Universitario, 7000-Tandil, Buenos Aires. E-mail: silvanas@vet.unicen.edu.ar

Fasciolasis is a parasitic disease produced by the trematode *Fasciola hepatica*, affecting both human and animals as well. Its treatment is based on the administration of triclabendazole, a benzimidazole. Currently, its indiscriminate use has determined the emergence of resistant strain. Hence, the need to know detoxification and resistant mechanisms in *F. hepatica* arises. In general, helminths evade antiparasitic effect by: i) mutation of target molecules, ii) overexpression of efflux pumps, i.e. glycoprotein P (pgP), and/or iii) overexpression of metabolic activity. The aim of the present work was to characterize the presence, distribution and expression of pgP in *F. hepatica* with immunoelectrophoretic and immunohistochemical techniques. Results were confirmed by SDS-PAGE and immunotransfering pgP present in microsomes. Optical microscopical immunohistochemical studies located the pgP mainly in the enterocytes and vitellogenic cells. These studies on protein systems related to drug disposition in parasites are a significant contibution to further understand anthelmintic resistance mechanisms.

97.

DEVELOPMENT OF AN ELISA TEST FOR THE DETECTION OF GH IN PEJERREY *Odontesthes bonariensis*

Scharrig ME[1], Kraemer MN[1], Sciara A[2], Canosa LF[1], Arranz SE[2], Somoza GM[1].
[1]IIB-INTECH. Chascomús. Buenos Aires. Argentina. [2]IBR. Rosario. Santa Fe. Argentina. E-mail: escharrig@intech.gov.ar

Despite the fact that pejerrey is considered a promissory species for aquaculture, the growth rates obtained in intensive culture are still very low with respect to other species traditionally produced. Therefore, it is crucial to study the endocrine regulation of growth hormone in this species. Pejerrey growth hormone (pjGH) has been recently characterized and expressed in a recombinant form. This has made possible to obtain specific antibodies and therefore the development of a quantitative method. In this context, the present work was directed towards the development of a specific ELISA test to determine pjGH levels.
In this way, we set up a competitive ELISA by using recombinant pjGH and the specific antiserum previously produced. Briefly, the ELISA plate was coated with 200 ng/ml of pjGH ON at 4ºC in carbonate buffer pH 9,6. The non-specific binding was blocked with PBS-Blotto. The antiserum was used in dilutions up to 1:60.000. The development of this test will allow us to study the regulation of GH with biotechnological aims to study how to increase the growth rates for this species in culture.

98.

EXTRA PITUITARY EXPRESSION OF GONDOTROPINS IN PEJERREY FISH. RELATIONSHIP WITH HIGH WATER TEMPERATURES.

Soria FN[1], Elisio M[1], Somoza GM[1], Strûssmann CA[2], Miranda LA[1]
[1]Laboratorio de Ictiofisiología y Acuicultura. IIB-INTECH. Chascomús, Buenos Aires. [2]Tokyo University of Marine Science and Technology. Tokyo, Japan. E-mail: lmiranda@intech.gov.ar

It is well known that pituitary hormones FSH and LH play a key rol in the regulation of vertebrate reproduction. However, it has been recently demonstrated the extra pituitary presence of these hormones. In this work, fragments of FSH and LH α and β subunits were characterized in the brain and gonads of adults pejerrey of both sexes. Their expression in the brain and gonads of fish kept at different water temperatures was also studied by Real Time-PCR. In all cases, the expression of gonadotropins (GtHs) subunits significantly diminished in fish kept in water from and above 23ºC when compared with fish kept at 19°C, an optimal for reproduction of this species. This difference was even higher in females than in males. In spite of extra pituitary GtHs functions are not clear yet, these results suggest that they can play novel roles acting as neuromodulators in the brain and as regulators in the gonads.

99.

PKCε/ERK1/2 PARTIPATION IN THE PROLIFERATIVE EFFECT OF ESTRADIOL IN INTERACTION WITH FGF-2 ON LACTOTROPHS

Sosa L, Gutiérrez S, Petiti J, Palmeri C, De Paul A, Torres A.
Centro de Microscopía Electrónica-FCM, UNC- Córdoba.

In previous studies we demonstrated that estradiol (E2) co-incubated with FGF-2 produced mitogenic effects on lactotroph cells. The objective of the present work was to evaluate the participation of PKC α, ε, δ and ERK1/2 on the lactotroph cell proliferation induced by E2/FGF-2 interaction. Primary pituitary cell cultures from female rats were treated with E2 (10nM) and FGF-2 (0.6 nM), alone or combined, for 4h. Lactotroph cell proliferation was determinate by ICQ (BrdU/PRL). Estrogen receptor (RE) α, PKC α, ε and δ, and ERK1/2 total and phosphorylated (p) expressions were determinate by WB. FGF receptor (RFGF) was identified by immuno-electron-microscopy (ICQe). Statistical analysis: ANOVA-Fisher. The individual treatment with E2 or FGF-2 did not modify the lactotroph cell number compared to control, whereas that E2/FGF-2 combination significantly increased the mitogenic activity of these cells. RFGF was detected in pituitary cells. The RE α and PKC β expression levels did not exhibit variation after treatments. PKC α levels increased in similar way ($p<0.01$) in all experimental models compared to controls. E2/FGF-2 co-incubation induced the greater PKCε expression and ERK1/2 activation. These results suggest that lactotroph cell proliferation induced by E2/FGF-2 interaction is mediated by PKCε and ERK1/2.

100.

REACTIVE OXIGEN SPECIES PARTICIPATION IN PORCINE OOCYTE *IN VITRO* MATURATION

Soto M, Alvarez G, Cetica P, Dalvit G.
Biochemistry, INITRA, School of Veterinary Sciences, UBA.

It has been previously suggested that a certain level of reactive oxygen species (ROS) is involved in the oocyte fertilization process. ROS influence on the mammalian oocyte *in vitro* maturation and their effect on the subsequent embryo development have not been clarified yet. The aim of the present study was to determine ROS participation in porcine oocyte *in vitro* maturation. Cumulus-oocyte complexes (COCs) were collected from ovaries of slaughtered gilts, then matured 48 h in 199 medium (control), added with generators of hydrogen peroxide (xanthine + xanthine oxidase) and superoxide anions (xanthine + xanthine oxidase + catalase), or scanvengers of hydrogen peroxide (catalase) and superoxide anions (superoxide dismutase + catalase). Meotic maturation was evaluated by presence of metaphase II and ROS production by fluorescence techniques. ROS production was lower in matured oocytes with all the treatments ($p<0.05$). ROS level increased when hydrogen peroxide and superoxide anions generators were present ($p<0.05$), but no difference was observed when the scanvengers were present, respect to the control group. Meiotic maturation rate was unaffected by any treatment. Oocyte ROS production increase during culture would not influence meiotic maturation, but their effect on fertilization and embryo development should be evaluated.

101.

TYROSINE KINASES INCLUDING C-SRC ARE ACTIVATED BY HEPARIN CAPACITATION IN CRYOPRESERVED BOVINE SPERM

Taminelli G[1], Satorre M[2], Santa Coloma TA[1], Córdoba M[2]
[1]Conicet-UBA. [2]Instituto de Investigación y Tecnología en Reproducción Animal (INITRA), Química Biológica, Facultad de Ciencias Veterinarias. UBA.

The aim of the study was to determine the variation of intracellular calcium concentration (Cai), and tyrosine kinase participation in heparin (H) capacitation induction. Shimatzu fluorescence spectrophotometer was used to measure Cai in samples loaded with Fura 2-AM. and confocal microscopy was used to determine intracellular calcium distribution using Fluo3-AM as calcium indicator. Capacitation was evaluated by chlortetracycline and the viability by trypan blue stain. Protein phosphorylation was determined by Western blot. Data were analyzed by ANOVA and Tukey test ($p<0.05$). Cai increased in H samples (400.0±45.2 nM) vs control ($p<0.05$). Genistein (150µM), specific inhibitor of tyrosine kinase provoked Cai (226.0±74.0nM) decrease and a capacitated percentage diminish in H induction, the same was observed with PP2 (specific inhibitor of C-src-tyrosine kinase) ($p<0.05$). Genistein and PP2 produced a differential inhibition in the protein phosphorylation pattern that was induced by heparin. The activity of C-src-tyrosine kinase is calcium dependent so it may be related to H-Cai variation. Heparin induces an increase in calcium concentration and the activation of tyrosine kinases including C-src, both required to bovine sperm capacitation.

102.

EFFECT OF TEMPERATURE ON GROWTH OF THE FRESHWATER CRAYFISH *CHERAX QUADRICARINATUS* (DECAPODA)

Tropea C, López Greco LS.
Departamento de Biodiversidad y Biología Experimental, FCEN, Universidad de Buenos Aires.

Cherax quadricarinatus is a freshwater crayfish native of Australia, which is cultured with commercial purposes. The culture strategies are aimed at the production of monosex all male populations and the increase of females somatic growth, in order to obtain greater meat yields. Temperature is an important abiotic factor that modulates many aspects of this species biology. The objective of the present study is to analyze the effect of a temperature higher than the one commonly used in culture on both males and females growth. Juveniles from both sexes were exposed to one of the following treatments: high temperature (30±1ºC) and normal culture temperature (27±1ºC). The animals were weighted and sexed twice a month, during 330 days. The specific growth rate and growth increment were not statistically different between treatments for females, but they were different for males, being higher for those exposed to the normal temperature. When comparing sexes, the approximate weight at which males growth becomes larger than females growth could be determined (15 grams). Both results are of great importance to optimize commercial exploitation of this species.
UBACYT X458 y PICT 2004-953.

103.

PARTICIPATION OF EPITHELIAL CADHERIN IN MURINE FERTILIZATION

Veiga MF[1], Choren MV[2], Cameo M[2], Vazquez-Levin MH[1].
[1]IBYME, CONICET-UBA. [2]Biología de la Reproducción; Buenos Aires, Argentina. E-mail: veiga@dna.uba.ar

Introduction: Mammalian fertilization involves gamete adhesion events. Epithelial cadherin (Ecad) is a transmembrane glycoprotein that participates in Ca^{2+} dependent cell-cell adhesion events. We have characterized the expression of Ecad in human spermatozoa and oocytes, and shown evidence of its involvement in fertilization. Its localization in murine gametes was later described by us. **Objetive**: To evaluate the participation of Ecad in murine fertilization. **Methods**: *In vitro* fertilization (IVF), as well as binding to *zona pellucida* (ZP) and fusion to the oolemma assays were done in the presence/absence of anti Ecad antibodies (H-108: cadherin 5 domain; ECCD1: cadherin 1 domain). **Results**: Sperm preincubation with H-108 (20 µg/ml) resulted in inhibition ($p<0.05$) of fertilization on IVF assays done with cumulus oocyte complexes (38% Inhibition Rate, IR) or denuded oocyte (54% IR). Moreover, sperm preincubation with H-108 antibody impaired sperm binding to ZP (32% IR). The ECCD1 antibody inhibited ($p<0.0001$) sperm fusion to the oolema (64% IR). **Discussion**: The findings from this report are in agreement with previous studies in humans and support the notion of Ecad participation in gamete adhesion events leading to fertilization.

104.

IMMUNOGENICITY OF TESTICULAR PROTEIN CRISP2 AND STUDY OF ITS RELEVANCE FOR FERTILITY

Weigel Muñoz M, Bluguermann C, Ernesto JI, Goldweic NM, Cohen DJ, Cuasnicu PS.
Instituto de Biología y Medicina Experimental (IBYME-CONICET)

Results from our group indicate that testicular sperm protein CRISP2 (*Cysteine Rich Secretory Protein 2)* is involved in gamete fusion. To study whether CRISP2, as its epididymal homologue CRISP1, is relevant for fertility, male and female rats (n: 68) were immunized with recombinant CRISP2 (100ug) coupled to MBP (Maltose Binding Protein), using recombinant epididymal CRISP1 and MBP as controls (4 injections). ELISA of sera collected at different intervals after immunization revealed that the administration of CRISP2 raised antibodies in both sexes, with levels that increased as a function of time. Western blot studies showed that, in contrast to the complete negative reaction observed for pre-immune and control groups, sera from CRISP2-immunized animals were capable of specifically recognizing the native protein in both testicular and sperm extracts. To evaluate the effect of the immune response on fertility, males from the three injected groups were mated with non-treated females. Results indicated that while the animals injected with CRISP1 exhibited the already reported decrease in fertility, those immunized with CRISP2 presented fertility rates not different from controls. Together, these observations confirmed the immunogenicity of CRISP2 and suggest that, differently from CRISP1, the presence of antibodies against CRISP2 would not compromise male fertility.