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60 years after the description of the DNA structure

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PLENARY LECTURE

A1
MOLECULAR BIOLOGY 60 YEARS AFTER THE DISCOVERY OF DNA STRUCTURE

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SYMPOSIA

SATELLITE SYMPOSIUM "DEVELOPMENTAL BIOLOGY"

A2
THE ROLE OF IHH/GLI CELL SIGNALING IN *Xenopus laevis* NEURAL CREST DEVELOPMENT

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Neural crest (NC) is a transient and multipotent cell population found only in vertebrates embryos. These cells differentiate into numerous diverse derivatives of great importance for the organisms including the peripheral nervous system, melanocytes and craniofacial cartilages. It has been demonstrated that the development of the NC is mediated by complex interactions of multiple signals and transcription factors. Recently we have shown that new cell signaling pathways are required for the early induction and subsequent NC development. In our laboratory we have been analyzing the Indian hedgehog/Gli (Ihh/Gli) pathway role during NC developmental processes. Using in situ hybridization and qPCR analysis of its components we have found that they are expressed in the neural plate border into the prospective NC. Gain- and loss- of function experiments demonstrated that the activity of Ihh/Gli pathway genes are required for the initial induction, specification and migration of NC tissue. Moreover, we found that this cell pathway may signal through both autocrine and paracrine mechanisms. In addition, the role and transcriptional activity of different intracellular components of Ihh/Gli pathway was investigated. Our results show new functions for this cell signaling pathway and confirm that diverse cell signals are necessary for the correct development of NC and the formation of its derivatives.

A3
FINE TUNING OF THE WNT SIGNALLING PATHWAY DURING THE EMBRYONIC DEVELOPMENT OF VERTEBRATES

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Cellular nucleic acid binding protein (CNBP) is a small protein and highly conserved that binds single-stranded nucleic acids and may act as a nucleic acid chaperone. Data collected so far suggests that CNBP participates in the control of cell proliferation and threshold levels of the protein are necessary for proper development of the embryonic anterior-most structures. CNBP seems to control the balance between cell proliferation and cell death of cranial neural crest cells; however, the molecular targets and cellular processes that involve CNBP have not yet been clearly established. Monohybrid assays in yeast using mouse and zebrafish genomic libraries allowed to identify and validate in vivo at tbx2, smarca5 and wnt5 as molecular targets of CNBP during zebrafish embryonic development. Besides, it was possible to identify the DNA-consensus binding site sequence of CNBP (CNBP-CBS). This CBS was subsequently used to search for genes reported as involved in vertebrate embryonic development present in the human, mouse, chicken, amphibians and fish genomes and that contain at least one CNBP-CBS in their promoter regions. Three of the 16 genes found were validated in vivo as CNBP targets during zebrafish development, and are involved in the Wnt signalling pathway. These findings lead us to propose that, through its nucleic acid chaperone activity, CNBP might "fine-tune" the Wnt pathway during the embryonic development of vertebrates.

A4
TRANSCRIPTIONAL CONTROL OF NEURONAL SUBTYPE IDENTITY

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The generation of cellular diversity in the embryonic nervous system is a prerequisite for the establishment of functional neuronal circuits. According to the proposed model, based on studies from the last decades, neural progenitor cells interpret extrinsic graded signals through the induction of genetic programs largely controlled by the combinatorial action of transcription factors. In this presentation I will analyze how initially equivalent progenitor cells of the developing neural tube give rise to distinct classes of postmitotic neurons of the hindbrain and spinal cord. The role of several HD- and bHLH-transcription factors in the specification of subtype identity was assessed using molecular genetics in the mouse and *in vivo* missexpression, in combination with gene expression analysis and genetic fate mappings.

A5
SONIC HEDGEHOG DRIVES CHEMOTAXIS OF NEURAL CREST CELLS, WHICH IS PERTURBED BY ETHANOL EXPOSURE

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Colonization of neural crest cells (NCCs) toward ocular region in Sonic hedgehog (Shh) gradients and its alcoholic perturbation were studied. Directional parameters showed chemotaxis of in vitro NCCs toward Shh, notochord cocultures or conditioned medium, which was inhibited by anti-Shh antibody, cyclopamine and anti-Smo morpholino. Ptch-Smo expression was also showed on NCCs. On whole embryo in situ hybridization and immunolabeling, we showed expression of Shh mRNA and protein at ocular field, as well as Ptch, Smo and Gli/Sufu on cephalic NCCs. Blocking of Shh>Ptch/Smo by transfection with anti-Smo morpholino, or negative dominant Ptch plasmid and electroporation shown lower density of NCCs at the ocular region. Implant of Shh or cyclopamine embedded microbeads support the guiding rol of Shh on NCC orientation. Ethanol exposure perturbed NCC chemotaxis toward Shh gradient, and embryos shown cranio-facial anomalies assigned to deficient distribution of NCCs, associated with abnormal in situ expression of Shh, supporting the involvement of cephalic NCCs in the Fetal Alcohol Syndrome. These in vitro and whole embryo results show a Shh guidance action through the Ptch-Smo system, independent of Gli; adding a new approach to the perturbations induced by non controlled toxic factors, and point out a new guidance function for the Shh morphogen, besides the other knew functions.

A6
A PACEMAKER CIRCUIT IN VERTEBRATE DEVELOPMENT

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During embryonic development, cells generate and process information to orchestrate the patterning of tissues and organs. Several fundamental patterning mechanisms have been identified. Among them, an interesting example is vertebrate segmentation. During vertebrate development, the axis that spans from head to tail is subdivided into regular segments that will later form the vertebrae and other tissues. Such segments form one by one, with a precise rhythm. The rhythm is controlled by a biological clock: at the cellular level, a gene regulatory circuit produces biochemical oscillations in the concentrations of some proteins. We use an interdisciplinary approach that brings together theory and experiment to unravel mechanisms that produce and control the rhythm of this biological pacemaker.

A7
MOLECULAR AND DEVELOPMENTAL ADAPTATIONS TO HYPOXIA IN *Drosophila*

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Adaptation to hypoxia involves a coordinated regulation of a wide range of genes involved in restoring oxygen homeostasis. This response is mainly directed by the hypoxia-inducible factor (HIF) family of transcription factors, which are heterodimeric proteins consisting of two subunits: a HIF-α subunit that is tightly regulated by oxygen and a constitutive HIF-β subunit. Oxygen negatively regulates HIF-α through proteasomal degradation, blockage of transcriptional co-activator

recruitment and subcellular localization. This hypoxia-responsive system is conserved in *Drosophila melanogaster*, being Sima and Tango the orthologues of HIF-α and HIF-β respectively. We have performed a genome-wide RNAi screen, aimed to the identification of genes required for Sima activity in hypoxic conditions. We have found more than 20 novel regulators that are required for this response in vivo and characterized some of their mechanism of action. Some of these genes turned out to participate in developmental adaptations to hypoxia, particularly in the ramification of the tracheal system. We found that this adaptive developmental response has features in common with mammalian angiogenesis, particularly, transcriptional induction of the FGF homologue Branchless as well as of its dedicated receptor Breathless. We also found that general body growth is inhibited in hypoxic conditions and that this inhibition is due in part to translational regulators of the transcription factor Sima, as well as general inhibitors of CAP-dependent translation, such as 4E-BP. We demonstrate an absolute requirement of these genes for fly survival and adaptation to hypoxia.

SYMPOSIUM "ANIMAL BIOLOGY AND BIOTECHNOLOGY"

A8
STATE OF THE ART OF REPRODUCTIVE BIOTECHNOLOGIES IN HORSES

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Since the second half of the 20th century the massive application of reproductive biotechnologies has grown exponentially in the equine industry worldwide especially artificial insemination (AI) and embryo transfer (ET) (Squires, 2006) generating a high impact on the equine industry. More than 90% of the pure breeds of horses have regulated its use by breeders. Argentina is the second world producer of equine embryos (15,000 embryos per year) then Brazil (42,000) to Latin America as the largest producer of equine embryos in the world. Considering that the first report of a foal born by ET in 1974 and the first births in Argentina in 1985, in less than 25 years this biotechnological tool has been positioned in the production systems, growing at a rate close to 20% a year. Assisted reproduction techniques of greater complexity such as ICSI and cloning, of very low efficiency and high operational cost, currently aimed at an elite of individuals of high genetic, economic or emotional value today offer tools to solve specific problems of infertility and undoubtedly its results will improve with the increase of ladders controlled experiments to real production systems. Today in Argentina are commercially offered a battery of reproductive biotechnologies that include, besides the above mentioned, cryopreservation of sperm, embryos and somatic cells; fetal sexing by ultrasonography; embryo sexing by PCR; sperm sexing; cloning by SCNT, being, in the latter, the country with as many equine cloning companies today.

A9
FOCUS ON *IN VITRO* PRODUCTION OF BOVINE EMBRYOS

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Scientific achievements in oocyte and embryo developmental biology have provided unprecedented opportunities for *in vitro* production (IVP) of bovine and human embryos throughout the last decades. Nevertheless, embryos generated *in vitro* still differ from their *in vivo* produced counterparts. Actually, it is possible to achieve blastocyst rates of up to 70% if *in vivo*-matured bovine oocytes are used. *In in vitro* maturation (IVM) the success is high (approximately 90%), but a considerable proportion of oocytes still fails to develop to the blastocyst stage following *in vitro* fertilization (IVF). In contrast, if oocytes are matured *in vitro*, blastocyst rates are only less than half that of those matured in vivo. In fact, only 30-40% of such oocytes reach the blastocyst stage, at which they can be transferred to a recipient or frozen for future use. This rather limited success may be attributed to the heterogeneous population of oocytes which are normally retrieved from follicles of 3–8 mm rather than from preovulatory follicles. We know now that the quality of the oocyte and its maturation are crucial in determining the proportion of immature oocytes that form blastocysts, while the post-fertilization culture environment has a major influence on the quality of the blastocyst. *In vitro* culture (IVC) conditions have been enhanced, mainly by adjustment of media formulations, whereas the IVM protocols wakes interest up recently. Mammalian embryos can be produced *in vitro* successfully using oocytes derived from ovaries of sexually immature but efficiency in IVF commercial programs is lower than that with oocytes from sexually mature animals. It was observed high male and female effects on the efficiency of IVP. Sexed and frozen bovine sperm can be used successfully in an IVF-programme. However, the high variability of the results demand to paying close attention to known a set of minimum standards of sperm quality to identify the sorted sperm that has the highest potential to fertilize under commercial conditions.

A10
IMPACT OF SPERM CHROMATIN QUALITY ON REPRODUCTIVE HEALTH

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The ultimate goal of spermatozoon is to deliver the paternal genome into an egg. This esential task for the surviving of any species is jeopardized by the numerous factors capable of damage the sperm DNA. Different factors can affect sperm morphology and function that negatively impact men fertility. Studies on humans and animals suggest that both radiation and chemotherapy alter sperm chromatin thus promoting significant damage on sperm DNA and, decreasing the level of protamination, thus altering DNA compaction. Although some components of the sperm chromatin structure are repaired differentially overtime after chemotherapy-induced damage, significant DNA damage remained even after many years of the end of treatment. Thus, cancer survivors face the posibility of infertility due to chemo-dependent long-term damage of their spermatozoa. In times where couples are delaying parenthood and the increased exposition to toxicants that impact fertility make artificial reproductive techines (ARTs) an increasing alternative sought by infertile couples. The ability of spermatozoa carrying DNA damage to fertilize oocytes makes these techniques, particulary the intracytoplasmic sperm injection (ICSI), a possible vehicle for transmition of defects to the offspring. It is necessary to develop new and complementary strategies are needed to better characterize the sperm chromatin quality for seletion of spermatozoa used in ARTs.

A11
STATE OF THE ART IN HUMAN REPRODUCTION: 1978 - 2013

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Human IVF begins in 1978 with the irrefutable evidence obtained from a single observational case report. The occurrence of one pregnancy (n = 1) was enough as an evidence to make a randomized controlled trial (RCT) redundant: today there are around 5 million children born in the world by this technique. From this date to the present we have witnessed numerous technological proposals: some very successful, as ICSI or vitrification, and others that have been forgotten due to their ineffectiveness or because they have been overcome. The history of reproductive medicine teaches us that very few of these new developments have been introduced in the medical practice after its corresponding clinical validation. Can we apply a new technique without previous RCTs and say -as an editorial in the *British Medical Journal* - that RCTs "are a dangerous innovation perpetrated by the arrogant to serve the cost cutters and suppress clinical freedom"? Or should we understand that there is a marked tendency to copy inventions without knowing clearly whether they are useful or not? During this presentation we will review the history of human assisted reproduction stressing the need to work with a commitment of responsible technological innovation. This means that we must be attentive to scientific reports that demonstrate the effectiveness of the new techniques without compromising, however, more simple studies or innovative ideas.

SYMPOSIUM "PLANT BIOLOGY AND BIOTECHNOLOGY"

A12
THE RELEVANCE OF WATER TRANSPORT THROUGH THE CELL TO CELL PATHWAY: GATING AND TRAFFICKING MECHANISMS IN PLANT AQUAPORINS

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Aquaporins -small transmembrane proteins that efficiently improve water exchange in biological membranes- have reopened the discussion of water homeostasis, particularly in plants. Their abundance, ubiquity and sophisticated regulatory mechanisms reflect their water exchange capacity to modulate water membrane permeability in almost all biological membranes. However, the main entrance to the cellular pathway is restricted by the plasma membrane. Particularly in plants, water exchanges at the level of the plasma membrane are controlled by plant aquaporins known as PIP. PIP can rapidly adjust membrane water permeability by means of two mechanisms: channel gating (closing the pore if cytosolic acidification occurs) and channel translocation of PIP subunits (PIP1 and PIP2, organized -or not- in mixed tetramers). Evidences indicate that these mechanisms are not only highly conserved among species but their juxtaposition enhances the dynamics of the response. Here we evaluate their consequences on the overall hydraulic conductivity, in order to understand the relevance of

the cell to cell pathway when considering not only physiological challenges but also the response to adverse plant environmental conditions.

A13
PLANT USE INFORMATION FROM THE LIGHT ENVIRONMENT TO MODULATE IMMUNE RESPONSES

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Solar radiation is a major regulator of plant immunity. In shade-intolerant plant species, perception of competition signals by informational photoreceptors activates shade-avoidance responses and reduces the expression of plant defenses against pathogens and insects. Recent experiments using model (Arabidopsis) and non-model plant species demonstrate that phytochrome B (phyB), a photoreceptor that plants use to detect the proximity of other plants, is a major modulator of defense signaling. Low ratios of red to far-red radiation (R:FR), which indicate the proximity of other plants and result in partial inactivation of phyB, cause a marked down-regulation of defense signaling. This down-regulation is caused by simultaneous reduction of plant sensitivity to the defense hormones jasmonate (JA) and salicylic acid (SA). Low R:FR appear to suppress JA responses by altering the balance between DELLA and JAZ (JA-ZIM domain) proteins, in favor of the latter. The regulation of JA responses by R:FR and phyB is likely to play an major ecological role, optimizing the distribution of limited resources between competing physiological functions, including growth and defense. A better understanding of these mechanisms will be useful to develop varieties of cultivated species with improved resistance to pests and diseases.

A14
THE ROLE OF CHLOROPLAST REDOX METABOLISM IN PLANT TOLERANCE TO ENVIRONMENTAL HARDSHIPS

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Part of the damage undergone by plants exposed to adverse environments is caused by faulty electron delivery in chloroplasts, resulting in transfer to oxygen and build-up of reactive oxygen species that inactive all types of biomolecules. Plants, and the microorganisms from which they evolved (algae and cyanobacteria), display different strategies to cope with such adverse situations. Plants resort to multigenic responses involving scavenging and repair, whereas microorganisms respond by replacing sensitive targets with iso-functional, stress-resistant counterparts. We describe a novel strategy involving introduction of a cyanobacterial gene encoding for a chloroplast-targeted flavodoxin (Fld) into plants, which led to transgenic lines with increased tolerance to multiple sources of stress. Fld is induced by stress in microorganisms but is absent in plants. The mechanism of tolerance was studied *in vitro* and *in vitro*, showing that Fld is able to interact with plastid systems and enzymes, despite eons of evolutionary divergence, restoring proper electron delivery. The dose and redox state of Fld were critical to tolerance, and further manipulation of the electron transfer machinery in Fld-expressing plants strengthened the protective effect, leading to even higher levels of tolerance with strong biotechnological potential.

"SYMPOSIUM OF THE SOCIETIES OF BIOLOGY"

A15
ADN: REPLICATION, ERRORS AND REPARATION. GENETIC STABILITY AND VARIABILITY IN BACTERIA.

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Living organisms have developed strategies capable to prevent, tolerate and repair DNA mutations. The prevention mechanisms are directed to the elimination of the mutagens; the tolerance mechanisms involve the action of alternatives DNA polymerases capable to bypass the structural barrier imposed by dna modifications, on the other hand the repair mechanisms eliminate the altered base allowing the reintroduction of the correct nucleotide. The mismatch repair system is a

posttranscriptional device to correct errors produced during the dna synthesis and to inhibit recombination between partially homologous (homeologous) DNA sequences. Deficiency of mrs in humans has been associated to the appearance of cancer, and in different bacterial species its deficiency generates hypermutator cells, with 100-1000 fold increase in the mutation rate. The presence of hypermutator cells is high in some clinical infections, though its role in this process is uncertain. In our laboratory different phenotypic and molecular aspects of the mrs are analyzed in *E. coli* and *P. aeruginosa*. During the last years we have studied the behavior of hypermutators cells in presence of antibiotics and the relationship among the chromosomal position, the replication fidelity and the efficiency of the repair process. Recently, we detected the existence of interactions between a mrs component and the replicative apparatus and postulate that it could regulate the accessibility of different dna polymerases to the replication process.

A16
MOLECULAR GENETIC IN HORTICULTURAL PLANT BREEDING: GLOBE ARTICHOKE

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Cynara cardunculus L. (*Asteraceae*) includes two domesticated *taxa*: the globe artichoke (var. *scolymus*) and the cultivated cardoon (var. *altilis*), as well as their common ancestor the wild cardoon (var. *sylvestris*). Both cultivated forms arrived to Argentina by Spanish and Italian immigrants in the 20th century and the edible parts are immature capitula (head) and left. In order to make available new commercial varieties adapted to the Argentinean climatic conditions, a *C. cardunculus* breeding program was promoting by the Agronomic Faculty (UNR). As results of this program, four clonal varieties ("Oro Verde FCA", "Esmeralda FCA", "Gurí FCA" and "Gauchito FCA") and an open-pollination variety ("Estrella del Sur FCA") were developed. Moreover, a germplams collection of the species was created with accession from different countries, which was morphological and molecular characterized. Recently, a new molecular linkage map of the species was developed by a cross between a genotype of wild cardoon and "Estrella del Sur FCA" globe artichoke. These linkage map included SRAP, AFLP, SSR, and SNPs molecular markers and three important agronomical traits (spiny head, spiny left and color head). Adult plants of the three *taxa* of *C. cardunculus* were evaluated for biomass production and aboveground growth suggesting that this species, after the heads or left collection, could be useful as energetic renewable sources. On the other hand, the wild cardoon is not considered as a crop in our region but will be incorporated into the culture system as an industry or energy crop.

A17
ANALYSIS OF GENOMIC DAMAGE IN THE LUNG EXPOSED TO ENDOTOXIN: MECHANISMS AND THERAPIES

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To measure DNA radicalization we use the immuno-spin trapping assay with the nitrone spin trap 5,5-dimethyl-1-pyrroline – *N* oxide (DMPO). Biochemical models showed that HOCl added as a bolus or continuously generated by myeloperoxidase (MPO) radicalizes purified DNA. Cell models showed that activated neutrophils can release MPO and epithelial cells can take it up. MPO inside epithelial cells exposed to H_2O_2 and in activated neutrophils can produce HOCl that radicalize genomic DNA. In addition, by trapping DNA radicals downstream effects, such as formation of 8-oxo-dG and mutation of the *HPRT* gene were prevented. Biochemical and cell models also showed that resveratrol crosses easily cell membranes and reacts faster with HOCl than with DNA and, thus protects against genotoxicity. *In vivo* models in mice shown that DMPO had a protective effect against neutrophilic inflammation triggered by intratracheal instillation of LPS. This protection was more likely due to interference with the retention of neutrophils caused by irritation of the airways by LPS. Indeed, DMPO interferes with the NF-kB signaling pathway triggered by LPS, thus decreased expression of adhesion molecules and pro-inflammatiory cytokines. Homing/activation of neutrophils, intracellular HOCl production and DNA radicalization are possible therapeutic targets against genotoxicity casued by neutrophilic inflammation of the airways.

A18
SEQUENCE VARIATIONS IN THE SOUTH AMERICAN CAMELIDS' GENOME

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Extant living South American Camelids (SAC) are represented by two wild species: the guanaco (*Lama guanicoe*) and the vicuña (*Vicugna vicugna*), that diverged from a shared ancestor two to three million years ago, and two domesticated species: the llama (*Lama glama*) and the alpaca (*Vicugna pacos*). There exists a whole-genome alpaca shotgun sequence database. The aim of the present work was the development of SAC species specific genetic markers. *V. v. vicugna* genomic DNA was used to generate (a) a set of random amplified DNA fragments by using arbitrarily sequence primers and (b) a mini-genomic library. RAPD and cloned fragments were sequenced and compared with sequences from the alpaca genome. PCR reactions with specific primers pairs amplified genomic DNA sequences from guanaco, vicuña, llama and alpaca DNA samples and confirmed these sequences as Sequence-Tagged Sites (STS) from different loci of the SAC genomes. Sequences comparisons identified repetitive DNA sequences (LINEs, SINEs, SSRs), potential Single Nucleotide Polymorphisms (SNPs) and insertion/deletion polymorphisms (Indels). These markers will provide a valuable resource for further genetic studies. An indel polymorphism consisted of a 95 bp deletion easily detectable by PCR. The allele with the deletion was absent in both *Lama guanicoe* and *Lama glama* species and should be a useful tool for species assignation.

SYMPOSIUM "YOUNG INVESTIGATORS"

A19
THE BARCODE OF LIFE IN ARGENTINA: OBJETIVES, APPLICATIONS AND ITS ROLE FOR STUDYING BIODIVERSITY AND EVOLUTION

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The Barcode of Life project is generating an identification system for life based on short DNA fragments that has diverse applications. This initiative is organized through the International Barcode of Life project (iBOL), an alliance of around 30 countries in which Argentina is a regional node. The barcode library currently includes about 3,000,000 sequences belonging to 200,000 species. This library also enables studies of biodiversity and evolution. In particular, it allowed us to study avian diversification in the Southern Cone of South America. Results have shown a complex assortment of patterns compared to other regions, including areas such as Patagonia and the Andes where glacial cycles had a significant influence and other areas, such as northern Argentina, where conditions have been more stable and other factors have been more relevant for avian diversification. In addition, 7% of avian species were shown to have high variation and in many cases diverging lineages that could be different, unrecognized species. The analysis of these cases in more depth is increasing our knowledge of avian diversity in the Southern Cone of South America.

A20
NEW NEURONS OF THE ADULT BRAIN AND ITS CONTRIBUTION TO PROCESSING OF STIMULI

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There are only two regions of the adult brain, where new neurons are generated throughout life, the olfactory bulb and the hippocampus. In particular granule neurons of the hippocampal dentate gyrus develop over several weeks and integrate into the preexisting network. Although adult hippocampal neurogenesis has been implicated in learning and memory, the specific role of new neurons remains unclear. This symposium will present experiments that examine the role of newborn neurons in the processing of stimuli in the adult hippocampus.

A21
HEPATOTOXICITY, RT-QPCR AND SANITATION.

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Hepatotoxicity and sanitation are associated to chemical pollutants. The activities in our working group are linked to the basic research, technology transfer and innovation.
We study the microRNA systems in rat hepatotoxicity models, evaluating their participation in the mechanisms of damage and repair of liver injury, as well as blood biomarkers. Our works propose that the decreased miRNA-122 expression (specific and abundant in liver tissue) during hepatotoxicity is the result, at least in part, of a diminished rate of transcription of the miRNA-122 precursor, due to a decrease in the expression of the transcription factors C/EBP□ and HNF4□. We propose that this phenomenon is associated with the liver response of self protection against the chemical exposure and recovery from the damage caused. We are also evaluating the use of the blood levels of miRNA-122 and miRNA-192 as liver damage biomarker. The quantification of microRNAs and their targets has provided us a deep understanding of the Real Time qPCR technique and a methodological development involving data normalization. The access to a suitable water supply and sanitation has been declared by the ONU as a human right. In Rosario City, at least 8% of homes lack an access to a formal drinking water supply. The Ríe Pibito group ("*Laugh, little child*", www.riepibito.com.ar, Fbioyf-UNR) works at the Villa Banana neighborhood promoting health, characterizing the actual situation and designing sanitation tools.

ORAL PRESENTATIONS

ANIMAL BIOLOGY

A22
CARBOHYDRASE ACTIVITIES IN THE HEPATOPANCREAS OF CRAB *Neohelice granulata*: DIFFERENTIAL LONG-TERM POST-INGESTA RESPONSE UNDER DIFFERENT SALINITIES

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Studies of possible differential long-term post-ingesta digestive adjustments at the biochemical level under hyper/hypo-regulation in euryhaline crabs are lacking. The aim was to study the effect of salinity on long-term post-ingesta amylase (Amy), maltase (Mal) and sucrase (Suc) activities in hepatopancreas of N. granulata. Male crabs were maintained (10 days) in 35 (osmoconformation), 37 and 10‰ salinity (S) (hypo and hyperegulation conditions, respectively). Immediately after ingestion (To) and at 24 (t24), 48 (t48) and 72 (t72) h activities in hepatopancreas were determined. The supernatant (10000xg 15min) (homogenate:0.1M Tris-HCl, pH 7.4) (4ml buffer x g of tissue-1) was used. Amy (µg maltose x min-1 x mg protein-1), Mal and Sac (µg glucosa x mg prot-1 x min-1) were assayed by hydrolysis of the corresponding substrate in 50mM phosphate 30ºC (Amy); 0.1 M maleate/OHNa 30ºC (Mal and Sac).In 35‰S at t48 and t72 Amy (22,8%;32,7%) and Mal (20,1%;35,8%) were lower than To (To=Amy: 12326± 2798; Mal: 1285±241).In 37 ‰S no activity changed. In 10‰S Suc was higher (207,7%) at t72 (To:473 ±113) The results suggest differential long-term biochemical digestive adjustments occurring in relation to osmoregulation status.

A23
EFFECT OF ANTHROPOGENIC ACTIVITY ON BLOOD PARAMETERS OF ANTARCTIC PENGUINS

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Blood biomarkers of oxidative stress and antioxidant defenses from *Pygoscelis adeliae* and *Pygoscelis papua* were analyzed in order to determine the physiological status of penguin populations living in high and low anthropogenic impact areas of Hope Bay (Antarctica). Samples were taken from chicks and adults in both species and area (with and without human impact). Levels of plasma uric acid, as well as activities of glutathione S-transferase (GST), glutathione peroxidase (GPx), and levels of reduced glutathione (GSH), lipid peroxidation (LPO) and protein oxidation (PO) from erythrocytes were measured using spectrophotometric techniques. Results from the high impact area showed: the activity of GST and LPO levels were increased

in adults of both species ($p < 0.05$); and activity of GPX was increased in Papua adults only ($p < 0.001$). No differences were registered for OP and GSH levels ($p > 0.05$) between the penguin populations from the studied areas. The uric acid levels were affected only in high impact´ Adelia penguins ($p < 0.001$). From this analysis it could be concluded that both species are affected by mechanical and/or chemical human activities´ pollution. The present study also provides oxidative stress baseline values, metabolic-health status of Hope Bay penguins' populations and the reference for future monitoring and / or comparisons with other Antarctic populations.

A24
THE "RED CHERRY" SHRIMP, *Neocaridina heteropoda* (CARIDEA, ATYIDAE) AS CANDIDATE FOR ORNAMENTAL CULTURE IN ARGENTINA

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The objective was to evaluate by means of laboratory analysis the potential for aquaculture of the shrimp *N. heteropoda* that is actually the first imported ornamental crustacean species in Argentina. The main features of the species as an excellent candidate are: gregarious species with no agonistic interactions sharing the same shelters in all developmental stages, rapid acceptance of commercial feeds, low fecundity with high survival at hatching and early juvenile stages, quick growth achieving sexual maturity nearby 2 months, dimorphic species with bigger and more coloured females ranging from pale orange to red that maybe determined by the substrate colour, no male morphotypes were recognized avoiding competitive interaction, direct and lecithotrophic development with synchronic growth at early stages and high starvation resistance and finally, this species could be culture under little water exchange and without commercial foods if biofilm is provided.

A25
HISTONE H3 MODIFICATIONS RELATED TO CHROMOSOME SILENCING AND ELIMINATION IN A BIRD

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In our laboratory we reported the first case of a germline-restricted chromosome in a bird (*Taeniopygia guttata*, zebra finch). This chromosome is eliminated during male meiosis and its chromatin is enriched with proteins related to gene silencing. In the present work we report a similar case of chromosome elimination in the Bengalese finch (*Lonchura domestica*), a related species to zebra finch. Using immunolabeling on chromosome spreads from the testis we analyzed molecular modifications of the chromatin related to chromosome condensation and segregation. We found that before elimination the chromatin of the restricted chromosome (RC) is enriched with di- and tri-methylated H3 at lysine 9. During metaphase I, the RC is devoid of H3K9me2 but H3K9me3 persists. In addition, the phosphorylated histone H3 at serine 10, a mark for chromosome condensation during mitosis and meiosis, is absent from the RC during metaphase I, while the chromosomes of the regular set are strongly labeled. It is proposed that the change of methylation status of the RC is part of the signals leading to its elimination during the first meiotic division. The deficiency in H3 phosphorylation may have a role to inactivate the RC centromere, in agreement with similar data reported in other organisms showing chromosome elimination.

CELULAR AND MOLECULAR BIOLOGY

A26
INFLUENCE OF EPIDERMAL GROWTH FACTOR ON CELL MOTILITY AND COLONY DYNAMICS

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In this contribution, Hela cell colonies in standard and epidermal growth factor (EGF) supplemented media, with different geometries and cell populations (N) were analyzed. Cell coordinates, their velocities, trajectories, directionality and persistence, and colony front dynamic geometric characteristics were evaluated. The EGF produced the decrease in the local cell density. The kinetics of q-radial colonies with low N fulfilled a first order exponential law in terms of N or the average

colony radius (<R>) with a kinetic constant that increased with EGF. For longer t (larger N) a constant front displacement velocity regime set in only for the colony spreading in S medium but not in the presence of EGF. Cell motility was guided by local crowding constrains, depending on N. Two cooperative transport mechanisms contributed to cell displacement: a conventional diffusion and a ballistic-like one. The presence of EGF increased cell and colony front velocities and the contribution of the ballistic mechanism. This correlates with changes in focal contact properties. Colony front geometric characteristics in the standard and in the EGF containing media were compatible with a Kardar-Pasisi-Zhang (KPZ) equation. With EGF roughness saturation was faster. Cells redistribution at the border region determining appears to be related to the KPZ curvature dependent term.

A27
ANALYSIS OF THE TRANSCRIPTIONAL ACTIVITY OF SYNONYMOUS MUTANTS OF TP53 IN YEAST

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The tumor suppressor TP53 is a transcriptional factor that regulates central pathways of the cell cycle, apoptosis and senescence. In cancer, the gene TP53 is mutated in approximately 50% of tumors. The 90% of these mutations are punctual and affect mainly the DNA binding domain of the protein. Silent mutations, that don't change the amino acid in the protein, are among 20 and 100 times more frequent than it's expected if they were neutral. The main purpose of our work is to understand the effect of silent mutations in the p53 function. In this project we aim to analyze the effect of 21 synonymous mutations in the transactivation activity of this factor, using the FASAY system, in *Saccharomyces cerevisiae*. Two yeast strains, which contain in their genome a p53 response element (p21 or RGC) that controls a reporter gene, were transformed with the cDNAs containing the different silent mutations. The results indicate that some mutations affect the transcriptional activity of p53, in comparison with the wild type protein. In some cases we detected variations in the expression level and the solubility of the protein, which suggest that diverse mechanisms might be involved in the alteration of functional activity.

A28
Trypanosoma cruzi PROLINE TRANSPORT: ITS IMPORTANCE IN OXIDATIVE STRESS AND TRYPANOCIDAL DRUGS RESISTANCE

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Trypanosoma cruzi has a metabolism largely based on the consumption of glucose and proline. L-proline is an important metabolite since it is involved in the differentiation of intracellular epimastigote to trypomastigote forms, which is required for the establishment of infection in the mammalian host. We generated a transgenic model of *T. cruzi* that overexpresses the proline transporter Tc069. These parasites showed a significant increase on the proline transport rate and also had a higher intracellular proline concentration than controls. This transport activity was inhibited by all the proline analogues tested, but no by other natural amino acids except D-proline, that competitively inhibited the transport. This lack of stereospecificity results interesting since *T. cruzi* has two functional proline racemases. We also tested the role of proline in oxidative stress responses (hydrogen peroxide and nitric oxide) and also in trypanocidal drugs resistance (nifurtimox and benznidazol). We found that Tc069 parasites were more resistant than controls in all the cases. These results suggest that decreasing intracellular proline levels, e.g. through blockage of proline transporter Tc069, could affect the survival ability against host natural defenses or pharmacological treatments.

A29
ALTERED METHYLATION LEVEL AT NUCLEAR GENES INVOLVED IN CARCINOGENESIS: THEIR CORRELATION WITH MITOCHONDRIAL GENOME INSTABILITY IN HUMAN BREAST CANCER

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MicroRNAs (miRNAs) are small noncoding RNAs that contribute to tumorigenesis by acting as oncogenes or tumor suppressor genes and may be important in the diagnosis, prognosis and treatment of cancer. Many miRNA genes have associated CpG islands, suggesting epigenetic regulation of their expression. Sixty-four pairs of tumoral/nontumoral breast

cancer samples were analyzed. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assays were developed for 11 miRNA loci that were chosen because all could be epigenetically regulated through the associated CpG islands and some could additionally modulate the epigenome by putatively targeting the DNA methyltransferases or their antagonist retinoblastoma-like 2 (RBL2). Mitochondrial Genome Instability (mtGI) was analyzed by two D-loop region markers, a (CA)n mtMS starting at the 514-bp position, and four informative MnlI sites between the 16,108-16,420-bp. Compared with the respective non-tumoral tissues, the predominant alteration in tumor tissues was increased methylation (hypermethylation) for the miRNAs 124a-1*, 124a-2*, 124a-3*, 148a** y 152**; decreased methylation (hypomethylation) for 18bI*, 208a* y 373*, being these differences statistically significant by *p<0.0001 y **p<0.001, respectively; and no major change for 1-1, 200a y let-7a-3. In particular, hypermethylation at miR-18bI was associated with mtGI (p:0.014). Moreover, miR-18bI methylation level has shown statistically significant differences depending the histopathological type of breast cancer analyzed, being higher in ILC than in IDC (p:0.04). Our results highlight the importance of alterations in the mitochondrial genome as well epigenetic changes of methylation at a gene promoter level, in particular for the miRNA analyzed loci, in human breast cancer.

ANIMAL BIOTECHNOLOGY

A30
IN VITRO MATURATION AND SUSCEPTIBLITY OF BOVINE OOCYTES TO BOVINE VIRAL DIARRHEA VIRUS

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There are structural differences of the ZP between embryos produced *in vitro* compared with those naturally conceived. There is evidence that indicates that such structural changes can modulate their interaction with Bovine Viral Diarrhea Virus (BVDV) in a different way facilitating the viral infection of *in vitro* produced embryos. These differences can also affect both the capacity and persistence of the infection into *in vitro* production systems. Mammalian oocytes are competent through their development and they can support BVDV replication after infection with the virus. *In vitro* maturation and the susceptibility of bovine oocytes to BVDV were assayed. In this study, no differences in the percentage of maturation rate between the experimental groups were detected. However, the viral isolation from ZP-intact oocytes demonstrated that BVDV was infectious during the *in vitro* maturation stage. These results indicate that the ZP itself does not guarantee full protection against BVDV. The infection model used not only demonstrates that oocytes are susceptible to BVDV during the *in vitro* maturation stage but also that the virus remains infectious in the ZP-intact oocytes, infecting susceptible cells when viral isolation was performed.

A31
AVIAN CYTOKINES AND TOLL-LIKE RECEPTORS AGONISTS, FOR THE PREVENTION OF INFECTIONS IN THE POULTRY INDUSTRY

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In order to analyze the protective effect of cytokines, bacterial flagellin and DNA on avian infections, an oral formulation was administered to different groups of chicks. From a total of 12 groups (15 chick per group), 2 were used as controls, 5 were infected without treatment, and 5 were infected and treated with the following pathogens: IBDv (Gumboro), GaHV-1 (Laryngotracheitis.), H_1N_1 (Avian influenza apathogenic), NDV (Newcastle Disease) and coccidia *(E. acervulina, E. maxima and E. tenella*). Comparing treated and non-treated groups, significant differences were observed in terms of macroscopic and microscopic lesions produced by all pathogens. However, only chicks infected with IBDv and coccidia produced significant differences in the body weight. The colony counts of *E. coli* per gram of ileum were significantly reduced in the treated last group. Our results suggest that cytokines and TLR agonist treatment could reduce the economic losses produced by avian pathogens.

A32
BLASTOCYST PRODUCTION BY INTERSPECIFIC ICSI IN FELIDS

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The ICSI is an assisted reproductive technique that can be used in wildlife to preserve the biodiversity. The aim of this work was to determine the fertilizing capacity of cheetah spermatozoa (*Acinonyx jubatus*) using the ICSI technique with domestic cat oocytes (DC, *Felis catus*). The oocytes were obtained from cats subjected to ovariectomy and matured for 22h. Mature oocytes were injected with a cheetah or DC spermatozoon (frozen/thawed) obtained from electroeyaculation and epididimis, respectively. Another group was injected without any spermatozoon (sham control). Injected oocytes were cultured immediately or were chemically activated with ionomycin (Io) before culture. The results were analyzed by Fisher test, $p<0,05$. Cleavage rates were: 66,3% (65/98 Cheetah); 73,6% (67/91 Cheetah-Io); 43,5% (37/85 DC); 69.7% (76/109 DC-Io); 19,2% (10/52 Sham) and 65,3% (47/72 Sham-Io). Interspecific embryos reached blastocyst stage equally as DC embryos, 32.6% *vs.* 20%, respectively. On the other hand, ionomycin assistance did not improve blastocyst rates in any of the groups, 21% *vs.* 17.4% for Cheetah-Io and DC-Io. The Sham control showed blastocyst formation only when Io was used (Sham-Io group, 6,9%). In summary, the ICSI technique without Io assistance was an efficient technique for embryo production with cheetah spermatozoa, which is an endangered species. This technique has great potential for poor quality sperm samples of endangered wild felids.

REPRODUCTION

A33
CHANGES IN IGF-1, IGF-2 AND IGF-1R EXPRESSION IN CANINE PLACENTAE OF DIFFERENT PREGNANCY AGE

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The IGF system is involved in human placental development. The aim of the work was to evaluate IGF-1, IGF-2 and IGF1R expression in canine early, mature and term placentae (EP, MP, TP, respectively). Placental samples from 7 dogs were obtained by Hysterectomy and processed by indirect immunohistochemistry for the detection of IGF1, IGF2 and IGF1R.
In EP fetal trophoblast and in labyrinthine cytotrophoblast both IGF were evidenced (IGF2>IGF1). Their expression decreased as pregnancy drawed on. IGF1R was evidenced in MP. IGF1R expression in syncytiotrophoblast was greater than that of both IGF. Labyrinthine and endometrial maternal endothelia were positive to IGFs and IGF1R, with stronger labelling in MP than in EP. Although gland endometrial cells were positive for all the studied markers, IGF2 detection was markedly polarised (apical pole in EP, apical and basal poles in MP), coinciding with IGF1R expression.
IGF are expressed in the main cellular populations of the canine endotheliochorial placenta. They may be involved in cell signalling and mediate endometrial invasion by the trophoblast. IGF-2 expression is stronger than IGF-1´s. IGFs expression is stronger in EP, while IGF1R increases later on. Endometrial gland cells communicate with maternal endothelia and with fetal cells.

A34
GLUCOSE FATE DURING BOVINE COC *IN VITRO* MATURATION

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The aim of this work was to estimate the relative fate of the consumed glucose by bovine cumulus-oocyte complexes (COCs) during in vitro maturation (IVM). Hexosamine biosynthesis pathway (HBP), Glycolysis (G) and pentose phosphate pathway (PPP) were analyzed. IVM was carried out in 199 medium, 5 % FBS, FSH + LH, 5 % CO_2, 39ºC for 22 h (control) supplemented with increasing concentrations of DON, NaF or 6-AN, inhibitors of the HBP, G and PPP, respectively. The degree of cumulus expansion, the lactate production and the ability to reduce Brilliant Cresyl Blue (BCB) were used as parameters for the evaluation of the HBP, G and PPP, respectively. Glucose uptake during COC IVM and oocyte meiotic maturation was determined in all cases. The addition of DON had a dose dependent inhibitory effect on cumulus expansion and glucose uptake ($p<0.05$), while no effect was observed on oocyte meiotic maturation. The supplementation with NaF inhibited in a dose dependent manner lactate production, glucose uptake and meiotic maturation ($p<0.05$). With the addition

of 6-AN a dose dependent inhibition on the ability to reduce BCB, glucose uptake and meiotic maturation rate ($p<0.05$) was observed. Of the consumed glucose, approximately 23 % would be fated towards HBP, 50 % to G and 13 % to PPP.

A35
SPERM MOTILITY IN ANURA CORRELATES WITH PKC DEPENDENT ATP-SYNTHASE PHOSPHORYLATION AND ACTIVATION OF CALCINEURIN

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An exposure of Amphibian sperm to low osmolarity solutions triggers its motility. This process is associated to phosphorylation events by PKA. We have recently identified a phosphorylation/dephosphorylation PCK dependent pathway, also associated with sperm motility and regulated by Calcineurin. The purpose of this study was to advance on the characterization of this pathway and its connection with the PKA dependent one. We observed phosphorylation of ATP-synthase enzyme during the hipoosmotic shock in correlation to a decrease of cellular ATP levels. Simultaneously, we detected dephosphorylation of PKC substrates in the tail region. Pharmacological assays with Ciclosporin-A enabled as to show that dephosphorylation is mediated by calcineurin and its inhibition impaires motility. Our results indicate that both, dephosphorylation of PKC substrates in the flagellum and phosphorylation of ATP-synthase, are necessary events for the activation of the sperm motility in *Rhinella arenarum.*

A36
ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR-D (VEGF-D) IN BOVINE OVIDUCTAL EPITHELIAL CELLS

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The oviductal microenvironment provides favorable conditions for initial events occurring in reproduction. Cytokines, proteases and growth factors were detected in oviduct, some of which are involved in events that occur in the oviductal lumen. The relationship between VEGF and proteases of the plasminogen-activation system (PAS) in certain biological processes, suggests that in the oviduct regulate their activities. The aim of this work was to analyze if VEGF-D in culture media of bovine oviductal epithelial explants affects the expression of PAS components. First examined the VEGF-D mRNA presence in bovine oviductal epithelium by RT-PCR and low expression levels was found. The expression of urokinase type plasminogen activator (uPA), its receptor (uPAR) and the inhibitor PAI-1 was evaluated in oviductal explants cultures incubated with VEGF-D, 1 ng /ml or 10 ng /ml for 0, 24 and 48 h by RT-PCR assays. An increase in the mRNA levels of uPA and uPAR at 24 and 48 h after stimulation was observed, whereas PAI-1 expression levels decreased after 24 h. These results support that the addition of VEGF-D affects the transcription of the genes studied in bovine oviduct. Its action is probably mediated through binding to VEGF receptors present on the oviductal epithelium.

A37
LIMK1/2 PARTICIPATES IN THE MECHANISMS ASSOCIATED TO SPERM MOTILITY BY AN INDEPENDENT ACTIN POLIMERIZATION PATHWAY

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LIMK plays a central role in the signaling pathway of the small GTPases Rho, Rac and cdc42. Its activation has been associated with different cellular events and mediated by the dynamic of the actin cytoskeleton through phosphorylation of cofilin. In this work, we investigated the role of LIMK in mouse sperm. By western-blot we detected that LIMK is phosphorylated (LIMK-P) in Thr508 during capacitation. This phosphorylation is downstream of PKA and would be independent of the Rho and Rac signaling pathway. By immunofluorescence, we observed that LIMK-P is localized mainly in the sperm flagella and is associated with the Triton X-100 insoluble fraction. Inhibition with specific inhibitor of LIMK-P (BMS-3) results in a dose-dependent manner decrease in progressive motility. The inhibition of LIMK-P does not decrease actin polymerization, suggesting that its mechanism of action is independent of the dynamics of the cytoskeleton in opposition of what is observed in other cell systems. In conclusion, LIMK phosphorylation downstream the activation of PKA is important for mouse sperm motility.

POSTER PRESENTATIONS

ANIMAL BIOLOGY

A38
PELVIC LIMB ANATOMY OF THE WILD BOAR AND ITS CORRELATION TO PRODUCTIVITY

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The wild boar - Sus scrofa - is an introduced species in the Country and has become a popular big game animal in the entire region of Patagonia Argentina. Its meat is highly prized, particularly its ham. This motivated the present study which aims to determine, in addition to accurately identify each of the muscles that make this cut, in what proportion they do. Left pelvic limbs of two adult male animals were isolated, measured and frozen in extended position at - 18 ° C to perform on them the corresponding cross sections. Right limbs, in turn, were dissected to identify the muscles - it was assumed a priori that they have the same structure than those present in the domestic pig, a fact to be confirmed. Transverse sections were made in a thickness of 2.50 cm, from the tarsus to the buttocks. Impressions were made of the proximal surfaces of each cut, imbuing them with black ink, then printing them on graph paper to determine exactly which dimension each muscle involved, depending on the region and the level of the cut. As a result, they were identified the muscles that make each of the parts into which it divides the wild boar ham.

A39
DESCRIPTION SEMITENDINOUS MUSCLE ANATOMY OF BOAR (*Sus scrofa*)

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The boar -*Sus scrofa*- is a wild animal native to Europe and belongs to the family of pigs (Suidae), introduced in Argentina around 1904. Previous studies focused on the myology of this species, evidenced by the particular structure of the semitendinosus muscle, which motivated to deepen their knowledge. Ten specimen were dissected, six females and four males, all adult animals. As is logical to assume, given the proximity taxonomic this species has compared the structure of the muscle, with the pig and then with other animals. From there came the results given below. In the boar, the semitendinosus muscle has the typical two heads of origin, vertebral and ischial, as in the pig, horse and rabbit among domestic mammals in marsupials, most phisipeds, insectivores and rodents, some prosimians and lagomorphs, the probosideos, perissodactyla, llama and giraffe. In the boar, the vertebral head drive distally, in search of the head takes origin in the ischium and the fascia that shares with biceps femoris muscle. In all of the species above, the vertebral-caudal-head disappears and only the ischial head remains. Vertebral head of the boar semitendinosus muscle is located between the diverging branches of the ischial head sits deeply the same, but easily separable by dissection. Finally, near to be inserted both bellies converge together in the proximal third of the tibial shaft.

A40
ROLE OF PASSIVE STRUCTURES IN THE BIOMECHANICS OF THE CAUDAL THORACIC REGION OF THE EQUINE SPINE

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The caudal thoracic region of the spine of the horse can be thought a priori as very particular from the functional point of view. In a biomechanical approach is necessary to initially focus on the shape and structure of the passive elements, such as the bony and ligamentous structures responsible for facilitating or limiting movement. To this end, six horses and four mares, adults and free from injuries in the forenamed region were dissected. It was stipulated that caudal thoracic region would

cover from 11th to 18th thoracic vertebrae. Once stripped of musculature, the vertebral set was subjected to manual forces to test movements of flexion, extension and laterality. These results led to the following conclusions:
a- the extension movements are primarily restricted by a mechanical limiting factor depending on the adjacent spinous processes
b- in flexion, play a leading role ligaments and the ventral part, cranial and caudal, of vertebral bodies
c- lateral movements are limited by the presence of the ribs. However, from 15th thoracic vertebra, the joint surfaces for the tubercle of the rib and the head of the rib begin to merge into a single cavity, which allows greater freedom of movement
From these results, it is clearly stated which passive elements play a facilitating or restricting role in movement.

A41
SALINITY TOLERANCE OF ENDEMIC CLADOCERANS IN BIOASSAYS WITH NATURAL SALTS AND PHREATIC WATER

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Daphnia menucoensis and *Moina eugeniae* are the most common autochthonous cladocerans in saline lakes of central Argentina. By means of bioassays, we began to generate ecophysiological information to help to explain their geographical and temporal distribution. Previous bioassays have verified that survival is higher when using natural salts instead of analytical grade reagents, and that the most suitable culture medium is water from the aquifer of Santa Rosa city (La Pampa). The aim of this study was to test that the combination of natural salts dissolved in phreatic water is optimum to determine the tolerance of both species. We determined the survival of neonates by 48-h acute bioassays (without food supply and medium renewal) at five concentrations of salts (5, 10, 15, 20 and 25 $g.L^{-1}$). Bioassays began with five neonates and four replicates were made. Survival was different ($H=6.01$; $p=0.0305$), since 100% of neonates of *M. eugeniae* and 50% that of *D. menucoensis* survived at 5 $g.L^{-1}$. While at intermediate salinities (10 and 15 $g.L^{-1}$) survival was practically complete, at the highest salinity (25 $g.L^{-1}$) 40% of the neonates of *M. eugeniae* but none of *D. menucoensis* survived. The results confirm that the combination of natural salts and aquifer water is the most appropriate, since survival was recorded at the known tolerance range for both species in the nature.

A42
EFFECT OF FEEDING ON GROWTH AND REPRODUCTION OF THE PARENTAL STOCK OF *Cherax quadricarinatus* (PARASTACIDAE)

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The objective of the present study was to determine the effect of the amount of food offered to the parental stock on their growth and survival, percentage of ovigerous females successfully hatched (OFH), and egg size. Three females were stocked with one male in glass aquaria with water at 26±1°C under continuous aeration. Each aquarium (n=12) was randomly assigned to one of the following treatments: Control and Restrictive feeding (feeding at 1.5 and 0.5% of body weight respectively). When an ovigerous female was detected, a sample of ten eggs was taken to determine their volume and weight. At the end of the experiment all the animals were measured and weighed, their hepatopancreas and gonads were removed and weighed to determine the hepatosomatic (HSI) and gonadosomatic (GSI) indexes. These variables were compared among treatments with one-way ANOVA. The specific growth rate, growth increment, final weight, total length, postorbital length, GSI, HIS, OFH and egg size were similar between treatments ($p>0.05$). Therefore, restrictive feeding seems to have no effect on growth, nutritional state and gonad maturation of the reproductive females. This may allow the reduction of production costs of the species.

A43
DIGESTIVE FLEXIBILITY IN RESPONSE TO HIGH ENVIRONMENTAL SALINITY AND TEMPERATURE IN THE NON-SYMBIOTIC SEA ANEMONE *Bunodosoma zamponii*

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Studies on digestive flexibility at the biochemical level in relation to high environmental salinity and temperature in non-symbiotic sea anemones are lacking. The aim was to study maltase (Mal) and proteolytic (Pr) activities in coelenteron mesenterial filaments (MF) in *Bunodosoma zamponii* from the intertidal area of Punta Cantera (Mar del Plata, Bs. As.) acclimated to 35 and 40 psu and after an acute enhancement (15 min) of temperature from 20°C to 30ºC. The supernatant (10000xg 15min) from MF homogenate (0.5M Tris-HCl, pH 7.4) (4ml buffer x g of tissue^{-1}) was used. Mal (µg glucosa x mg prot^{-1} x min^{-1}) was assayed by hydrolysis of 28.0 mM of maltose in 0.1 M maleate/NaOH buffer (pH 6.4) at 37°C and Pr (U.h^{-1}.mg protein^{-1}) by hydrolysis of 1% w/v azocasein in 0.1 M Tris–HCl buffer (pH 7.5) Mal activity was higher in 40 psu (80%) (35psu: 21.58±5.18,40 psu=38.90±7.89, T=34 P=0.017) and upon the increase in temperature (99%) (20ºC: 21.58±5.18, 30ºC 43.13±9.14, T=25 P=0.026). Pr was not affected in any case (p>0.05). The results suggest the occurrence of specific digestive adjustments in relation to high salinity and temperature.

A45
DISTRIBUTION OF THE INTRAMUSCULAR CONNECTIVE TISSUE IN PIG BICEPS FEMORIS WITH REGARD TO THE INSTRUMENTAL TENDERNESS.

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The muscle heterogeneity was demonstrated by the presence of neuromuscular compartments (CNM), which vary its structure, the fiber characteristics and intramuscular connective tissue organization (TCIM). However the NMC have not been considered when the muscle anatomical characteristics are related with meat quality traits. Fibers characteristics were described in different CNM of the semitendinosous and biceps femoris muscles in pig (BFC) although there is no documentation about TCIM distribution in them. The TCIM is considered one of the most influential factor on tenderness. The goal was to measure the area occupied by TCIM in each CNM of BFC. Our hypothesis is that there are significant differences between the values of the area occupied by the endomysium and perimysium in each CNM in BFC. In frozen sections of each CNM we identified the perimysium and endomysium using a lectin (1:30, immunohistochemical protocol and revealed by DAB-peroxidase). Using digital images and an analyzer was measured the TCIM in each area in each CNM. The results indicated no significant differences among CNMs (ANOVA, p <0.05). Given that in previous studies it were obtained different meanings of instrumental tenderness, the studies to determine the qualities of TCIM should continue.

A46
CYTOGENETIC CHARACTERIZATION OF THE SHEEP FLY *Lucilia sericata* MEIGEN (DIPTERA: CALLIPHORIDAE)

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Calliphoridae has much interest since it is predominant at the Fresh and Bloated stages of succession on carcasses, causes myiasis, and has a forensic and health importance in determining the *post-mortem* interval and in maggot therapy, respectively. Cytogenetic data on 9 species of this family have shown a noticeable karyotypic uniformity (2n=12=10+XX/XY). In this work, we study the mitotic karyotype of *Lucilia sericata* by conventional staining and C-banding in brain chromosome preparations of third instar larvae. This species has 2n=10+XX/XY and somatic pairing in the homologous chromosomes. Two largest metacentric autosomal pairs, the large submetacentric X and the smallest Y were distinguished. Both one largest autosomal pair and the X have a secondary constriction. This species has scarce and pericentromeric C+ heterochromatin. We conclude that *L. sericata* show similarities in karyotype, and content, location and distribution of constitutive heterochromatin to the nine species of Calliphoridae previously studied. Therefore, the family Calliphoridae is cytogenetically homogeneous. From data of *L. cuprina* Wiedemann, we propose that in *L. sericata* the secondary constriction on the X chromosome would correspond to a nucleolus organizer region.

A47
EFFEC OF DIETARY CAROTENOIDS IN THE SHRIMP BROODSTOCK *Artemesia longinaris*

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The aim of this study was to determine the effect of dietary carotenoids over bioacumulative capacity of carotenoids in different organs, total antioxidant activity, growth and maturation. Immature females (with unilateral eyestalk ablation) were held in 3m diameter tanks with aeration (33‰ salinity, 18°C, pH 7, 12:12 h photoperiod). Three diets were tested, two supplemented with β-carotene or astaxanthin (300mg carotene/kg diet) and other without supplementation. After 60 days ovaries, midgut gland and integument were removed. Nonpolar carotene (β-carotene) and polar (astaxanthin) was determinate by using UV-visible spectroscopy. Antioxidant activity of midgut gland was analyzed by magnetic resonance spectroscopy. In all treatments 100% of females have ripen ovaries. Carotenoids enhanced weight gain in both treatments related to the control. Both, the integument and ovary showed higher bioaccumulation of β-carotene than free astaxanthin. Carotenoids were not detected in the midgut gland. Either with or without addition of carotene, the midgut gland exhibited an antioxidant protection, evidenced by the ability to react with the stable radical 2,2-diphenyl-1-picrylhydrazyl.

A48
EFFECT OF DIFFERENT TEMPERATURES ON COMPENSATORY GROWTH IN THE FRESHWATER CRAYFISH *Cherax quadricarinatus*

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The objective of the present study was to evaluate the compensatory growth in juveniles of the freshwater crayfish *Cherax quadricarinatus* reared under two feeding regimes and three different temperatures. The temperatures were: **23± 1°C**, **27± 1°C** and **31± 1°C**. The feeding regimes for each temperature were: **control** – in which animals were fed once a day over a 90-day period; and **cyclic feeding** – in which animals were fed once a day for 4 days followed by 4 days of food deprivation in repeated cycles, over a 45-day period, and fed once a day for the following 45 days. Fifty 1-g juveniles were distributed in individual plastic containers (500 cm^3, 350 ml of dechlorinated water with continuous aeration) for each feeding regime. The one-way ANOVA results showed that growth of juveniles cyclically fed was lower than daily fed , at day 45, at all temperatures assayed. However, at day 120, these juveniles achieve the same size as control, at all temperatures. It is concluded that the compensatory growth is not affected in the temperature range tested.

A49
EFFECT OF CYCLIC AND CONTINUOUS FEEDING ON ENERGY RESERVES OF *Cherax quadricarinatus* JUVENILES REARED UNDER DIFFERENT DIETS

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The objective of the present study was to evaluate changes in energy reserves in the hepatopancreas and abdominal muscle of juveniles of the freshwater crayfish *Cherax quadricarinatus* reared under two feeding regimes and two different diets. The diets were:
diet A (49% of crude protein) and diet B (38% of crude protein). The feeding regimes for each diet were: **control** – in which animals were fed once a day over a 120-day period; and **cyclic feeding** – in which animals were fed once a day for 4 days followed by 4 days of food deprivation in repeated cycles, over a 45-day period, and fed once a day for the following 75 days. Sixty 1-g juveniles were randomly distributed in 12 aquaria for each diet. At day 45 three aquaria were randomly selected and the juveniles were sacrificed to determine total protein, lipid and glycogen contents in their hepatopancreas and abdominal muscle. The same procedure was applied at day 120. The one-way ANOVA results showed that these variables were similar between the feeding regimes for each diet, both at day 45 and 120. It is concluded that although cyclic feeding resulted in a lower juvenile growth it did not affect reserve mobilization.

A50
HYPOXIA INCREASES TOLERANCE TO OXIDATIVE STRESS IN THE BLOOD CELLS OF *Themiste petricola*

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Themiste petricola is a coelomate marine worm that inhabits the intertidal zone, exposed to periodic hypoxia and re-oxygenation, producing high amounts of oxygen reactive species. It does not have a true circulatory system and the coelom contains cells that have oxygen transport and immune functions (coelomocytes). The aim of the work was to study the response of coelomocytes to H2O2 in normoxic and hypoxic conditions (O2<0.1%). Coelomocytes were collected from the coelomic cavity and were cultured in vitro exposed to several doses of H2O2. Cell death was evaluated by fluorescein-di-acetate /propidium iodide, mitochondrial potential was evaluated with TMRE probe, and glutathione was evaluated by 5-chlormethyl-fluorescein, and superoxide anion with dihydrothidine, in all cases by flow cytometry. At 24h cell death was increased dose-dependently by H2O2 exposure, being 1.5 mM the H2O2 50% cytotoxic dose (EC50). Mitochondrial membrane depolarization using the EC50 dose of H2O2 had a peak of 20% at 3h. The H2O2 dose that depolarizes 50% of cells was 3.67 mM. Reduced glutathione decreased and superoxide anion increased dose-dependently with H2O2. Finally we evaluated the cytotoxic response of coelomocytes to hypoxia, resulting an EC50 dose of 4.7 mM H2O2. We conclude that coelomocytes of *Themiste petricola* tolerate a higher level of oxidative stress under hypoxic conditions.

A51
EFFECT OF DIETS WITH DIFERENT LIPID CONENT IN THE CRAB *Neohelice granulata* FROM DIFFERENT HABITATS

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The animals undergoing dietary changes among habitats are expected to exhibit metabolic adjustments. However intraespecfics studies in estuarine euryhaline crabs are lacking. Objective: To study the effect of diets with different lipid proportions on triglycerides (TG) and protein (Prot) content in hepatopancreas (HP) of individuals of *N. granulata* from mudflat (MF) and saltmarsh (SM) of the Mar Chiquita lagoon (Pcia. of Bs As). Methods: Adult male crabs were fed 3 times a week (diets with 3,5 (D1) or 5 % (D2) of lipids) and starved 48 h before determinations. Before (t0) and after 30 (t30) days, TG (mg tissue xg-1) and Prot (mgtissuexg^{-1}) were determined in HP homogenate (0.1M Tris-HCl, pH 7.4, 4mlxgtissue^{-1}) by enzyme kit TAG Wiener-Lab.AA and Bradford method, respectively. Results: Individuals from MF: D1 (TG: 460.33 ± 285.54; Prot: 23.61 ± 2.58) and D2 (TG: 242.88 ± 35.01; Prot: 26.08 ± 3.38) did not affect TG or Prot content. In individuals from SM: Prot was higher (660%) than t0 (18.27 ± 1.5) in subjects fed with D1. TG did not change. There was no change with D2. Conclusions: The differential responses in Prot content in HP regarding diets with different lipid proportion suggests the existence of differential metabolic adjustments relative to the habitat and compensatory adjustments and interrelation within different metabolic pathways.

A52
GLP-1, GLYCAEMIA, INSULINEMIA AND HOMA THROUGH ORAL GLUCOSE TOLERANCE TEST IN DOGS WITH CUSHING'S SYNDROME

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Objectives: The aim of this study was to assess GLP-1, glucose and insulin concentrations, $HOMA_{\beta\text{-cell function}}$ and $HOMA_{insulinsensitivity}$ in dogs with Cushing's Syndrome (CS), and compare these values with those in normal and obese dogs. **Methods:** Three groups were formed (n=6 each group): CS-group, Obese-group and Control-group. They performed the Oral Glucose Tolerance Test (OGTT) to dose GLP-1, glycaemia and insulin levels while baseline, 15, 30, 60 and 120 minutes (m). Statistical analysis was performed using ANOVA Friedman test followed by Dunn's multiple-comparison. Values are expressed as median and interquartile ranges (P<0,05). **Results:** GLP-1 levels were statistically elevated in the CS-group compared to the other groups, both at basal time (P <0.01) and at 15, 30 (P <0.001), 60 and 120m (P <0.05 vs. Obese-group and P <0.01 vs. Control-group). Insulinemia followed similarly behavior to the variations of GLP-1 and their concentrations were higher in CS-group (P <0.01 at baseline, 30, 60 and 120m and P <0.001 15m vs Control-group; P <0.05 basal, 15, 30 vs. Obese-group). The glycaemia was higher in CS-group vs. Obese-group and Control-group (ANOVA P<0.01). $HOMA_{insulinsensitivity}$ was statistically decreased in CS-group vs. the other groups (P <0.01). $HOMA_{\beta\text{-cell function}}$ was statistically increased in CS-group vs. the other groups (P <0.01). **Conclusions:** Dogs with CS have higher concentrations of GLP-1,

glucose and insulin, reduced insulin sensitivity and increased functionality of pancreatic β-cell. The obese have an intermediate behavior. The highest concentrations of GLP-1 might overstimulate pancreatic β-cell, predisposing its depletion and subsequent development of Diabetes Mellitus.

A53
N-AMINOPEPTIDASE ACTIVITY IN HEPATOPANCREAS OF EURYHALINE CRAB *Neohelice ganulata*

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Little is known about N-Aminopeptidase activity (APN) in hepatopancreas (H) of euryhaline crabs. We studied the occurrence, biochemical characteristics and responses to salinity of APN in H of *N. granulata.* Adult males (n=5) were acclimated 10 days in 35, 10 and 45‰ salinity (S) (osmoconformation, hyper and hypo-regulation, respectively). The supernatant (10000xg 15 min) (homogenate:Tris-HCl 0.1M, pH 7.4 ; 4mlxg of tissue^{-1}) was used. APN (µmolesxmin^{-1}xmgprot^{-1}) was colorimetrically quantified (curve substrate: 0.083-0.500 mM in Tris/HCl 50mM, pH 7.6, 37ºC); (pH curve: buffers pH 6.6-9: Tris/HCl; 10.0: Glycine; L-alanine 0.42 mM, 37ºC); temperature curve: 4-50 °C; L-alanine 0.42 mM, pH 7.6). APN was higher at pH 7.6 and 8.5-9.0 and (% of maximal activity): 25,41,40 at pH 6.6, 8.0 and 10.0. APN was higher at 37 °C and (% of maximal activity): 45(4 ºC), 35 (20º and 30 ºC), 98 (45 ºC), 75 (50ºC). APN showed Michaelis-Menten kinetics (Km= 0.11 mM) (pH 7.6, 37 ºC). APN was higher in 10‰ (3184±328) and 37‰S (5720±1242) than in 35‰S (2202±268).The results suggest the role of APN in digestive adjustments upon hyper and hyporegulation.

A54
LIPASE AND PROTEOLYTIC ACTIVITIES IN HEPATOPANCREAS OF *Neohelice granulata:* BIOGENIC AMINES EFFECT

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Little is known about the regulation of digestive enzymes activities in euryhaline crabs by chemical messengers (i.e. biogenic amines). Aim: to study the *in vitro* effect of serotonin (5-HT) and histamine (Hist) on lipase and proteolytic activities in hepatopancreas of *N. granulata*. Adult male crabs were maintained for 30 days at 35 ‰ salinity. Hepatopancreas slices were incubated in the absence and in the presence of 5-HT or Hist 10^{-4}M (100mg/2ml medium mM: 400 NaCl, 13 KCl, 10 $MgCl_2$ 8.8 H_3BO_3, pH 7.6, 30ºC). Enzymatic activities were determined after 0, 45, 60 and 90 min of incubation in homogenates of the tissue slices (Tris-HCl 0.1M pH 7.4 4mlxg^{-1}). Lipase was measured by pNPpalmitate hydrolysis (0.8mM, A_{410} nm). Proteolytic activity was measured by azocasein hydrolysis (1%w/v, A_{440} nm) (n=5, paired t-test, $p<0.05$). Lipase activity was 2.4 fold higher in the presence of 5-HT between 60-90 min of incubation and 71% higher at 45 min with Hist incubation, than t=0 without agent. Proteolytic activity was not affected by 5-HT or Hist. These results show the differential effect *in vitro* of 5-HT and Hist on lipase and proteolytic activities in hepatopancreas, suggesting the occurrence of differential mechanisms of regulation of these digestive enzymes activities and the direct effect of these biogenic amines on the hepatopancreas.

A55
FATTY ACID COMPOSITION AND LIPOPEROXIDATION OF OVARY AND LIVER MICROSOMES FROM YOUNG LAYING HENS. DIET ANALYSIS

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The unsaturated fatty acids are susceptible to peroxidation. The aim of this study was to evaluate the influence of diet on the composition of fatty acids (FA) and sensitivity to lipid peroxidation in microsomes obtained from ovary and liver of young laying hens. FA profile was analyzed by gas chromatography and lipid peroxidation was quantified by means of chemiluminescence. In microsomes of both organs C16: 0 and C18: 0 were the most common FA unsaturated, while C18: 1n9 (22.27 ± 1.21 liver, ovary 23.9 ± 1.30) and C18: 2n6 (liver 7.49 ± 0.6, 8.23 ovary ± .71) were the most abundant unsaturated FA. The FA composition of food was 42% of saturated FA and 58% of unsaturated FA, with a prevalence of C18: 1n9. The unsaturation index of the food was approximately 1.30 times lower than in microsomes. The liver and ovarian microsomes were not affected by the lipid peroxidation process. Light emission originated from microsomes of both tissues was not statistically different when comparing controls and peroxidized: in liver 393.83 ± 27.56 vs. 374.06 ± 46.4, in ovary

276.2 ± 17.56 vs 261.99 ± 24.06. The results indicated that in microsomes of ovary and liver of chiken prevailed unsaturated FA with low number of doublé bonds. FA composition of food did not influence the content of double bonds of the microsomes. The low sensitivity to lipoperoxidation of these organs could be related in part to its composition of AG, protecting them against oxidative damage and preserving its function.
Key words: fatty acids, lipid peroxidation, ovary, laying hen.

A56
NUMBER AND SIZE OF JUVENILES IN RELATION TO MOTHER WEIGHT FOR THE FRESHWATER SHRIMP *Neocaridina heteropoda*

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The objective of the present study was to determine whether the mother weight affects the number and size of juveniles I (JI) in a decapod crustacean of importance as ornamental species. Once detected, ovigerous females were placed in individual plastic containers at a temperature of 28±1°C, with constant aeration and Java moss. They were fed with balanced food for tropical fish. After hatching, the number of JI was recorded (actual fecundity), a sample of 10 JI was taken to determine their size, and the mothers were weighed. The relationship between these variables was analyzed using simple regression. No significant relationship ($p>0.05$) was found between JI size (1.26±0.02 mm of carapace length) *versus* mother weight (range: 50-110 mg) and between JI size *versus* actual fecundity (24.4±1.62 JI/female). This variable tended to increase with the mother weight, but this relationship was not statistically significant ($p>0.05$). It is concluded that the mother weight does not affect the number and size of the juveniles hatched, at least for the weight range considered. The present study represents a first approach to this topic for a shrimp with direct development.

A57
EFFECT OF TEMPERATURE ON THE INCUBATION PERIOD, FECUNDITY AND JUVENILE SIZE IN THE FRESHWATER SHRIMP *Neocaridina heteropoda*

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The objective of the present study was to evaluate the effect of temperature on the incubation period, and number and size of juveniles I (JI) in a decapod crustacean of importance as ornamental species. Once detected, the ovigerous females were randomly assigned to one of the following treatments: High Temperature (HT): 32±1ºC; Normal Temperature (NT): 28±1ºC; Low Temperature (LT): 24±1ºC. Females were maintained in individual plastic containers with continuous aeration and Java moss. They were fed with balanced food for tropical fish. After hatching, the number of JI was recorded (actual fecundity) and a sample of 10 JI was taken to determine their size. These variables were compared among treatments using one-way ANOVA. Even though the incubation period decreased with increasing temperature ($p<0.05$; 12.4, 14.6 and 20.8 days for HT, NT and LT, respectively), the actual fecundity (24.2±0.09 JI/female) and JI size (1.27±0.00 mm of carapace length) were similar among treatments ($p>0.05$). It is concluded that the accelerated embryonic development observed as a result of increasing temperature does not affect negatively the number and size of the juveniles hatched.

A58
THE USE OF BIOFILM AS NATURAL FOOD SOURCE ON THE “RED CHERRY” SHRIMP *Neocaridina heteropoda* CULTURE.

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Neocaridina heteropoda is a freshwater omnivorous shrimp with potential as an ornamental species. The study was aimed at evaluating the contribution of biofilm associated to different substrates as a natural food source on its culture. The experiment was run for 60-d in a zero water exchange system in tanks with 20 L of aerated dechlorinated water and 174 adults shrimps/m^2. Three substrates were tested: (PET) plastic bottles, (PN) plastic net (1 mm mesh size) and (AV) agrovelo (non-woven tissue). Higher values ($p>0.05$) of survival were registered in all substrates (100% for AV and PN, while 82% for PET). Specific growth rate showed no differences ($p>0.05$) between groups, while biomass was higher ($p<0.05$) for AV and PN substrates. Total lipids, proteins and carbohydrates (as mg/g biomass) were analyzed to determine the nutritive value of

the biofilm revealing higher values ($p<0.05$) in AV compared to both PN and PET groups. However, total lipids in the shrimps did not show differences ($p>0.05$) among substrates. The results showed that the biofilm in a zero water exchange system could be considered a good tool for *N. heteropoda* culture. Even though AV shown best results, recycled material (such as PET) was also appropriated for this purpose, thus reducing environmental impact and production costs.

A59
COMPARISON OF THREE CULTURE MEDIA FOR BIOASSAYS WITH ENDEMIC CLADOCERANS (CRUSTACEA)

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Bioassays with cladocerans are used in ecotoxicology. Introduced easy-to-breed species (*Daphnia magna* and *Moina macrocopa*), are frequently used and cultured with standardized media. In the UNLPam, ecophysiological bioassays are being developed to determine the salinity tolerance of *Daphnia menucoensis* and *Moina eugeniae*, two autochthonous halophilic cladocerans. The aim of this work was to compare the efficiency of three culture media at different salinities. Acute survival bioassays (48-h, without food supply or medium renewal) were performed using neonates younger than 24- h of both species. Five replicates were made with solutions using demineralized water, phreatic water and EPA medium, with 5, 10, 15, 20 and 25 $g.L^{-1}$ of analytical-quality NaCl. Differences were found between the culture media for both species (*D. menucoensis*: H=19.38; p=0.0001 and *M. eugeniae*: H=12.67; p=0.0315). In both cases, survival was higher in the water from the aquifer. In the case of *D. menucoensis*, most neonates survived up to 20 $g.L^{-1}$, whereas those of *M. eugeniae* survived up to 15 $g.L^{-1}$. Although EPA is one of the most used media, it showed to be deficient for both species. Few neonates of *D. menucoensis* survived up to 10 $g.L^{-1}$ and although in nature *M. eugeniae* is a widely tolerant species, none of its neonates survived, even at the lowest concentration of NaCl.

CELLULAR AND MOLECULAR BIOLOGY

A60
CHARACTERIZATION OF *Toxoplasma gondii* CLONES EXPRESSING H2B.Z HISTONE

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Toxoplasma gondii is an obligate intracellular parasite, capable of producing toxoplasmosis, affecting primarily immunodepressed patients and newborns. Because of the importance of chromatine in gene expression modulation and DNA replication, in this work we focalized in variant histone H2B.Z. This histone is acetylated in lysines K3, K8, K13, K14 and K18, modifications that could be relevant for its function. Stable lines of *T. gondii* expressing wild type H2B.Z, and a version with all lysines interchanged by alanine (aminoacid not able to acetylate) fused to c-Myc tag, were obtained using pTUB8mycGFPftailTy/H vector. The constructions were transfected in *T. gondii* RH□HXGPRT□UPRT strain by electroporation. This strain may be cultivated in metabolic stress conditions without CO_2 to obtain bradyzoites. Stably transfected parasites were identified by indirect immunofluorescence assay (IFA) using antibodies against c-Myc tag, and also by Western blot. Two clones were obtained for each line (Myc-H2Bv 1 and 2 and Myc-H2BvK3,8,13,14,18A 1 and 2) and were characterized. The first step of phenotipic characterization was the replication assay. Clones from W.T. line were similar to parental line, while clones from the mutant line showed a minor deficiency in replication. Acetylations in H2B.Z histone would be important to conserve the parasite phenotype.

A61
CLOCK GENES AND XENOBIOTIC METABOLIZING GENES EXPRESSION IN A MURINE MODEL OF CHRONIC JET LAG AND TUMOR DEVELOPMENT

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Most of physiology is affected by the circadian clock. Its disruption is associated with an accelerated growth of tumors. Xenobiotic metabolizing genes (XMG) metabolize endo-/exogenous compounds, and are also under circadian clock regulation. We analyze mRNA expression of clock genes Per1 and Per2, and XMG Nat1 and Cyp1a1 in suprachiasmatic

nuclei, liver and tumor samples, obtained from C57BL/6 mice, in a chronic jet lag model (CJL). We have four groups of animals: 1) healthy mice under L/D 12:12, 2) healthy mice under CJL, 3) mice inoculated with B16 tumor cell line, under L/D 12:12; 4) mice inoculated with B16 tumor cell line under CJL. mRNA relative quantification was performed with the $2^{-\Delta\Delta Ct}$ method, using Hprt as normalizer. So far, the results show a significant decrease in the expression of Per1 and Per2 ($p < 0.01$) and Nat1 and Cyp1a1 ($p < 0.05$) in tumor tissues of those animals under CJL, compared to those under L/D 12:12. Regarding liver tissue, there are no differences in the expression levels for any of the genes. The data suggest a clear alteration of rhythms in tumor tissues under CJL.

A62
LXRB EXPRESSION IN ALTERED IN THE HYPOTHALAMUS OF ANIMALS SUBMITTED TO HYPERGLYCEMIA DURING GESTATION.

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We have recently demonstrated that the LXR expression is altered in a rat model of glucose intolerance. The relationship between the hypothalamic LXRβ levels and the area under the curve (AUC) obtained from a glucose tolerance test (GTT) was also modified in these animals (Kruse 2012). Here we studied the LXR expression and its relationship with AUC in rats born to diabetic mothers. Gestational diabetes was induced after a single dose of streptozotocin (30mg/Kg i.v) to mothers and the LXR expression was analyzed by Western blot. Adult males and females born to diabetic dams (DO) were compared to controls (CO). The AUC was calculated using trapezoidal method. Male DO presented a decreased LXRβ expression compared to CO. Females did not show any LXR differences between DO and CO. When we analyzed the correlation between LXRβ and AUC we found two different populations among DO. Animals with AUC<300, as CO, showed a negative correlation in males and females. In contrast, DO with AUC> 300 showed a positive correlation in males, a behavior previously observed in a different experimental model of glucose intolerance. Female DO with AUC> 300 did not show any correlation at all. Altogether these results suggest that male DO may be more sensible to develop glucose intolerance related to alter LXRβ expression in the hypothalamus.

A63
GLUCOSE AFFECTS THE EXPRESSION OF LXR IN RAT HYPOTHALAMIC AND HIPPOCAMPAL EXPLANTS.

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LXR expression is altered in the hypothalamus of glucose intolerant animals. Here we study some of the factors that regulate the expression of LXR in vitro. We used slice-explant cultures of rat hypothalamus and hippocampus as experimental models. LXR expression was analyzed by Western blotting and the LXR changes induced by treatment with glucose (5.5, 8.5, 15.5 or 25.5 mM) or insulin (1, 2.5, 5 or 10 nM) at different times (2, 4 and 6 h) were examined. At 2h no changes were observed in the hypothalamus. In the hippocampus, 15.5 and 25.5 mM glucose increased LXRaexpression (30 and 35%, $p<0.05$). 4h of these two glucose concentrations decreased LXRb expression in the hypothalamus (50% and 60%, respectively; $p<0.05$) and increased the expression of LXRb in hippocampus (17% and 61%, respectively; $p<0.01$). 6h of incubation with 25.5 mM glucose decreased LXRb in the hypothalamus (20%, $p<0.01$). LXRa showed no changes in any of these treatments. No effects with insulin on both subtypes were found in either explant. The LXR activation products, ABCA1 and Glut2, were unaffected in hypothalamus while there were increased in the hippocampus (ABCA1 35% with 15.5 mM glucose, $p<0.05$; Glut2 27% with 25.5 mM glucose, $p<0.05$). All these results indicate that expression of LXR in the hypothalamus and hippocampus is sensitive to glucose and not to insulin. The LXR expression changes are tissue-dependent a response previously observed in a different experimental model.

A64
ALTERNATIVE SPLICING: ANCESTRAL MECHANISM FOR THE HEXOSAMINIDASE SYNTHESIS?

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The N-ac-glucosaminidase (Hex) has been linked to several metabolic processes. In mammals, three Hex isoforms are originated by combinations of two different subunits (a and b), encoded in two different genes. Their specific activities depend on the identity of two amino acids of the 12 that compose Hex active site. In our laboratory we characterized the putative amphibians Hex gene. Our results show that a and b subunits can be generated from only one gen by alternative

usage of 2 exons. One of the transcripts originated of this gene was cloned and sequenced (GenBank: JN127371; *X.laevis*). The deduced protein presented an active site corresponding to an a subunit. We aimed to express JN127371 and measure Hex activity in order to characterize its identity. JN127371 was subcloned in a pcDNA3 vector and transfected in CHO cells. Western-blot experiments demonstrated the expression of a protein of the expected molecular weight. Using substrates capable of discriminating between the a and b subunit, a significant increase in a activity was observed in these extracts. Our results demonstrate that the characterized gene correspond to Hex and that JN127371 identifies as an a subunit, evidencing the possible common evolutionary origin of the a and b Hex genes present in mammals.

A65
GENE EXPRESSION OF THE TELOMERIC SHELTERIN COMPLEX IN A MURINE MELANOMA MODEL

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The shelterin protein complex regulates telomeric stability. Functional alterations can lead to telomeric instability and tumoral cell development. The pattern of expression of genes trf1, trf2, pot1a and tin2, members of the complex, was analyzed in a model of murine melanoma. B16 melanoma murine cell line was employed subcutaneously inoculated into the flank of C57BL/6 mice. Samples of hepatic, splenic and melanoma tissues were extracted from every mouse. Liver, spleen and skin tissue samples of normal mice inoculated with D-MEM medium were used as control samples. Liver and spleen tissue was employed as their expression profile is diminished in liver samples and up regulated in spleen cells. The expression of these genes was analyzed by qPCR quantification using GADPH as housekeeping gene. Statistical analysis was performed according to the $2^{-\Delta\Delta Ct}$ method. We found no alteration of pot1a, trf2 and tin2 expression in melanoma tissue in relation to normal skin. However, trf1 expression was in fact diminished in melanoma tissue samples ($p<0.05$). The expression levels found in liver and spleen are in agreement with literature. Our data show a significant trf1 down regulated expression. However, trf2, pot1a and tin2 expression is not altered in murine melanoma in relation to normal skin tissue.

A66
DIFFERENT FAT DIETS EFFECTS ON LXR EXPRESSION IN RAT HYPOTHALAMUS

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In previous studies developed on glucose intolerant animals, we observed a link between increased plasma triglycerides (TG) and the expression of hypothalamichepatic receptors (LXR).These receptors are also involved in reverse cholesterol transport. To assess the *in vivo* effects on hyperlipidemia hypothalamic LXR, we generated several experimental models.Male Sprague Dawley45-50 daysrats, were fed for two weeks with normal diet alone (control) or with the addition of 38% bovine fat (F)+ 2% fat cholesterol (FC), fat + 0.2% cholic (FA) and fat + cholesterol + cholic acid (FCA). Serum cholesterol levels (determined by colorimetric method) showed a significant increase ($p<0.05$) with: FA (7%), FC (22%), FCA (63%) and TG levels with: F (19%)<FCA (57%).Preliminary analysis of LXRexpression (using western blot and specific antibodies for LXRα and LXRβ) in F and FCA diets, indicated a subtype β increase of 47.2% ($p<0.05$) in hypothalamus, and 36.3% ($p<0.05$) in liver, only with F.While FCA diet produces the largest increase in plasma parameters, the presence of cholic acid, which can be metabolized to an LXR agonist, appears to be influencing the expression of LXR receptors.(CONICET-PIP860).

A67
MODULATION OF APOPTOSIS IN EQUINE HERPESVIRUS-1 INFECTED CELLS.

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Cellular apoptosis is used as a protective mechanism against infectious agents. Several viruses have developed different modulatory mechanisms to regulate it for stimulate their replication. Up to now it is unknown whether *Equid herpesvirus type 1* (EHV-1) may exert a modulatory effect on the apoptotic process of the infected cell. We aimed to study the effect of EHV-1 in a heterologous cell culture line. The Madin-Darby bovine kidney (MDBK) cells infected with EHV-1 and then, were induced to apoptosis with sorbitol at 3, 9, and 18 hs posinfecion (pi) (Inf+/ Apo+). Apoptosis was studied by orange acridine and ethidium bromide staining, as well as by flow cytometry using an annexin V/propidium iodide apoptosis detection kit. In addition, ultrastructural changes were evaluated. We found a significant reduction in the apoptotic rate of (Inf+/Apo+) cells at 9 hs pi in comparison with the non infected and apoptosis induced group (Inf-/Apo+). Viral particles and

changes induced by apoptosis were ultrastructurally recognized. We conclude that EHV-1 may delay the apoptosis of the infected cells during the first stages of their cycle, an effect that may improve its replication.

A68
STUDY OF A NOVEL TRANSLATIONAL REGULATOR OF THE HYPOXIA INDUCIBLE FACTOR (HIF) IN *Drosophila*

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Hypoxic response is mediated by a family of evolutionary conserved alpha-beta heterodimeric transcription factors HIFs (hypoxia-inducible factors) that elicit the expression of a broad range of genes in response to low oxygen tension. Molecular mechanisms that mediate HIF regulation operate at the level of the alpha subunit, controlling protein stability, subcellular localization, and transcriptional co-activator recruitment. The present study shows for the first time the characterization of a novel translational regulator of *Drosophila* HIFα homologue, Sima. Insertion of Sima 3´UTR in a luciferase reporter gene cause luciferase activity reduction, but not mRNA levels, in normoxia. Point mutations in the 3´UTR of Sima prevent this translational repression. Furthermore, loss-of-function mutations of the RNA binding protein leads to upregulation of Sima inducible reporters as well as Sima's target genes in cells and flies. In addition, this regulator protein interacts genetically with *Fatiga*, the prolyl-4-hydroxilase that controls Sima protein stability. Interestingly, larvae mutant for the novel regulator or *Fatiga* present melanotic masses, a phenotype dependent on Sima and associated to abnormal hematopoiesis in the larval hematopoietic organ, the lymph gland. Taken together, our data provide the first evidence for a role of an evolutionary conserved RNA binding protein in Sima translational regulation.

A69
THERAPEUTIC EFFECT OF *Salmonella* Typhi Ty21a IN A MOUSE MODEL OF T-CELL LYMPHOMA.

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We have previously demonstrated that the attenuated *Salmonella* Typhi vaccine strain CVD 915 can be used as a cancer immunotherapeutic agent. The aim of this study is to investigate whether immunization with the *Salmonella* Typhi commercial vaccine strain Ty21a can induce an antitumoral effect on T-lymphoma-bearing mice by reducing tumor growth. In addition, we studied the direct oncolytic effect of this strain on tumor cells. EL4 tumor cells were inoculated subcutaneously (SC) into the C57BL/6J mice. When tumors became palpable, tumor-bearing mice were inoculated with either *Salmonella* or PBS, both intratumorally and SC within the draining lymph node areas. Unlike PBS-treatment, *Salmonella* inoculation promoted a decrease in the mean tumor volume as early as 5 days after treatment ($P<0.05$). When the oncolytic effect of bacteria was tested in 18 or 48 h *in vitro* assays, significant differences in the viability of EL4 cells cultured with bacteria were found, as compared with those treated with culture medium ($P<0.001$, after 18 h). These findings demonstrate the antitumor effect of the Ty21a vaccine and support its potential use as a cancer therapeutic agent.

A70
GENERAL POLYAMINE METABOLISM IS ACTIVATED IN *Pseudomonas syringae* DURING PLANT COLONIZATION.

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Polyamines are important for virulence of pathogenic bacteria and its levels are modulated by the regulation of its biosynthetic and catabolic routes. In this work, our aim was to explore the regulation of polyamine metabolism in the plant pathogen *P. syringae.* Our analysis demonstrated the existence of two genes encoding the polyamine biosynthetic enzymes arginine and ornithine decarboxylase. In turn, several genes involved in three catabolic routes were also identified. qRT-PCR studies showed that biosynthesis is repressed in polyamine-amended medium whereas catabolism is induced, a process that might prevent polyamine accumulation. Further experiments demonstrated that both metabolic pathways are activated after contact with Arabidopsis leaves, suggesting that this regulation might be a key step in plant colonization. Moreover, some of the catabolic genes maintain high expression levels during later stages. Interestingly, growth of bacteria in T3SS-inducing media provoked only a mild induction of polyamine catabolism, indicating that polyamine metabolism is regulated by a different mechanism than key virulence processes.

A71
ALLATOTROPIN AND ALLATOSTATIN-C: TWO MYOREGULATORY PEPTIDES ACTING IN THE AORTA AND CROP OF KISSING-BUGS

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In the present study we present a partial characterization of the Allatotropin (AT) and Allatostatin-C (AST-C) receptors, also showing their miorregulatory activity in the anterior midgut (crop) and aorta in males of the kissing-bug Rhodnius prolixus. Ovary, Malpighian tubules, aorta, midgut and rectum cDNA were prepared from a pool of fed and unfed individuals. Using specific primers base on the corresponding sequences found inVectorbase, fragments of 900 and 600 bp of RpATr and RpAST-CR were sequenced. Their expression was found in every organ analyzed. For physiology assays we analized the heart rate in starved males, who were sequentially inyected with Serotonin10-9M, AT 10-9M to induce a stimulation of the frequency of contractions and AST-C 10-6 M. To further analyze the activity of these peptides, we essayed the effect of different doses of AST-C (from 10-14M to 10-6M) during post-prandial diuresis. Differences between treatments were analyzed by Multifactorial Analisys of Variance. In the group of starving insects we found that AT have a mioestimulatory funtion, causing increased heart rate, which decreased more than 20%, after applying AST-C 10-6 M. Dose-response assay in fed insects, shows that the contractions of the aorta and the crop decreased significantly during post-prandial diuresis (approximately 50% and 20%, respectively). Finally, our results suggest that the peptide AST-C would inhibit the muscle contractions of both organs,probably antagonizing the stimulatory action of AT.

A72
REGULATION OF cAMP IN *Giardia lamblia*

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Giardia lamblia is an anaerobic protozoan parasite that inhabits in the small intestine of humans and other higher animals, that ingested water or food contaminated with *Giardia's* cyst. It has been described that the beginning of the infection (excystation) would be mediated by the second messenger cAMP. However, the mechanisms of the cAMP regulation in *G. lamblia* has not been described. In order to enhance our knowledge of the molecular mechanisms that regulate the infection process of this parasite, we tried to to analyze how *G. lamblia* is able to regulate its own cAMP levels. As first step, during an bioinformatic analysis of the complete genome of *G. lamblia*, we identified two sequences that encode for enzymes that regulate cAMP: Adenylyl Cyclase (gAC) and phosphodiesterase (gPDE). The expression of these genes in *G. lamblia* was later confirmed by an RT-PCR study performed from trophozoites cultured in vitro. With the purpose of characterizing these putative enzymes, the sequences encoding gPDE and gAC were amplified by PCR and then inserted in expression vectors.
At the moment, we expressed and purified from bacteria three constructs of gAC truncated variants (gAC118, gAC268 and gAC301) containing its catalytic domain. The capability to syntheses cAMP of these constructs of gAC was subsequently confirmed by enzymatic assay in vitro. These results suggest that the regulatory enzymes of cAMP, gPDE and gAC are present in *G. lamblia* and that gAC would be directly involved in the synthesis of cAMP in this parasite.

ANIMAL BIOTECHNOLOGY

A73
CHOLESTEROL INCORPORATION INTO BOVINE OOCYTES PRIOR TO VITRIFICATION FAVORS RECOVERY OF GM1 LEVEL AT THE PLASMA MEMBRANE

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Methyl-β-cyclodextrin (MβCD) saturated with cholesterol or alone was used to modulate plasma membrane cholesterol level of metaphase II bovine oocytes. Membrane biophysical state of oocytes and cumulus cells was evaluated at decreasing temperatures and changes in the localization of the raft marker GM1 were analyzed in Cryotop vitrified oocytes. After 2 hours of incubation with 15 mM MβCD saturated with cholesterol, fluorescence intensity of BODIPY-cholesterol at the plasma membrane increased. Oocytes that were incubated with MβCD alone showed no differences in membrane

fluorescence intensity but evidenced changes in the distribution of cytoplasmic lipid droplets. Laurdan generalized polarization revealed that membrane fluidity of oocytes is lower than that of cumulus cells at temperatures closer to the physiological but this relation is inverted when temperature decreases below 20°C. GM1 loss caused by vitrification was restored when cholesterol was incorporated into oocytes before vitrification and removed after warming, thus indicating that membrane raft integrity can be preserved by cholesterol modulation.

A74
THE EFFECT OF DIMETHYLUREA ON *IN VITRO* MATURATION OF PORCINE OOCYTES

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A balanced redox environment is important for oocyte quality. Oocyte viability is affected by the increase of reactive oxygen species. Dimethylurea (DMTU) is a chemical antioxidant that captures free radicals derived from oxygen. The effect on *in vitro* maturation (IVM) of the addition of DMTU during follicular aspiration was evaluated. Porcine COCs were obtained by follicular aspiration from slaughter ovaries. In control, TCM 199 was used for aspiration and supplemented TCM for washing, whereas in treatments 2µM and 20µM of DMTU was added in both media. After IVM, denuded oocytes were stained with Hoechst 33342 to evaluate the percentage of nuclear maturation. Those oocytes who reached Metaphase II were considered mature. It was used an n=97 (control), n=123 (treat. 2µM) and n=137 (treat. 20µM); three replicates. Comparison of proportions (Infostat) showed no statistically significant differences. In future, the citoplasmatic maturation and the apoptosis rates will be evaluated in order to determine if the addition of DMTU as an antioxidant is convenient.

A75
ESTABLISHMENT OF A REVERSE GENETIC SYSTEM FOR THE STUDY OF AN INFLUENZA A VIRUS FROM RED-WINGED TINAMOU

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In 2008 an H1N1 influenza virus (A/red-winged tinamou /Argentina/MP1/2008) was isolated in Buenos Aires but so far it has not been studied at the molecular level. The goal of this work was to obtain the 8 segments of the virus by reverse genetics, with the aim of generating infectious viral particles to allow its molecular and biological characterization. After reverse transcription, the different fragments of the strain were cloned by a first PCR reaction using primers that recognize conserved sites within the non coding sequence. These fragments were used as megaprimers in a second PCR reaction using as target the pHW2000 vector, which was then treated with DpnI and transformed into DH5alpha bacteria. DNA was extracted from positive colonies and analyzed using the restriction enzyme BglI. Positive clones were already obtained for the HA fragment, while putative ones corresponding to fragments NS, M, NP and NA colonies are currently being analyzed. We have thus established a reverse genetics system to study this novel influenza virus.

A76
ARTIFICIAL INSEMINATION IN PIGS: CERVICAL VS DEEP INTRAUTERINE. INCREASING EFFICIENCY OF THE BOAR

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Deep intrauterine artificial insemination (AI) is a method that optimizes boar performance with respect to the cervical AI given that it maximizes the number of possible inseminations with equal volume of semen. This technique is not still used in production and is not recommended in gilts. However, to date there are no reports of trials of this technique in gilts. In this study we compared two techniques of AI in sows: 1) cervical and 2) deep intrauterine. Both techniques were applied with cooled semen to 8 sows of similar characteristics grouped into two categories: multiparous (7 repeats, 2 inseminations per heat) and nulliparous (8 repeats, 2 inseminations per heat), to form the experimental groups some sows were inseminated more than once. There were a total of 7 cervical AI and 8 deep intrauterine AI (these last with a half of the semen volume). We compared the pregnancy rates obtained after insemination. Data were analyzed with the Fisher exact test ($p < 0,05$). No significant differences in pregnancy rates were observed, or between methods or between categories (75% in gilts with both methods and 100% in sows with both methods). The depth of semen deposition in nulliparous is lower than in multiparous.

We conclude that deep intra-uterine AI is a good technique, both in nulliparous and in multiparous, and increases the efficiency of the boar. Key words: AI- porcine- efficiency.

A77
VITRIFICATION OF BOVINE OOCYTE WITH CRYOTOP REDUCES CASPASE ACTIVATION COMPARED TO OPEN PULLED STRAW

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Exposure of phosphatidylserine and caspase activation of oocyte vitrified in two devices Cryotop and Open Pulled Straw (OPS) were evaluated. In vitro matured oocytes were randomly assigned to 2 experimental groups: vitrified with Cryotop and OPS. Exposure of PS was detected with Annexin V-FITC. Activated caspases were evaluated using a specific inhibitor (VAD-FMK-FITC). No differences were registered in PS exposure (Cryotop, 46 % and OPS, 55%). Vitrification with OPS increased dead oocytes (18%) with respect to Cryotop (7 %). Oocytes vitrified with Cryotop showed a decrease of activated caspases with respect to OPS (16% vs 29%, respectively). Vitrification with both devices induced translocation of PS to the external leaflet of plasma membrane. On the other hand, vitrification with Cryotop reduces activated caspases compared to OPS, suggesting that there is a partial recovery of oocytes vitrified with this device. Hence, Cryotop is an advantageous device with respect to OPS in terms of survival post-cryopreservation.

A78
BOVINE ANDROGENETIC EMBRYO PRODUCTION WITH ZONA PELLUCIDA OR ZONA-FREE

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The production of androgenetic embryos (AE) is a useful tool to obtain several copies of one sperm genome, enabling pre-cigotic genetic screening. In this work, developmental capacity of AE constructed with ZP or zona-free (ZF) was compared. Firstly, ZP and ZF embryos were produced by IVF. Then, the female nucleus was aspirated using Hoetch staining and the resulting AE were cultured for 7 days. ZF enuclated embryos show higher cleavage rates (see Table; a, b $p<0,05$ Fisher´s Test; SHAM: ZF embryos to which only cytoplasm was aspirated). This may be due to the fact that there is no visual interference caused by spermatozoa attached to the ZP, presumably reducing UV exposure. AE´s blastocyst rates are lower than controls´ due to their haploid condition. In conclusion, it is possible to construct AE both with ZP or ZF, but the latter is simpler and results in higher cleavage rates.

A79
EVALUATION OF ANGIOGENIC EFFECTS OF LITHIUM-MODIFIED 45S5 BIOACTIVE GLASS

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The controlled and localized release of lithium (Li) ions from bioactive glasses (BGs) might prove a promising therapeutic alternative to initiate angiogenesis in tissue repair procedures. The aim of this work was to assess the angiogenic effects of the ionic dissolution products (IDPs) of new BGs based on 45S5-type glass doped with 1% (45S5.1Li) or 5% Li_2O (45S5.5Li). The IDPs were obtained by incubating 1% w/v particles (<5 µm) of BGs in egg water at 37ºC for 72 h. The determination of soluble ions was conducted through ICP-MS. Angiogenesis assays were carried out on zebrafish embryos (*Danio rerio*) at 48 hpf. The embryos were incubated for 24 h at 28.5°C in 6-well culture plates containing either 5 mL egg water (control) or egg water enriched with the IDPs. We carried out 2 replicates with n=30 embryos per treatment. The embryos were processed for the subsequent morphometric analysis of the subintestinal vascular plexus, through enzyme-histochemical identification of endogenous alkaline phosphatase. In all cases we observed the embryonic development expected for the incubation time assessed. The results obtained showed that only the IDPs of 45S5.5Li BG exhibited pro-angiogenic capability. The positive effects can be attributed to the release of 0.20 mM Li from 45S5.5Li microparticles.

PLANT BIOLOGY AND BIOTECHNOLOGY

A80
NITRIC OXIDE IMPAIRS ZINC UPTAKE AND MOBILIZATION IN WHEAT PLANTS

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In this study, the effect of nitric oxide (NO) on net Zn-uptake and root-to-shoot translocation rates was evaluated in wheat plants (*Triticum aestivum*) cv. Chinese Spring. The plants were hydroponically cultured under supra-optimal and deficient Zn availabilities. S-nitrosogluthathione (GSNO), as NO donor, was applied in the nutrient solution at a final concentration of 100 µM. After 17 days of culture, the Zn-well-supplied plants exposed to NO showed lower concentration of Zn in tissues than those not exposed. The Zn concentration in roots decreased from 92±16 to 54±8 nmol g^{-1} FW, while in shoots it declined from 117±9 to 79±7 nmol g^{-1} FW. In Zn-deprived plants, this effect of NO was not observed. In addition, Zn-deprived plants were transferred to a complete nutrient solution for 48 h and Zn net uptake (NUR) and root-to-shoot translocation rates were estimated. The NUR was 4-fold higher than that measured for plants maintained in a complete nutrient solution. The exposition to NO for 48 h repressed this increase. Root-to-shoot Zn-translocation rate was also affected by NO. It declined from 3.1 to 1.5 nmol Zn g^{-1} FW h^{-1}. Our results suggest a reversible action of NO in the modulation of uptake and root-to-shoot translocation in plants cultured under different Zn availability.

A81
THE EFFECT OF POLYAMINES ON THE GERMINATION OF *Lotus japonicus* UNDER LOW TEMPERATURE STRESS.

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Polyamines (PAs) are polycationic compounds with a recognized role in plant growth, development and biotic and abiotic stress responses. We evaluated the germination percentage (GP) for 7 days at different temperatures (22, 12, 8 and 4 ± 1 °C) of MG-20 and Gifu ecotypes of *L. japonicus*. GP of both ecotypes was reduced at low temperatures; however, Gifu was more affected at 12 and 8°C than MG-20.
Considering these results, 8°C was selected for further analysis of the GP and PAs levels in others *L. japonicus* ecotypes. A positive correlation between the GP and putrescine (Put) levels was found on the germinated seeds (r = 0.82). The levels of PAs at days 0, 3 and 7 were measured for MG-20, Gifu, MG-1, MG-4 and MG-83 (contrasting ecotypes in their GP at 8°C); significant results between them were only observed at day 7. In other assays, 1mM of Put and DFMA (a Put anabolism inhibitor) were added at day 0 to the seeds of the sensible ecotype MG-1. Put increased GP compared with control, while DFMA partially reversed this effect. As a conclusion, the *L. japonicus* ecotypes evaluated present differential GP under low temperature stress; that could be due to different Put levels in the seeds. Despite the fact PAs have been related to different abiotic stresses, their role in the germination process under low temperatures is still unclear.

A82
REGULATORY SMALL RNAS INVOLVED IN THE SYMBIOSIS BETWEEN *Phaseolus vulgaris* AND *Rhizobium etli*

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Common bean (Phaseolus vulgaris) establishes a symbiotic interaction with Rhizobium etli, resulting in the formation of root nitrogen-fixing nodules. Interestingly, beans from the Mesoamerican center of genetic diversification are more efficiently and preferentially nodulated by strains that are predominant in the same geographical region. Our group has identified several transcription factors that are determinant for this preferential interaction. One of them encodes an A subunit of the heterotrimeric transcription factor NF-Y named PvNF-YA1. PvNF-YA1 is strongly expressed at early stages of the interaction with the highly efficient strain of R. etli, but not with the less efficient one, suggesting a special function in strain specificity. In silico analysis revealed that PvNF-YA1 could be regulated by two microRNAs (miR169b and miR169d) through their putative target sites, which are present in the 3'UTR of the PvNF-YA1 transcript. Overexpression of a miR169 resistant version of PvNF-YA1 resulted in an increase in the number of nodules formed by the less efficient strain. RT-PCR analysis revealed that miR169d precursor is specifically and highly expressed in young nodules formed by the highly efficient strain, where two different spliced variant where identified. Simultaneously, in order to get access to global changes in small RNAs (sRNAs) abundance during this symbiotic interaction, we have constructed sRNAs libraries of roots inoculated with the more

or the less efficient strain that will be subjected to Illumina sequencing. This approach will allow us to identify new sRNAs involved in the preferentially association

A83
NFYC1, A TRANSCRIPTION FACTOR REQUIRED FOR NODULE ORGANOGENESIS, INTERACTS WITH A RECEPTOR LIKE KINASE AND A GRAS TRANSCRIPTIONAL REGULATOR

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Common bean (Phaseolus vulgaris) establishes a nitrogen fixing association with its partner Rhizobium etli. In this interaction, host-dependant competitiveness has been observed, in which accessions from the Mesoamerican region are more efficiently and preferentially nodulated by strains that are predominant in the same geographical region. A C subunit of the heterotrimeric nuclear factor Y (NF-Y), PvNF-YC1, was identified as a gene required for nodule organogenesis and bacterial infection that contributes to this preferential association. In order to identify proteins that can physically interact with this transcription factor, a yeast two hybrid screening was performed using PvNF-YC1 as a bait and a cDNA library from root tissue inoculated with R. etli. A total of eight clones that potentially interacts with NF-YC1 were isolated and sequenced. Among them, one encodes a receptor-like kinase and another clone encodes a transcriptional regulator of the GRAS family. Interaction of PvNF-YC1 with these gene products has been confirmed by retransformation of yeast, and it is being evaluated in planta by bimolecular fluorescent complementation assays in Agrobacterium-infiltrated Nicotiana benthamiana leaves and co-immunoprecipitation assays. Function of these PvNF-YC1 interacting proteins in nodulation efficiency and bacterial infection is being evaluated by both RNAi and overexpression. This will help to elucidate the signal transduction pathway specifically activated in Mesoamerican common bean in response to its cognate R. etli strain.

A84
DISSECTING THE MOLECULAR RESPONSE OF COMMON BEAN TO *Rhizobium etli* WITH NEXT-GENERATION SEQUENCING TECHNOLOGIES (NGST)

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Legume plants establish a mutualistic symbiosis with soil bacteria that convert N2 to forms that can be directly incorporated into the metabolism. Understanding the molecular basis of this process is key to alleviate the use of chemical fertilizers, preventing the environmental pollution associated to modern agricultural practices. In this work, we used next generation sequencing technologies to unravel two aspects of the symbiotic interaction, the root transcriptional response modulated by bacterial signals and the molecular basis of the selection of the most efficient strains by the plant. To achieve the first objective, mRNA from root tissue of common bean plants inoculated with the wild type strain of R. etli or with mutant strains that are impaired in the synthesis of the Nod Factor, the exopolysaccharide and, the lipopolysaccharide I were used to construct Illumina libraries that have been sequenced. For the second objective, we took advantage of the evolution of common bean in two centers of genetic diversification, the Mesoamerican and the Andean regions, which resulted in the capability of Mesoamerican accessions to preferentially associate with R. etli strains that are prevalent in Mesoamerican soils. This specific association is correlated with a more efficient nodulation and nitrogen fixation. Illumina libraries were prepared to compare the transcriptome of roots inoculated with a more efficient strain with that of roots inoculated with the less efficient one. Expression of selected genes was confirmed by RT-qPCR. Our current progress in NGST analysis, as well as further implication of our findings, will be discussed.

A85
THE ROLE OF CHLOROPLAST-GENERATED REACTIVE OXYGEN SPECIES IN THE RESPONSE TO PLANT-PATHOGEN INTERACTIONS.

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Plants react against pathogen challenges by deploying complex responses that involve expression of multiple genes and the hypersensitive reaction (HR). As a result, there is a concomitant production of reactive oxygen species (ROS) in various cellular compartments. In this work we used tobacco plants expressing a cyanobacterial flavodoxin in chloroplasts, which

showed increased tolerance to multiple adverse environmental conditions and lower ROS accumulation because flavodoxin acts as a general antioxidant. We used these plants to study if the HR light requirements are linked to ROS production in chloroplasts. We report here the phenotypic and biochemical characterization of the interaction between these plants and pathovars of *Pseudomonas syringae* that represent the non host, virulent and avirulent interactions, in the presence or in the absence of light. ROS quantification by fluorescent microscopy indicated that the transgenic plants accumulated lower levels of ROS and suffered less damage than the wild type, but only if light was present. The results lend support to models that indicate the participation of plastid-generated ROS in the defense response of plants against pathogens.

A86
MICRORNA390 AND TAS3-DERIVED TASIRNAS REGULATE THE DEVELOPMENT OF NITROGEN FIXING NODULES IN THE MODEL LEGUME MEDICAGO TRUNCATULA

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The development of nitrogen fixing nodules depends on the activation of two highly coordinated genetic programs in leguminous plants: the nodule organogenesis and the rhizobial infection. MicroRNAs (miRNAs) have emerged as major post-transcriptional regulators of gene expression during development or in response to environmental stimuli. In a previous work, we have identified miRNAs that change their abundance in roots of the model legume Medicago truncatula at early stages of its interaction with Sinorhizobium meliloti. One of these miRNAs, the miR390, dramatically decreased (about 80%) at early stages of the symbiotic interaction. miR390 targets the non-coding RNA TAS3 and triggers the production of the trans-acting small interference RNAs (tasiRNAs). In turn, TAS3-derived tasiRNAs control the stability or translatability of transcripts encoding the Auxin Response Factors ARF2, 3 and 4. Concomitantly with the change on miR390 abundance, inoculation with S. meliloti lead to a decrease in tasiRNA production and a higher accumulation of ARF2 3 and 4 transcripts. Overexpression of a precursor of miR390 resulted in a reduction in the number and size of the nodules, as well as in the number of infection events. On the other hand, expression of a mimicry (MIM390), which sequestrates miR390 blocking its action on TAS3 transcript and reducing tasiRNAs levels, increased the number of nodules; but affected negatively the size of the nodules and the number of infection events. These results suggest that the miR390 and tasiRNAs pathway is involved in the fine-tune regulation of nodule development and rhizobial infection in M. truncatula.

A87
COMBINING IMMUNOPURIFICATION OF RIBONUCLEOPROTEIN COMPLEXES AND RNA-SEQ TO CHARACTERIZE POSTRANSCRIPCIONAL GENE REGULATION IN THE SYMBIOTIC INTERACTION *Medicago truncatula- Sinorhizobium meliloti*

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Several studies have characterized the steady-state levels of transcripts at different stages of root legume symbiosis. However, this appoach does not distinguish transcripts that are targeted for degradation, sequestered in ribonucleoprotein complexes or undergoing translation. The distribution of mRNAs on these cytoplasmic complexes plays a crucial role in the regulation of gene expression during development or in response to environmental stimuli. Here, we characterized changes in the association of transcripts to polysomes in roots of the model legume *Medicago truncatula* during the symbiotic interaction with *Sinorhizobium meliloti*. Polysomes were isolated by immunopurification (IP) from root tissue expressing a FLAG-tagged ribosomal protein L18. This methodology was combined with Illumina RNA sequencing technology (RNA-seq) allowing a quantitative analysis at genome scale of inoculated and control roots. Approximately 3500 transcripts were classified as up-regulated at translational level, whereas 5770 transcripts were found as down-regulated. A comparison of fold change levels in response to rhizobia of 12 transcripts encoding proteins involved in the nodulation signalling pathway obtained previously by qRT-PCR showed a good correlation with data generated by RNA-seq. Current experiments are being conducted to immunopurify other cytoplasmic complexes involved in storage or degradation of mRNAs, such as storage granules or P Bodies. This experimental approach constitutes a valuable tool to shed light on the mechanisms and significance of post-transcriptional regulation in *M. truncatula* roots during its association with *S. meliloti*.

A88
ENDOPHYTIC COLONIZATION AND PLANT PROTECTION AGAINST *Botrytis cinerea* MEDIATED BY OXALIC ACID-DEGRADING BACTERIA

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Oxalic acid (OA) is a virulence factor produced by the phytopathogenic fungi *Botrytis cinerea* and *Sclerotinia sclerotiorum*. OA-deficient *B. cinerea* strains are hipovirulent and transgenic plants that degrade OA are more resistant to this fungus than wild-type plants. In this work, the existence of rhizospheric bacteria able to degrade OA and in turn endophytically colonize plants was evaluated, also analysing their potential for plant protection against *B. cinerea*. Two strains (OxA and OxB) able to use OA as the sole carbon source were isolated from rhizospheric soil. OxA and OxB inoculation on leaves of *Arabidopsis thaliana* demonstrated that they are able to endophytically colonize plants. In addition, colonization by OxA and OxB reduced leaf damage caused by exogenous potassium oxalate. Moreover, leaf colonization by these bacteria attenuated disease symptoms caused by *B. cinerea* infection. Thus, OxA and OxB strains are potential biological control agents of plant diseases caused by *B. cinerea*. Current work aims to the taxonomic identification of OxA and OxB and confirming that protective effects are mediated by OA-degradation.

A89
BIOCHEMICAL ANALYSIS IN CONVENTIONAL AND TRANSGENIC SOYBEAN BY CHEMOMETRIC

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The present work aim to determine the behavior of near parental soybean varieties: DM48 - DM4800RG and Msoy7501 - Msoy7575RR (conventional - transgenic respectively) through biochemical analysis and chemometric. Plants were grown during 5 weeks before the application of glyphosate. Catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APX) activities, lipid peroxidation (MDA) and total amino acid (TAA) were determined in leaves and roots at 0 and 72 h after glyphosate application. Data were analyzed by principal component analysis (PCA). Loadings plot determined that CAT and APX were the most influent variables for grouping. In leaves, scores plot grouping conventional and transgenic varieties at 72 h after glyphosate treatment by CAT influence. In roots at 0 h, PCA analysis grouping the four varieties by influence of APX and at 72 h conventional and transgenic was grouped under the influence of CAT. As conclusion, differential behavior of biochemical parameters in transgenic and conventional soybean was noticeable through PCA analysis.

A90
OVEREXPRESSION OF A CBM ON *Arabidopsis thaliana* CELL WALL INCREASES PLANT RESISTANCE TO *Botrytis cinerea*

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A carbohydrate binding module (CBM) is a contiguous amino acid sequence within a carbohydrate-active enzyme that provides the ability to bind cell wall polysaccharides. Most of cell wall enzymes contain CBMs. Here we present an approach to modified the cell wall by overexpressing a strawberry (*Fragaria x ananassa*) EXPANSIN 2 CBM (CBMFaEXP2) in *Arabidopsis* cell wall. In order to direct the CBM to the secretory pathway, a fusion was made between CBMFaEXP2 and the signal peptide of AtEXP8. Once transgenic plants were obtained, the subcellular localization was confirmed by confocal microscopy. Real Time PCR assays revealed that expression levels of a number of genes involved in cell wall catabolism were down-regulated in transgenic plants regarding wild type (WT). Considering that in a previous work we reported that overexpression of CBMFaEXP2 confers an increase in total cell wall content and in cell wall thickness; the resistance of CBM-expressing plants to *B. cinerea* was evaluated. Results showed a significant decrease of the necrotic area in transgenic plants after inoculation in comparison to WT. We conclude that overexpression of CBMFaEXP2 alters cell wall genes expression and increases resistance to damage caused by the necrotrophic fungus *B. cinerea*.

A91
MEASURING PLANT CELL WALL CREEP AND EXPANSIN ACTIVITY WITH A COMMERCIAL TEXTURE ANALYZER

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Plant growth comprises an irreversible extension of preexistent cell wall. This extension is mediated by phyto-hormones (majorly auxins and giberellins) and by light. Also, cell wall experiments a transient extension when under tension at acidic pH. This extension would not be mediated by cell wall synthetic processes. Cosgrove (1989) analyzed the cell wall creep in cucumber (*Cucumis sativus*) hypocotyls and showed its behavior under different treatments, concluding that the hypocotyl creep is an enzyme dependent process. In 1992, McQueen Mason et al. proved that this creep activity is indeed mediated by non-hydrolytic enzymes called "expansins". To the moment, expansin activity measurement requires "*ad hoc*" instrument construction, which has considerably limited this study to a few laboratories. The objective of this work was to adapt the Texture Analyser TA.XT plus in order to develop a measuring system for cell wall creep and expansin activity. In order to demonstrate cell wall creep and optimize the methodology, pH shifting assays were performed. The influence of different chemicals agents was also assayed. Expansin activity was determined in a reconstitution of the cell wall creep assay, using a thermally denatured cucumber hypocotyl and a total cucumber hypocotyl protein extract. This optimized methodology will allow analyzing both parameters in a broad variety of vegetables tissues.

BEHAVIOR

A92
DIFFERENT NEURAL ACTIVATION OF THE STRIATUM AND MEDIAL PALLIUM DURING APPETITIVE AND AVERSIVE LEARNING IN TOADS (*Rhinella arenarum*)

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The Striatum and Medial Pallium are two telencephalic areas of amphibians, considered homologous to the mammalian amygdala and hippocampus, respectively. Amygdala is related with aversive learning and fear expression, and hippocampus is associated, among other, with spatial memory. The functional equivalence hypothesis stands that the function of these structures should be preserved both in amphibians as in mammals.
We conducted three experiments in which animals were trained using appetitive or aversive reinforcers. In each case toads began trials at 80% of their weights. In Experiment 1, animals were trained in a passive avoidance task in a shuttle box. They had to avoid a dark compartment, contrary to their natural tendency, where had an hypertonic solution as aversive stimulus. In Experiment 2, toads were trained in a runway situation, measuring the running latency to reach the goal box, where was a container with deionized water, used as reward. In Experiment 3 animals were trained in a spatial procedure using an open field with four water containers available, but only one with accessible water signaled by a visual cue. In all three experiments, after acquisition animals were sacrificed and their brains were processed with the AgNor technique in order to measure the striatum and medial pallium neural activity. Striatum showed more neural activation in Experiment 1 (an aversive procedure), but not in the others. Medial pallium neural activity was enhanced only in Experiment 3 (where was required a spatial task). All these results are in agreement with the functional equivalence hypothesis.

A93
SYNTHETIC STEROID ACTION ON ANIMAL BEHAVIOR IN OPEN FIELD AND FORCED SWIMMING TESTS IN RAT

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In previous studies we have analyzed the properties of different synthetic steroids (SS) analogues to allopregnanolone (A) and pregnanolone (P), developed as a more stable therapeutic alternatives on several in vitro models. Based on the anxiolytic properties of natural steroids, in this work, we evaluated the in vivo effect of intracranial administration (in dorsal hippocampus), of two synthetic analogues, Epoxy 3 and Epoxy 5, and natural steroids (A and P; 2 μg), using two anxiety tests: open field (OF) and forced swimming. We saw only effects in OF test where natural steroids were able to produce

anxiolytic effects evidenced by increased number of crossed lines (114% (A) and 136% (P)), reduced dwell time still (14% (A) and 11% (P)), increased exploratory activity (225% (A) and 430% (P)) and grooming (75% (A) and 350% (P); $p < 0.05$). While SS were not able to reproduce the same effects as natural analogues, these do not mean they are not able to produce other effects. Future trials are needed to determine the intrinsic properties of these SS. (CONICET-PIP860).

A94
EFFECT OF THE SQUID MEAL AS ATTRACTANT ON FEEDING BEHAVIOR OF THE CRAYFISH *Cherax quadricarinatus*

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Chemoreception plays a significant role in feeding behavior, thus it is logical to assume that inclusion of attractant in pellet will improve consumption time avoiding nutrients losses, since the food is one of the most important cost in aquaculture. This study analyzed the effect of the inclusion of squid meal on feeding behavior in C. quadricarinatus. The composition of base feed (BF) was: 37.98% of protein, 6.05% of lipid and 16.05% of ash and the attractant (squid meal) concentration was: 0, 0.25; 1; 2.5 and 10% (p/p). Individual behavior of 20 juveniles and group behavior of 4 juveniles with 5 replicates per treatment were observed. It was evaluated their first choice (attractant or not attractant) and the time of residence in each compartment for 10 minutes. The results showed that the inclusion of squid meal did not improve BF attractivity for this species unlike other crustacean culture. These results would be reinforcing the concept that C. quadricarinatus is an omnivorous opportunistic species of broad spectrum.

A95
THE HIND-PAW REFLEX IN TOADS WITH SPINAL CORD INJURY

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Use of the spinal cord injury model in toads (Rhinella arenarum) could be parallel to the injury profile described in other species. We hypothesize that spinal dorsal lesion would induce the development of reflex hypersensitivity. Animals were anesthetized by MS222 injection into lymphatic circulation. The dorsal aspect of the spinal cord segment was then exposed by performing a laminectomy. The meninges were cut with a gauge needle. A spinal dorsal injury (SCI) was then made with a clip. After the SCI electrophysiological tests were repeatedly performed: The hind paw nerve of the hind paw of the toads was stimulated. Sensitivity to electrical stimuli was tested by assessing the muscular responses. Stimuli with sequentially increasing or decreasing intensity were used to determine the withdrawal threshold. Latency and amplitude of the responses were also recorded. The average intensity of the stimuli that leads to a hind paw withdrawal was recorded and analyzed to be expressed as the average withdrawal threshold. Results show that, when exposed to nerve stimuli, muscular responses started to develop 6 weeks post SCI and were gradually getting more marked over time. In comparisons made with sham surgeries, the decreased threshold to hind paw withdrawal following probing with electrical stimuli indicated reflex hypersensitivity. Increases in the amplitudes of muscles responses and shortening of their latencies were also seen. These results seem to be promising for future experiments as the SCI mechanisms could be studied with this preparation.

DEVELOPMENT

A96
EMBRYONIC DEVELOPMENT OF *Sympterygia bonapartii* (ELASMOBRANCHII, RAJIFORMES) IN CAPTIVITY.

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Descriptions of the stages of embryonic development of skates, including the progressive appearance of the morphological features of Batoids (body flattening, ventralization of gill slits, expansion of the pectoral fins and their fusion with the trunk) are scarce in the literature. The oviparous species of elasmobranch fishes are of great interest as oviparity offers an excellent

opportunity to study the development of the embryo and its interactions with the environment, particularly after the pre-hatching event. The reproductive success achieved for the species *Sympterygia bonapartii* at the Aquarium of Temaikèn Biopark (with high fertilization, oviposition and birth rates), enabled a description of its embryonic development regarding external and internal morphological characteristics and body growth. Embryos of different ages were obtained from their egg cases, anesthetized and observed under the microscope. Optic and otic placodes, as well as pharingeal pouches and a tubular heart are observed on day 10. Buds of gill filaments are observed from day 17 while the external gills become fully developed on day 60. Pectoral fins begin to develop on day 25 and become completely fused with the trunk towards day 70. Pre-hatching occurs towards day 50, while the incubation period extends up to day 115, when a fully formed juvenile hatches from the egg case.

A97
ANALYSIS OF MUCINS EXPRESSION DURING POSTNATAL DEVELOPMENT OF RAT MAMMARY GLAND.

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The aim of this study was to analyze Muc1, Muc2 and Muc5ac mucins expression in different stages of rat mammary gland postnatal development. 64 rat mammary gland samples in the following stages: juvenile, prepubertal, pubertal, adult virgin, pregnant, lactating, post-lactational involution and old mammary gland, were included. Inmunohistochemistry (IHC) and RT-PCR techniques were employed. By IHC, Muc1 expression was observed in lactating mammary gland, post-lactational involution and old mammary gland. Lactating mammary gland showed an intense staining with a high percentage of reaction. In post-lactational involution and old mammary gland, a moderate reaction with a lower percentage of expression was detected. Muc5ac was found at all stages analyzed, except during lactation. Muc2 reaction was not observed. By RT-PCR, Muc1 was amplified at all stages analyzed, Muc5ac was observed in all phases, except during lactating while Muc2 always showed a very low expression. These results would suggest a possible role of Muc1 and Muc5ac in differentiation and glandular morphogenesis during mammary gland postnatal development.

A98
FUNCTIONAL ANALYSIS AND EXPRESSION OF ENDOTHELIN RECEPTOR B IN *XENOPUS* NEURAL CREST DEVELOPMENT

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The emergence of the neural crest cell population involves the integration of several signaling pathways as well as embryonic tissue interactions. Despite recent progress in this area, there are many aspects of neural crest development such as the genes involved in this process which are not very well understood. The aim of this work was to increase the knowledge of the signaling pathway mediated by the Endothelin Receptor B (Ednrb) in the formation of *Xenopus laevis* neural crest. We performed the molecular cloning of Ednrb cDNA and we subsequently did an analysis of the spatiotemporal expression pattern of this gene. We analyzed embryos at different developmental stages and it was found that the transcripts of *Ednrb* in *Xenopus laevis* became visible from stage 21 in expression zones located in the dorsal region of the neural tube, in the notochord, otic vesicle, paraxial mesoderm and melanoblasts. We carried out functional studies through microinyection of Ednrb mRNA in *Xenopus* embryos. The overexpression of *Ednrb* increased *Snail2* gene expression and decreased *FoxD3* expression. These results suggest that the signaling pathway mediated by *Ednrb* could not be involved in the induction of neural crest cells and it could not exert any early function in the development of this cell population. Overall the results presented here suggest that Ednrb could be active in the migration and differentiation phase of neural crest development.

A99
SONIC HEDGEHOG DRIVES CHEMOTAXIS OF NEURAL CREST CELLS, WHICH IS PERTURBED BY ETHANOL EXPOSURE

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Colonization of neural crest cells (NCCs) toward ocular region in Sonic hedgehog (Shh) gradients and its alcoholic perturbation were studied. Directional parameters showed chemotaxis of in vitro NCCs toward Shh, notochord cocultures or conditioned medium, which was inhibited by anti-Shh antibody, cyclopamine and anti-Smo morpholino. Ptch-Smo expression was also showed on NCCs. On whole embryo in situ hybridization and immunolabeling, we showed expression of Shh mRNA and protein at ocular field, as well as Ptch, Smo and Gli/Sufu on cephalic NCCs. Blocking of Shh>Ptch/Smo by transfection with anti-Smo morpholino, or negative dominant Ptch plasmid and electroporation shown lower density of NCCs at the ocular region. Implant of Shh or cyclopamine embedded microbeads support the guiding rol of Shh on NCC orientation. Ethanol exposure perturbed NCC chemotaxis toward Shh gradient, and embryos shown cranio-facial anomalies assigned to deficient distribution of NCCs, associated with abnormal in situ expression of Shh, supporting the involvement of cephalic NCCs in the Fetal Alcohol Syndrome. These in vitro and whole embryo results show a Shh guidance action through the Ptch-Smo system, independent of Gli; adding a new approach to the perturbations induced by non controlled toxic factors, and point out a new guidance function for the Shh morphogen, besides the other knew functions.

REPRODUCTION

A100
ANGIOTENSIN II INDUCES MARKERS OF UTERINE RECEPTIVITY *IN VITRO* IN RAT ENDOMETRIAL STROMAL CELLS.

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The requirement for Ca2+ mobilization, increased prostaglandin production, and the involvement of angiotensin II (Ang II) for maximal decidualization in rats, was reported. We demonstrated previously that Ang II activated Ca^{2+} / calcineurin / NFAT pathway and COX-2 expressión in rat endometrial stromal cells (ESC). Ang II also modulated ESC proliferation and metalloprotease activity, that could be involved in the effect of Ang II in the decidualization of ESC. To determine whether Ang induced decidual reaction and uterine receptivity *in vitro*, we cultured ESC isolated from rat uterus with Ang II (or estrogen plus progesterone as positive control) during different times, and then measured desmine mRNA expression (as a marker of decidualization) and the mRNA and protein expression of HB-EGF and IGF-1 (as markers of uterine receptivity), by RT-PCR and western blot. To assess whether calcineurin activity was involved in the effect of Ang II, we pretreated ESC with CsA. ESC cultured with Ang II showed increased desmine expression and morphology compatible with decidualized cells. Ang II induced HB-EGF and IGF-1 expression. CsA inhibited the increase in desmine as well as HB-EGF and IGF-1 expression in ESC cultured with either Ang II or the estrogen plus progesterone combination. Induction of markers of decidualization and uterine receptivity by Ang II *in vitro*, appears to require calcineurin activity.

A101
GLYCOLYSIS, PENTOSE PHOSPHATE PATHWAY AND MEIOTIC PROGRESSION OF PORCINE OOCYTES DURING IN VITRO MATURATION

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We have previously observed the inhibition of glycolysis by sodium fluoride (NaF) and ATP and the inhibition of the pentose phosphate pathway by 6-aminonicotinamide (6-AN) and NADPH in porcine cumulus-oocytes complexes (COCs). Our aim was to determine the influence of these inhibitors on the meiotic progression of porcine oocytes during in vitro maturation. COCs were cultured during 44 h at 39ºC in medium 199 with gonadotropins (control) + 5 mM NaF, 10 mM ATP, 0.025 mM 6-AN or 0.125 mM NADPH. Oocyte nuclear stage was determined at 0, 24, 32, 40 and 44 h of maturation by Hoechst 33342 stain. Inhibition of glycolysis by NaF or ATP decreased the percentage of oocytes reaching metaphase II ($P<0.05$), staying in metaphase I with NaF and in germinal vesicle stage with ATP. Inhibition of pentose phosphate pathway by 6-AN or NADPH decreased the percentage of oocytes reaching metaphase II ($P<0.05$), staying in metaphase I with both inhibitors.

Inhibition of glycolysis by NaF and pentose phosphate pathway by 6-AN and NADPH would allow meiosis resumption, but it inhibits the completion of maturation. On the other hand, inhibition of glycolysis by ATP seems to inhibit meiosis resumption.

A102
ANALYSIS OF EXTRINSIC AND INTRINSIC PATHWAY OF APOPTOSIS IN MOUSE DIABETIC TESTES INDUCED BY STREPTOZOTOCIN

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Type I diabetes mellitus (T1D) is a multifactorial autoimmune disease, which causes infertility in male population. The molecular mechanisms underlying this dysfunction are not completed understood. The aim of this study was to analyze the dynamics of apoptosis in diabetic mice testis induced with streptozotocin (STZ). 25 BALB/c adult mice were used. Mice received 5 injections of at a dose of 60 mg/kg body weight STZ. Three diabetic and 2 control animals were sacrificed at day 15, 20, 40, 70 and 80 post-treatment. The testes were weighed and measured; one of them was fixed for IHQ/ IF and the other one was frozen for qPCR, in both cases were analyzed Fas, Fasl, c-BID/Bid, Bax, Bcl2, Caspase-3/Active Caspase-3 and Atf6. Caspase-3 showed its highest level at days 15 and 80, while, Active Capase-3 was positive in all the time-points, Bcl2 showed the same expression pattern as Active Caspase 3 and Caspase-3. Bax was observed mainly at day 70. Fas and Fasl showed their highest level from day 40 to the day 80, c-BID showed a strong expression at days 15, 70 and 80. The highest level of Atf6 was observed at day 40. These results suggest a differential dynamics of the apoptotic proteins at early and late stages in the diabetic testis, with the extrinsic pathway as the main player. This pathway would be stimulated by endoplasmic reticulum stress.

A103
PARTICIPATION OF CA^{2+} IN HUMAN SPERM CAPACITATION

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Recent studies from our group revealed that the increase in tyrosine (Tyr) phosphorylation during human sperm capacitation involves both PKA activation and Src family kinase (SFK)-induced inactivation of serine/threonine (Ser/Thr) phosphatases (PP1γ2). Considering that in the absence of extracellular Ca^{2+} in the capacitating medium, human sperm exhibit an increase in Tyr phosphorylation, in the present work we investigated the role of Ca^{2+} as a modulator of kinases and/or phosphatases. Results showed that a decrease in extracellular Ca^{2+} did not modify PKA substrate phosphorylation. Moreover, using specific inhibitors, we observed that the effect of the absence of Ca^{2+} in Tyr phosphorylation depended on the activity of Ser/Thr phosphatase PP2B and calmodulin, a Ca^{2+} binding protein, and occurs independently of SFK/phosphatase. In conclusion, our results support the participation of different pathways in human sperm Tyr phosphorylation: one depending on PKA activation, one involving Ser/Thr phosphatase down-regulation and one regulated by extracellular Ca^{2+}, which cross-talk at different levels. These results will contribute to a better understanding of the molecular mechanisms involved in human sperm capacitation.

A104
EFFECT OF MEMBRANE ADENYLATE CYCLASE INHIBITION ON OXIDATIVE METABOLISM IN CRYOPRESERVED BOVINE SPERM CAPACITATION

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Hyaluronic acid (HA) and heparin (H) are glycosaminoglycans present in genital tract of bovine female. It has been identified an adenylate cyclase presents in membrane (mAC) that produces intracellular cAMP and induces capacitation and protein tyrosine phosphorylation. The aim of this study was to evaluate mAC participation on bovine sperm capacitation induced by HA or H, through oxygen consumption, lactate dehydrogenase (LDH) activity and variation of lactate concentration in incubation medium. 2´,5´-dideoxyadenosine (100 μM) was used as a mAC inhibitor. Capacitation was evaluated by chlortetracycline epifluorescent technique and viability and acrosome integrity by Trypan blue stain/DIC. LDH activity and lactate concentration in incubation medium were determined spectrophotometrically and oxygen consumption polarographically. Data was analyzed by ANOVA and Tukey test ($P<0.05$). mAC inhibition blocked capacitation induction and the respiratory burst produced by H. LDH activity decreased in samples incubated with HA and mAC inhibitor

(0.40±0.12 U/10^8esp). Samples incubated with HA or H evidenced a significant decrease in lactate concentration in incubation medium when samples were incubated with 2´,5´-dideoxyadenosine respect to samples without inhibitor ($P<0.05$). H and HA are capable of inducing bovine sperm capacitation involving different enzymatic pathways and intracellular signalling dependent on membrane adenylate cyclase.

A105
SPERM CHANGES AND ABNORMALITIES DURING EPIDIDYMAL TRANSIT OF ARMADILLO IN BREEDING PERIOD

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The aim of this work was to identify abnormalities, sperm patterns of nuclear integrity and tyrosine protein phosphorylation during epididymal transit and to evaluate testicular activity by histological sections of *Chaetophractus villosus* male in breeding period. The large hairy armadillo belongs to Dasypodidae family. Its reproductive season starts in late winter and ends in early spring. The male armadillo studied weights 3.67kg with a testis mass of 16.72g. Sperm smears from different regions of epididymis were analyzed. Tyrosine protein phosphorylation was analyzed by immunohistochemical technique and nuclear damage by Toluidine Blue stain, showing different patterns. Histological research showed an active seminiferous epithelium and plenteous epididymal sperm concentration. The percentage of nuclear integrity pattern was 8, 26 and 46% corresponding to epididymis head, body and tail, respectively. Immunofluorescent patterns showed no significant differences. Mid-piece cytoplasmic droplet, abaxial tail attachment and simple bent tail abnormalities were identified showing the following percentages: 14, 24 and 0 for head; 8, 40 and 60 for body and 6, 40 and 0 for tail, respectively. This study proposes male reproductive parameters for *Chaetophractus villosus*, which may contribute to increase knowledge about the reproductive behavior of the armadillo.

A106
TEMPORAL AND SPATIAL EXPRESSION PATTERN OF ESTROGEN RECEPTOR ALPHA, PROGESTERONE RECEPTOR AND wnt7a IN *Caiman latirostris* OVIDUCTS

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C latirostris oviduct maturation is a postnatal event. In mammals, wnt7a is involved in oviductal radial differentiation patterning and adenogenesis. There is no information about wnt7a role on reptilian reproductive tract development. The aims of our study were to determine in *C latirostris* oviducts, the temporal and spatial expression patterns of wnt7a, estrogen receptor alpha (ER), and progesterone receptor (PR); and to assess histo-morphological-related changes. Caiman females were euthanized at different ages from hatchling to juvenile. Oviductal histo-morphological features were assessed in trichromic stained sections and quantified by a 1 to 10 score scale. ER, PR and wnt7a were evaluated by IHC. Epithelial ER expression showed increasing levels from hatchling to 90 days of age and decreased on juvenile oviducts. Histological changes from pre-adenogenic to adenogenic oviducts positive correlated with the expression of, estrogen target molecules, PR and wnt7a. The endocrine environment modulates oviductal ER, PR and wnt7a levels determining, on early postnatal developmental stages, the luminal to-glandular epithelium differentiation program. Exposure to xenoestrogens could alter this process.

A107
VCP (VALOSIN CONTAINING PROTEIN) LOCATED IN THE EQUATORIAL SEGMENT OF MURINE SPERM ACROSOME IS RELEASED DURING ACROSOMAL EXOCYTOSIS.

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VCP belongs to the ATPase AAA type II family and participates in fusion of membranes. Given the importance of this event in sperm physiology, we aimed to characterize the localization and function of VCP during capacitation in murine sperm. By using transgenic mice expressing EGFP in the acrosome and RFP in mitochondria we evaluated the acrosomal state together with VCP localization by immunofluorescence. Using DBeQ, a specific VCP inhibitor, vitality, capacitation and relocalization of VCP were evaluated. By immunoprecipitation, VCP phosphorylation was evaluated during capacitation. As a result, we observed the presence of VCP in the tail and the equatorial segment in capacitated and non-capacitated sperm. Interestingly, VCP was lost after acrosomal exocytosis. In the presence of DBeQ, a decrease in motility and vitality, plus an

increase in the spontaneous acrosomal reaction were observed. VCP is not phosphorylated in tyrosine residues. So far, VCP would be the only protein known to be associated to the equatorial segment of murine sperm that is released after acrosomal exocytosis.

A108
APOPTOSIS IN FETAL AND NEONATAL HUMAN OVARY: EXPRESSION OF C-BID, CASPASE 8 AND ACTIVE CASPASE 3

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During prenatal period 7 million of oogonia are originated, 85% of them will be eliminated at birth trough apoptotic BCL2 and CASPASES families. The balance between BCL2 and BAX determines survival or germ cell death. Concerted action of proapoptotic cleaved BID (c-BID) and initiator and effector CASPASES 8 (C8) and 3 (C3) actives were analyzed in 49 human fetal and neonatal ovaries from week 8 of gestation until 16 days of life, by IHQ and IF. Cleaved BID and C8 were detected from week 12 (w12); its expression increased during the second trimester and decreased by the end of pregnancy and neonatal period with a cytoplasmic localization in germ cells, primordial and primary follicles. Active C3 was observed from the end of the first trimester of pregnancy, with the highest expression at w20, decreasing at the end of pregnancy and postnatal period in follicles in different stages. The highest detection of c-BID, C8 and C3 in germ cells, primordial and primary follicles were observed at mid gestation, coinciding with the highest rate of apoptosis, its decreased towards the third trimester and neonatal period, at this moment C3 was detected in secondary follicles. These results suggest a concerted action between BCL2 family component (c-BID) and CASPASES with a specific spatio-temporal expression pattern in human fetal and neonatal ovary.

A109
GLUTATHIONE ROLE IN REACTIVE OXYGEN SPECIES PRODUCTION IN BOVINE OOCYTE MATURATION

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Reduced glutathione (GSH) is a key antioxidant in normal cell function, involved in oocyte developmental competence. The aim of this work was to determine GSH role in reactive oxygen species (ROS) production during bovine oocyte *in vitro* maturation (MIV) and its aftereffect on *in vitro* fertilization (FIV). Cumulus-oocyte complexes (COCs) were obtained from slaughtered cow ovaries and matured in medium 199 (control) or supplemented with cysteine (GSH precursor) or chloro-dinitro-bencene (CDNB, GSH removal) at 39°C, 5% CO_2 for 22h. Nuclear maturation percentages were determined by the presence of metaphase II and FIV was determined by cleavage rate. ROS production was measured by DCHFDA at 0, 6, 12, 18 and 22h. COCs matured with cysteine showed a significant decrease in ROS levels at 12 and 18h ($p<0.05$) with no modification in meiotic maturation or FIV rates. In the presence of CDNB no significant differences were detected in ROS levels, but all oocytes were arrested at germinal vesicle stage. The antioxidant action of GSH when removed by CDNB could be compensated by other oocyte antioxidant systems. However, its role in the maintenance of the meiotic spindle would be essential for bovine MIV.

A110
PARTICIPATION OF SDH IN CAPACITATION AND ACROSOME REACTION IN PORCINE SPERM.

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The aim of this study was to determine the activity of succinate dehydrogenase (SDH; 1.3.5.1) and evaluate its participation in capacitation and acrosome reaction (AR) in porcine spermatozoa. The activity of SDH was determined spectrophotometrically at 600 nm, during 10 minutes, at 37°C. Enzime unit (U) was defined as the amount of SDH that catalyzes the reduction of 1 µmol of DCPIP/min. Capacitation and AR were determined, in the presence or absence of malonate (competitive inhibitor of SDH; 1, 5 and 10 mM), by CTC technique and trypan blue combined with DIC, respectively. Sperm viability was evaluated by the eosin-nigrosin technique and motility was evaluated by optic microscopy, with a thermal stage. Enzime activity (with or without malonate) data were analized with the paired t student test. The results of capacitation and AR were analysed by ANOVA and Bonferroni test. The activity of SDH without the inhibitor was $0,44\pm0,16$ U/10^{10} spermatozoa and in the presence of the inhibitor it was $0,05\pm0,01$ U/10^{10} spermatozoa. Capacitation and

AR were significantly diminished by the addition of 5 mM malonate, without affecting motility or sperm viability. Our results demonstrate the activity of SDH and its participation in capacitation and AR in porcine spermatozoa, indicating the pivotal role of the Krebs cycle in the ATP production required for these processes.

A111
IDENTIFICATION OF HEPARAN SULFATE IN HUMAN OOCYTES AND PRELIMINARY EVIDENCE ON ITS POSSIBLE ORIGIN.

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Oocyte decondensing ability varies with oocyte maturation. We have proposed that HS present in ooplasm acts as human sperm decondensing agent *in vivo*. The aims of this study are: **1)** to determine the presence of HS at different stages of oocyte maturation and **2)** to characterize GAG synthesis in mature (MII) oocytes, **C**umulus **C**ells or **C**umulus **O**ocyte **C**omplex. **1)** Immunocytochemistry of **G**erminal **V**esicles, MII and CC revealed that HS is present in MII (65510±1582 **A**rbitrary **U**nits vs 8579±742 AU (control), t-test, $p<0.05$, n=11), GV (186000±8065 AU vs 1996±4 AU, t-test, $p<0.05$, n=26) and CC (31010 ± 3971 AU vs 10900 ± 5985 AU, t-test, $p<0.05$). **2)** MII, COCs or CC were cultured 24h with [^{35}S]-sulfate and [^{3}H]-glucosamine. Guanidine hydrochloride extracts were chromatographed and eluted and radioactivity was measured. Preliminary results showed GAG/sulfated proteoglycan synthesis in MII, CC and COCs, but not in equal magnitude. **1)** HS ubiquitous location could be reassuring sperm decondensation. **2)** Preliminary metabolic results indicate that HS synthesis requires COC integrity.

A112
ACTIVATION OF FIBROBLAST GROWTH FACTOR RECEPTORS (FGFRs) AND RELATED SIGNALING PATHWAYS IN HUMAN SPERM

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In somatic cells, FGFRs modulate several cellular functions by the activation of signaling pathways that involve the phosphorylation of FGFRs, MAPK/ERK and PI3K/Akt. Previous studies from our laboratory have shown the presence of FGFRs in human sperm, although there is no evidence of their functionality. The aim of the present study was to analyze FGFRs, ERK and Akt activation in human sperm in response to the ligand (FGF). Sperm were incubated under capacitating conditions for total 4 h and exposed to FGF (1, 10 or 100 ng/ml) during the last 15 min. FGFRs, ERK and Akt phosphorylation was evaluated by immunocytochemistry and Western immunoblotting using specific antibodies against the phosphorylated forms. The 27±4 % of the 4-h capacitated sperm showed phosphorylation of flagellar FGFRs and the exposure to 10 and 100 ng/ml FGF led to a significant increase ($P<0.05$) in the percentage of immunoreactive cells (44±2 % and 57±3 %, respectively; n=3). In sperm treated with FGF, an increase in flagellar ERK and Akt phosphorylation was also observed (n=6). These effects were blocked when sperm were preincubated for 15 min with a FGFR specific inhibitor (BGJ398). In conclusion, sperm FGFRs are functional since exposure to the ligand induces their phosphorylation and the activation of the ERK and Akt signaling pathways. Supported by grants from Fundación Fiorini (2012, CIMB) and CONICET (PIP2120, MHVL).

A113
DYS/FXYD5 EXPRESSION IN HUMAN AND MURINE TESTIS AND SPERMATOZOA. CO-LOCALIZATION STUDIES WITH EPITHELIAL CADHERIN AND ACTIN

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Dysadherin (Dys) is a cell membrane glycoprotein overexpressed in human tumors. It acts as a negative modulator of epithelial cadherin (Ecad), at least in part, by competing for the actin cytoskeleton. The murine homologue, FXYD5, is expressed in normal adult mouse tissues. Our group has reported Ecad expression in the male reproductive tract and spermatozoa in both human and murine species, and its participation in gamete interaction. The aim of the study was to evaluate Dys/FXDY5 expression in human and murine testis and spermatozoa and its co-localization with Ecad and actin in the male gamete. Methods involved 1)RT-PCR to detect transcripts, 2)SDS-PAGE/Western-Immunoblotting to immunodetect protein forms in cell and tissue extracts, 3)Immunohistochemistry to assess protein expression in the testis, 4)Fluorescence microscopy to characterize Dys/FXYD5 localization and co-localization with Ecad and actin/filamentous

actin using antibodies and probes. Dys/FXYD5 was detected in both species: mRNA expression in testis, protein forms in testis (human= 91KDa; murine = 75-66 KDa) and sperm (human= 91KDa; murine= 35 and 66 kDa). A specific signal was found in the acrosome of testicular spermatids, the acrosome and flagellum of ejaculated (human) and *cauda* epididymis (murine) spermatozoa. Dys/FXYD5-actin and Dys/FXYD5-Ecad co-localized in the sperm acrosomal region. Based on these findings, we propose Dys/FXYD5 as a modulator of Ecad-mediated adhesion by mechanisms involving competition for the actin cytoskeleton. *Supported by a grant from CONICET (PIP2120) to MHVL.*

A114
ACTIVITY AND REGULATION OF KEY ENZYMES OF GLYCOLYSIS AND PENTOSE PHOSPHATE PATHWAY IN PORCINE CUMULUS-OOCYTE COMPLEXES

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The suboptimal results in porcine oocyte in vitro maturation (IVM) are in part due to the scarce knowledge about cumulus oocyte-complexes (COC) metabolic requirements. The aim was to study the activity and regulation of key enzymes of the glycolytic pathway (Phosphofructokinase 1, PFK) and the pentose phosphate pathway (Glucose 6-phosphate dehydrogenase, G6PDH) in porcine COCs. Immature COCs were resuspended in distilled water, sonicated (4 min, 50%), centrifuged (17000xg, 20 min, 4 °C) and the enzymatic activities were determined by spectrophotometry in the supernatants. For PFK an enzymatic unit (U) was defined as the amount of enzyme that produces 1 µmol of fructose 1,6-bisphosphate/min, measured as the oxidation of 2 µmol NADH/min. For G6PDH a U was defined as the amount of enzyme that catalyzes the decrease of 1 µmol of NADP/minute. The U for FFK and G6PD were 2.13±0.18 and 1.52±0.52 x10-5/COC, respectively. The addition of modulators (AMP, ATP / NADP, NADPH) significantly modified the activity of both enzymes. FFK activity was higher respect to G6PDH activity, suggesting a higher participation of glycolytic pathway in the metabolism of glucose. Enzymatic regulation was similar to that observed in other cell types. Our results contribute to elucidate the metabolic profile of porcine COCs, determining their nutritional requirements for IVM.

TOXICOLOGY AND ECOTOXICOLOGY

A115
IN VITRO BIOACTIVITY OF ESSENTIAL OILS OF FEMALE AND MALE PLANTS OF *Schinus areira* AGAINST *Ascosphaera apis*

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Ascosphaera apis is an entomopathogenic fungus that affects only the larvae of the honeybee. Were isolated strains of this fungus in naturally infected apiaries in the province from San Luis. The essential oil of *Schinus areira* was obtained from fresh leaves by hydrodistillation during 4 h of female and male plants, were harvested August 14 and 15, 2012,. The oil yields were for the male and female plants 0.68% and 0.60% v/w, respectively. Strains of *A. apis* were incubated in MY20 standardized culture medium at 30ºC ± 1ºC. Was used to determine oil bioactivity, the method dilution at different concentrations: 1.5; 1.0 and 0.5 µl/ml and a test without oil. In the center of each Petri dish 9 cm diameter, a agar disk of 5 mm diameter from the border of colonies of *A. apis* of six days of growth was sowed to observe the development of fungus. The growth halos were observed at 2; 3 and 6 days time that the mycelium of test touches the edge of the Petri disch. It can inferred that the six days of treatment all experienced oil concentrations significantly inhibited the growth of *A. apis*, analyzed by factorial ANOVA (p-value <0.01%).

A116
HEXAVALENT CHROMIUM EFFECT ON PROLIFERATION AND DIFFERENTIATION OF 3T3-L1 FIBROBLASTS.

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Heavy metals contamination has become an important risk factor for public health and the environment. Chromium is a frequent industrial pollutant and, in our country, high concentrations of this heavy metal were found in the river Matanza-Riachuelo. Since hexavalent chromium was reported as genotoxic and cancerigenous in different systems, to further evaluate

its cytotoxicity, we wanted to investigate the effect of this heavy metal in the proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts. These cells, after the addition of a mixture containing insulin, dexamethasone and methylisobutylxanthine, first proliferate, a process known as mitotic clonal expansion (MCE), and then differentiate to adipocytes. In this differentiation process a key transcription factor is induced: peroxisome proliferator activated receptor gamma (PPARg). We found that treatment of 3T3-L1 fibroblasts with potassium chromate (1-10 μM) inhibited proliferation in exponentially growing cells and ECM as well as differentiation. A decrease in PPARg content, evaluated by western blot and immunofluorescent, was found in cells differentiated in the presence of chromium. On the other hand, after inhibition of differentiation with chromium, when the metal was removed, differentiation was recovered, which would indicate that this is a reversible effect. We also found an increase in micronucleus in the cells treated with hexavalent chromium which is associated with genotoxic effects. These results suggest that hexavalent chromium is able to inhibit proliferation and differentiation of 3T3-L1 fibroblasts.

A117
EMBRYONIC AND FETAL ALTERATIONS AND CHANGES IN CARBOHYDRATE PLACENTAL PATTERN IN CADMIUM INTOXICATION

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Cadmium is a metal with detrimental effects on prenatal development. The aim of this work was to study alterations in placental and embryonic-fetal growth, and uterine and placental carbohydrate expression. Wistar rats were administered a single subcutaneous 10 mg/kg body weight cadmium dose at 7, 10 or 15 days of gestation and euthanized at 7, 10, 15, and 20 days of gestation. Control animals received sterile saline. Dams were divided into ten experimental and ten control groups with respect of day of injection/day of euthanasia, e.g. group 4/7. Samples of uteri, embryos and fetuses were collected for Alizarine red S, histology and lectin histochemestry techniques. Many fetuses showed gross morphological alterations. Placental weight decreased in dams of groups 7/20, 7/15, and 10/15 when compared to controls. Reactivity to lectines resulted altered in uterine epithelium and decidua in dams of groups 4/7, 4/10, and 7/10 and also in placentas of dams of all groups euthanized on days 15 and 20 of gestation. Changes in the pattern of carbohydrates found would affect cellular interactions and thus contribute to prenatal alterations.

A118
HISTOPATHOLOGIC ALTERATIONS OF THE SHRIMP *Pleoticus muelleri* BY EFFECT OF THE ENVIRONMENTAL NITRITE

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The aim of this work was to evaluate the effects of the nitrite on the histology of gills and hepatopancreas of *P. muelleri*. Shrimp were exposed to nitrite (0 to 1000mg/l) by 96hs. Histological techniques were performed on the cephalothorax of each individual. In the hepatopancreas, the changes were detected since 200mg/l of nitrite, consistent with an increase in the number of cells B and hyperplasia zones. Upper concentrations of nitrite caused distortion of the tubular structure and a high degree of cytoplasmic retraction in the cells. To concentration of 500mg/l, the cells showed abundant vacuolization (foamy cells) and only the F cells can be identified, to 1000mg/l all cells lost their cell identity. In the gills, the treatment with nitrite caused disorganization epithelial and folding of the cuticle, both effects were more prominent with the increase of this poluent. Upper concentrations of environmental nitrite caused distortion of the marginal channel, occurrence of subcuticular lagoons and collapse of the lamella. To 1000mg/l was detected disruption of the cuticle with the consequent deterioration of the gill lamella. In conclusion, the histological analysis of the hepatopancreas and gills of *P. muelleri* can be used as a biomarker by nitrite pollution.

A119
DETECTION OF PESTICIDES IN MULTICOMPONENT MIXTURES, ALCOHOL BIOINDUSTRY RESIDUES

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The purpose of this work is to detect Organochlorine Pesticides (OP) in vinasse (7,4 Brix) resulting from both the distillation of alcohol industry wine and in 49,8 and 61 Brix concentrates obtained in series connected evaporators that produce mulch

and vapor. Samples from the centre and the southeast sugar-cane factories of Tucuman were analyzed. The evaporation grade of vinasse was measured through the content of dissolved solids, using refractometry. Eleven OP were researched by means of gaseous chromatography, capillary column and electron capture detector before the liquid-liquid extraction. Calibration curves were prepared. Monitoring of the results was carried out with samples of poisoned vinasse achieving 80% of recovery. Two forbidden pesticides were detected: Lindane (0,08, 0,10 y 0,13 μg/L) and heptachlor epoxide (0,11, 0,14 y 0,15 μg/L) in vinasses of 7,4; 49,8 and 61 Brix grades, respectively. Little increase of OP content in the concentrated product is observed. The OP grams decrease in the concentrates and are detected in the vapor phase. The vapor of the first evaporator shows a greater content of pesticides, in grams, than its concentrate. This can be seen in the heptachlor epoxide graph. Similar results are found for Lindane. You should pay particular attention to the concentration of PO in the steam to reduce the risk of contamination.

A120
POSTNATAL EXPOSURE TO XENOESTROGENS ALTERS wnt7a EXPRESSION AND OVIDUCTAL RADIAL PATTERN IN *Caiman latirostris*.

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Wnt7a is a glycoprotein involved on mammalian reproductive female tract radial patterning differentiation. Wnt7a is target of xenoestrogen action. The aim of our study was to assess whether postnatal exposure to 17β-estradiol (E2) or to, the xenoestrogen, bisphenol A (BPA) alters wnt7a expression or radial pattern on *Caiman latirostris* oviduct. Two injections (s.c), 7 days apart from each other, of E2 (1.4 and 0.014 ppm), BPA (140 and 1.4 ppm) or vehicle were administered to one month old female caimans. Seven days after the last injection, oviducts were paraffin-processed. Wnt7a and smooth muscle α-actin expression were evaluated by immunohistochemistry. Wnt7a positive cells and proportion of subepithelial area occupied by muscle or *lamina propria* were assessed. Wnt7a expression was nuclear and epithelium-restricted. All treated groups but BPA 1.4ppm, showed decreased wnt7a expression. Moreover, all treatments increased the proportion of subepithelial area occupied by muscle. Postnatal exposure to xenoestrogens alters the oviductal radial pattern. Changes in subepithelial muscular area and in wnt7a levels could be considered as potential biomarkers of xenoestrogen action.

A121
ALTERATION OF THE MICRORNA-122 REGULATORY NETWORK IN RAT MODELS OF HEPATOTOXICITY

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MicroRNAs (miRNAs) are small RNA molecules that post-transcriptionally regulate gene expression. MiRNA-122 is the most abundant and specific liver miRNA. Thioacetamide (TA) and carbon tetrachloride (CT) are two known hepatotoxic widely used as models in rats. The aim of this work was to evaluate the miRNA-122 regulatory network in experimental models of liver damage induced by TA or CT.
Adult male Wistar rats received either i.p. TA (150 mg/kg rat), CT (1 ml/kg rat) or vehicles, and were sacrificed 24 hs later. The liver injury was studied by serum ALT levels and histological analysis. MicroRNA and mRNA were measure using RT-qPCR from total RNA purified by Trizol method. We observed that hepatotoxicity decreases miRNA-122 levels and increases the expression of its target genes Cyclin G1 and CAT-1. We also observed a decreased expression of the miRNA-122 precursor (pri-miRNA-122) and of the transcription factors that specifically bind its promoter C/EBP and HNF4 Cell proliferation was increased, as indicated by the PCNA and Cyclin D levels. We conclude that miRNA-122 expression levels are under transcriptional control during hepatotoxicity. We propose that this miRNA-122 alteration is associated with the liver response to cope with the injury caused by the hepatotoxins, likely through a cell proliferation process to repair the damaged tissue.

A122
CORRELATION BETWEEN SERUM ALT AND BLOOD MIRNA-192 LEVELS IN PATIENTS WITH LIVER INJURY

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Drug-induced liver injury is an epidemiologically important pathology. Its early diagnosis is essential to avoid the strengthening of the damage caused. The serum activity of alanine aminotransferase (ALT) is the main hepatotoxicity biomarker. The micro(mi)RNA-192 is a small (22 nucleotides) non-coding RNA highly expressed in the liver that has been postulated as a potential biomarker of liver injury in experimental toxicity studies. The objective of this work was to study the correlation between the blood miRNA-192 levels and the serum activity of ALT.
According to the protocols approved by the Bioethics Commission, patient samples with standard and altered serum ALT activities were obtained, on which total RNA was purified through the Trizol method (Invitrogen). Expression levels of miRNA-192 were determined through Stem Loop RT-qPCR, using miRNA-16 as reference gene. Since the sample size for each dosage group was inadequate to test the assumption of normality, then both Pearson product-moment (Pe, parametric) and Spearman's rank (Sp, nonparametric) correlation coefficients were determined. The variables were expressed relative to the levels of the healthy patients. We observed a significant and very strong correlation between miRNA-192 and ALT (Pe: $r = 0.78$, $p<0.001$; Sp: $rs = 0.65$, $p<0.001$). This result supports the potential use of miRNA-192 as blood biomarker of hepatotoxicity in humans.

A123
DOPAMINE EFFECT IN HUMAN MONOCYTE-MACROPHAGE CELLS AND ITS INTERACTION WITH BISPHENOL-A.

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Previously we showed that dopamine (DA) increases the IL-6 and IL-8 production in keratinocytes, by acting on β-adrenergic and dopaminergic receptors. Bisphenol-A (BPA), a chemical endocrine disruptor, didn't affect neither the basal nor the DA-induced cell proliferation and cytokine production. In this study we evaluated the effect of DA in the absence and presence of BPA and propranolol, on IL-8, IL-1β production (ELISA), activity of metalloproteinases (MMP, zymography), NFkB activation (Western blot) and IkB-inhibitor, in a human monocytic-macrophage cell line THP-1. The cells were incubated with DA (10^{-6}, 10^{-5}, 10^{-4}M) in the presence and absence of BPA (10^{-5}, 10^{-7} M) or the β-adrenergic antagonist propranolol (10^{-5}). We observed that DA stimulated the dose-dependent production of IL-8 (10^{-4}M, $p < 0.05$), which was reduced by propranolol. BPA didn't modify basal levels neither of IL-8 nor the stimulated by DA. IL-1β production was not affected by DA, BPA, or DA/BPA. There was a modulatory effect of DA and BPA on MMP-9 activity. DA didn't induce nuclear NFkB while cytosolic IkB increased. Results indicate that DA may be involved in the inflammatory immune response mediated by cytokines and MMPs without activating the NFkB signaling pathway. However BPA could not affect the inflammatory effects induced by DA.

A124
EFFECT OF GLYPHOSATE ON THE SYMBIOSIS *Rhizobium-Lotus tenuis* IN THE FLOODING PAMPA

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In the Flooding Pampa (FP), glyphosate is frequently used to eliminate weeds which compete and affect the productivity of *Lotus tenuis*. This legume establishes a symbiotic association with nitrogen-fixing bacteria, and its presence in pastures increases the nitrogen content and forage quality for livestock activity. Some studies indicate that glyphosate affects soil bacteria. For this reason this study is intended to assess the impact of this herbicide on viability, nodulation capacity and symbiotic nitrogen fixation of *L. tenuis* symbionts. We analyzed the genetic diversity of a collection of rhizobia isolated from nodules of L. tenuis in soils of the FP with history of glyphosate by rep-PCR. We also studied the effect of glyphosate on the free living conditions and the symbiotic ability of *L. tenuis* isolates in soils amended with different concentrations of the herbicide.It was observed a low genetic diversity among rhizobia tested. The application of glyphosate in soil did not affect the ability of rhizobia to form nodules in *L tenuis*. However, symbiotic efficiency was significantly decreased with concentrations above 1.5 mM equivalent glyphosate. The application of glyphosate at concentrations higher than 1.5mM affects both the viability and symbiotic efficiency of *L. tenuis* symbionts.

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