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LECTURES

A1
UPDATE OF KEY MOLECULES IN THE BRAIN INVOLVED IN THE CONTROL OF PUBERTY AND REPRODUCTION.

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The neuroendocrine reproductive axis is controlled by various hormonal and neural pathways that converge upon and regulate forebrain gonadotropin-releasing hormone (GnRH) neurons. The status of the reproductive axis and GnRH secretion differs between various life stages, including puberty and adulthood. However, until recently, many of the key neural circuits underlying the control of GnRH neurons at different life stages were only poorly understood. The recently-identified neuropeptides kisspeptin (encoded by the *Kiss1* gene), neurokinin B (NKB; encoded by *Tac2*), and RFRP-3 (GnIH; encoded by *Rfrp*), have been implicated as important regulators of GnRH neurons in numerous species. In mammals, these reproductive neuronal populations are located in several discrete brain regions, including hypothalamic nuclei and the amygdala. Accumulating evidence indicates that kisspeptin and NKB neurons are potent stimulators of the reproductive axis at different life stages, including puberty and adulthood, whereas RFRP-3 neurons inhibit the reproductive axis. Studies have now begun to examine how these circuits are themselves regulated, both in adulthood and development. Findings from multiple mammalian species and both sexes indicate that *Kiss1, NKB,* and *Rfrp* neurons are each regulated by sex steroids (estrogen and testosterone), to differing degrees. Intriguingly, the manner in which sex steroids regulate these neurons (stimulatory or inhibitory) is both gene and region-specific within the brain, a finding which may illuminate the cellular mechanisms of steroid-mediated positive and negative feedback regulation of GnRH secretion. Moreover, certain *Kiss1* populations undergo sexual differentiation, a developmental process driven by sex steroids during critical postnatal periods. Thus, compounds the mimic or antagonize sex steroid signaling, such as phytoestrogens, can have notable effects on reproductive brain circuits, and can alter both puberty and fertility, as well as sexual differentiation. These topics will be summarized and discussed, with particular emphasis on rodent models.

A2
PHARMACEUTICAL SOUP: THE NEW ENVIRONMENTAL REALITY.

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A vast array of industrial pollutants and agricultural pesticides, are now present in the environment. Hundreds of human and veterinary pharmaceuticals are also present in detectable and biologically active concentrations in every environment where they have been assessed. The main sources contributing to this pharmaceutical soup are human sewage effluents and agricultural run-off. The difference with pharmaceuticals compared to other pollutants is that the vast majority was specifically designed to be highly active in vertebrate systems at low concentrations. Research across vertebrate taxa is revealing significant effects of such pollutants on the nervous system in addition to peripheral organ systems. More specifically, it is now clear that many pharmaceuticals can affect neuroendocrine neurons and the physiological processes they control. I have proposed that 'neuroendocrine disruption' extends the concept of endocrine disruption to include the full breadth of integrative physiology-that is, neuroendocrine disruption is more than just hormones. It is possible that pharmaceutical pollutants disrupt numerous other neurochemical pathways, upsetting diverse physiological and behavioral processes. The impacts of the antidepressant Prozac and the contraceptive steroid ethinyl-estradiol on fish reproductive and metabolic physiology will be presented as examples of neuroendocrine disruption as they are now widely found in aquatic ecosystems, and can be detected in tissues of wild-caught fish.

SYMPOSIA

Endocrine Perturbation

A3
ENDOCRINE DISRUPTORS IN AQUATIC ENVIRONMENTS OF ARGENTINA. IS BIOTA AT RISK?

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Endocrine disruptors include a diverse group of environmental pollutants that share the property of interfering with the endocrine system. Because of this, they are characterized by inducing adverse effects at very low concentrations, sometimes even below the detection limits of the most sophisticated instrumentation. There are numerous cases worldwide

that highlight the environmental relevance of these compounds. In particular natural and synthetic estrogens are a major responsible for the observed in estrogenic activity sewage. In Argentina very little is known about the occurrence of these compounds in the environment and even less about the consequence of their effects on biota. Recent studies show concentration of E2 and EE2 in sewage and surface waters relatively high compared with other regions of the world. Studies with indigenous fish species indicate that observed concentrations would exceed those levels that alter the expression of genes involved in metabolism and intracellular signaling of steroids, as well as in gonadal development and sexual differentiation, and the development of secondary sexual characters. These findings raise concern on the potential existence of risk to fish and other aquatic organisms, but further studies are still needed to confirm these effects in natural environments.

A4
EXPOSURE OF *Caiman latirostris* TO ENDOCRINE-DISRUPTING CHEMICALS: MORPHOLOGICAL AND MOLECULAR CHANGES IN TESTES.

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Caiman latirostris is a reptilian species that exhibits temperature- and hormone-dependent sex determination (TSD and HSD, respectively). In gonad-adrenal-mesonephros complexes of TSD-males, TSD-females and HSD-females at 10 days of age, we evaluated the expression of genes associated with sex determination/differentiation: *amh*, *sox9* and *sf-1*. We found a sexually dimorphic pattern of *amh* and *sox9* and that *sox9* expression was different between TSD-females and HSD-females. We also found that *in ovo* exposure to endocrine-disrupting chemicals (EDC) - endosulfan (END), bisphenol A (BPA) or atrazine (ATZ)- disrupted the histo-functional features of the testis (tortuous seminiferous tubules, emptied tubular lumens, high frequency of apoptosis) of TSD-males. END exposure caused an increased expression of all the genes evaluated. Our results provide new tools to understand the mechanisms that lead to abnormalities in caiman gonadal biology and show that *in ovo* exposure to EDC affects molecular and morphological levels in testes.

A5
ENDOCRINE DISRUPTING COMPOUNDS AND FERTILITY: POSTNATAL EXPOSURE ALTERS UTERINE DEVELOPMENT AND FEMALE FERTILITY IN THE RAT.

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Postnatal development is a critical period and the endocrine disrupting compounds (EDCs) can be associated with reproductive pathologies, such as infertility. Our research efforts are focusing on EDCs effects on uterine development and their consequences later in life. Using a rat model of postnatal exposure we observed that low doses of EDCs disrupt the uterine development. Then, we studied long-term effects on: 1) reproductive performance, 2) implantation and post-implantation processes, 3) epigenetic marks of endocrine-dependent genes. We detected a decrease of implantation and an increase of resorption sites. To evaluate molecular effects, we observed a mis-regulation of implantation and decidualization-associated genes and an impaired endometrial proliferation. In addition, changes of estrogen receptor alpha DNA methylation were detected in adult rats. The EDCs exposure might contribute to the impaired fertility noted over the past decades.

A6
IMPOSEX IN MARINE SNAILS IN ARGENTINA: ENVIRONMENTAL EFFECTS.

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The introduction of TBT compounds into the international market of antifouling paints occurred during the 1960s and rapidly expanded due to the reduced cost and efficiency of these materials. The undesirable effects in natural environments were discovered a few years later, including the imposition of masculine characters over female gastropods, termed imposex. Imposex in caenogastropods is the most studied negative effect of TBT. Other effects of this contaminant in biota include malformations, mortality, and hormonal imbalance in dolphins, crabs, lobsters, oysters, invertebrate larvae, sea grasses, and algae. Imposex was found in Argentina in 2000. The imposex incidence and TBT pollution were investigated along 4,700 km of Argentinean coast, including city harbors and proximal zones without marine traffic. 12 gastropod species where studied, and found the imposex phenomenon for the first time in six species. In high marine traffic zones, TBT pollution was registered and the percentage of imposex was high, while these occurrences were null in areas without boat traffic. The species that best reflect the degree of imposex were those inhabiting sandy/muddy or mixed bottoms. TBT

determination and imposex incidence indicate that pollution was focused mainly near ports with high marine traffic or in areas where ship hulls are painted.

Ecotoxicology

A7
MONITORING OF WILD POPULATIONS OF NATIVE REPTILE SPECIES UNDER ENVIRONMENTAL STRESS PRODUCED BY PESTICIDES.

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The effects of pesticides on health include a wide range of damages. Among them, genotoxicity and oxidative stress (OS) are considered biologically relevant and highly informative as early warnings of the impacts on natural populations. The aim of this work is to evaluate the environmental situation of wild populations of two native reptile species living in areas highly exposed to pesticides in the central-east region of Argentina. We use biomarkers of: 1) genotoxicity: Comet assay (CA) and Micronucleus test, 2) OS damage to DNA by the CA modified with FPG and ENDO III enzymes, 3) OS damage to lipids by TBARS, and 4) antioxidant defense capacity by Catalase and Superoxide dismutase. Samples from broad-snouted caiman and tegu lizard (hatchlings and adults) were taken from areas highly exposed to pesticides and from a control area, and the techniques applied as previously adapted for these species by our group. Animals from the exposed regions showed genotoxicity, oxidation to DNA, lipid peroxidation and alteration in antioxidant enzymes, in comparison with controls. This study demonstrate the worrying effects that pesticides may cause at different levels on native reptile species environmentally exposed, giving more information on the possible mechanisms concerning pesticide toxicity on wildlife.

A8
THE MODERN PESTICIDES: NEW CHALLENGES IN ECOTOXICOLOGY.

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Pesticides are the only man-made contaminants deliberately released into the environment. Fifty years ago, the obvious environmental impacts of legacy pesticides (organochlorines and organophosphates) were key elements in promoting the emergence of ecotoxicology as a scientific discipline. The first decades of ecotoxicology studies led to the establishment of standardized test protocols, toxicity endpoints and risk assessment procedures that are nowadays essential for evaluating new pesticide molecules. Nevertheless, although the procedures currently employed in pesticide ecotoxicology are useful, they remain far from being ideal. For example, while laboratory exposures are continuous and to a single chemical, ecosystems are normally exposed in pulses and to various pest products simultaneously. Classical test designs are also ineffective at predicting situations where trophic interactions and indirect effects may operate, and the detection of negative effects caused by low-level persistent contamination remains a challenge. Pesticide ecotoxicology needs to get beyond its actual limitations and incorporate a greater level of field realism into its assessments. In the meantime, ecotoxicologists risk assessors, industrials, and governments need to be conscious of our actual limitation to truly assess the impacts pesticides may have in the environment once they are released.

A9
ACCUMULATION AND TOXIC EFFECT OF CYPERMETHRIN AND CHLORPYRIFOS ON NATIVE FISH *Jenynsia multidentata (Anablepidae).*

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Cypermethrin (CYP) and Chlorpyrifos (CPF) are insecticides broadly used in Argentina for agriculture and domestic purposes. Both pesticides have been detected in local surface waterbodies and could have an adverse impact on the health of non-target biota. The aim of the present study was to evaluate the bioaccumulation and toxic effects of CYP and CPF when the native fish *Jenynsia multidentata* was exposed to these pesticides singly as well as in technical and commercial mixtures. Thus, adult female fishes were exposed over 96 h to 0.04 µg/L of CYP; 0.4 of CPF; 0.04 µg/L CYP + 0.4 µg/L CPF in a technical mixture; and 0.04 µg/L CYP + 0.4 µg/L CPF in a mixture of commercial products. Treatment-dependent tissue accumulation was observed by GC-ECD. CYP was detected in muscle after single exposure, in liver and gut after technical mix exposure and in gut and gills after commercial mix exposure. Moreover, CPF was detected in liver, gut and gills after single exposure and in liver, gut, gills and muscle after commercial mix exposure. Tested concentrations of CYP, CPF, and both technical and commercial mixtures, produced behavioral changes, oxidative stress and inhibition of brain

aromatase expression in *J. multidentata*. Since similar levels have been detected in aquatic systems, pesticides occurrence should be monitored in natural environments to prevent biodiversity effects.

Symposium of the Societies of Biology of Argentina

A10
EMERGING ROLE OF MITOCHONDRIA AND OXIDATIVE STRESS IN RENAL INFLAMMATORY PROCESSES.

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Chronic kidney disease involves both programmed cell death and fibrosis. Both phenomena may be closely related to the recently described dysregulation, associated with oxidative stress, of the mitochondrial machinery in patients with chronic kidney disease. Injured tubule cells, attached to interstitial macrophages and myofibroblasts, release cytokines and growth factors that promote an inflammatory state, induce tubular cell apoptosis and lead to the accumulation of extracellular matrix. Angiotensin II plays a central role in renal fibrogenesis, leading to a fast and unrelenting progression of chronic kidney disease. High angiotensin II levels lead to increased expression of NF-KB, adhesion molecules, chemokines and growth factors, with release of inflammatory cytokines and oxidative stress. All current evidence suggests that angiotensin II increases mitochondrial oxidative stress, leads to the induction of apoptosis and allows the build-up and perpetuation of a chronic inflammatory state. Since mitochondrial dysfunction and oxidative stress have a major role in the pathogenesis of renal inflammatory processes, a set of anti-inflammatory tools against the mitochondrial oxidative stress causing apoptosis and perpetuating inflammation may be advanced as a novel therapeutic approach. This may open new perspectives of treatment for inflammatory kidney disease and related conditions.

A11
BIOMOLECULES SECRETED BY AMPHIBIAN OVIDUCT. THEIR ROLE IN FERTILIZATION.

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In *Rhinella arenarum*, the oviductal pars convoluta (PC) synthesizes and secretes the jelly that is deposited and arranged in layers surrounding the oocytes during their transit through this zone. In these envelopes, which are indispensable for fertilization, are present diffusible components, between them proteins, glycoproteins and ions, which constitute the diffusible factor (DF). We determined that Ca^{2+} is a partial inducer of the acrosome reaction (AR), suggesting the existence of other associated factors that participate in the event. Starting of DF, we purified a 74-kDa acid glycoprotein (gp74) highly diffusible in the insemination medium. Its role in fertilization is not known at present. The aims of our investigation were to study the biological activity of gp74 and their probable association with Ca^{2+} in gamete interaction. The results indicate that oocyte fertilizability progressively decreases depending on the extraction time of the DF from the jelly coats. The addition of the previously purified diffusible proteins partially restored the fertilization percentages, which increased significantly by the addition of 4 mM Ca^{2+}. It was demonstrated that both, DF and gp74, causes characteristic modifications on the oocyte surface identical to those induced by the acrosomal lysins that are physiologically released during the AR. This effect on the oocyte surface, which is enhanced by the addition of Ca^{2+}, was not observed in the absence of spermatozoa. No lytic effect was observed either when insemination was carried out with gp74 treated sperm in the presence or absence of Ca^{2+}. The results demonstrate for the first time in anurans that a glycoprotein of the DF would act on the spermatozoon together with Ca^{2+}, promoting the release of the acrosomal content.

A12
POTENTIAL HEALTH RISKS OF DENTAL MATERIALS EMPLOYED IN DAILY PRACTICE.

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Most dental materials employed in restoration and oral rehabilitation are not toxic free and may generate reactions at local and systemic levels. Materials for external use may cause allergies and hypersensitivity when they come in contact with support tissues, as it is the case with acrylic prostheses, adhesive fillings and other substances. Locally, the continued use of acrylic prosthesis may cause inflammation of the mucous membranes, hyperkeratosis or dyskeratosis congenital, and

while not necessarily malign, they are nevertheless classified as precancerous. Esthetic restoration materials have a component of a plastic called bisphenol A, which is stable after hardening, but it is released with the wear. In addition, bisphenol A reacts spontaneously with chlorinated substances in drinking water, generating new bioaccumulative compounds. Other external use materials are found in oral hygiene products, such as toothpaste and mouthwash, some

containing parabens or triclosan added as preservatives and inhibitors of bacterial growth of fungi and mold which can be absorbed through the mucous membranes, causing sensitivity in some individuals. Considering that new dental products are launched every year, it is important to be informed about their components and to follow the recommendations for use indicated by the manufacturers and researchers in order to minimize the risks to our patients' health.

A13
REGULATION OF ENZYMATIC ACTIVITIES NA^+, K^+-ATPASE AND ALDOSE REDUCTASE BY TUBULIN: IMPACT ON DIABETES AND HYPERTENSION.

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Tubulin, the protein unit of microtubules, is associated primarily with other membrane proteins interacting triggering multiple regulatory functions. In mammalian cells, tubulin is associated with Na^+,K^+-ATPase (NKA) inhibiting its enzymatic activity in a reversible process regulated by the presence of glucose and glutamate in the culture media. In our laboratory showed that tubulin is present in human erythrocytes and that this tubulin is distributed in three intracellular locations: membrane fraction and soluble fraction sedimentable. It was also shown that acetylated tubulin is increased in erythrocyte membrane from hypertensive patients and in experimental hypertensive rats. We demonstrate that the increase in the membrane tubulin reduces erythrocyte deformability and therefore hinders blood circulation with consequent increase of pressure. In diabetic patients erythrocyte membrane acetylated tubulin is also increased, it causes the inhibition of NKA. Since diabetic patients blood glucose is elevated, we studied the effect of glucose on the tubulin/NKA complex in cultured cells. Finding that glucose induces increased in microtubules, acetylated tubulin and tubulin/NKA complex. This correlated with increased of aldose reductase (AR) activity and content of sorbitol. Based on these results, we hypothesize that there is a synergistic effect between AR and microtubules. This will stimulate the formation of acetylated tubulin which joins the NKA and increases the amount of tubulin/NKA complex inhibiting the enzymatic activity. Our results indicate that glucose is reduced to sorbitol by AR and this metabolite induces MT formation. This in turn increases the formation of acetylated tubulin, by action of tubulin deacetylase on microtubules.

Young Scientists Symposium

A14
AUTOPHAGY IS INDUCED BY HEMIN IN HUMAN CHRONIC MYELOGENOUS LEUKEMIA CELL LINE.

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Autophagy is a normal degradative pathway that involves the sequestration of cytoplasmic components and organelles in a vacuole called autophagosome, which finally fuses with the lysosome to degrade the sequestered material. This pathway has been associated with several physiological processes such as erythroid maturation which involves the clearance of intracellular organelles such as mitochondria. LC3 is a protein present in the autophagosomal membrane, therefore is considered as a bonafide marker for this structure. Our results indicate that in K562 cells, hemin (an erythroid maturation inductor) lead to an increased number and enlargement of GFP-LC3 positive vesicles. These vesicles were labeled with lysotracker and DQ-BSA, markers of lysosomal compartments. In addition, we have assessed by Western blot the processing of LC3 protein upon hemin incubation, showing an increased amount of LC3-II. On the other hand, we have demonstrated that hemin induces mitochondrial membrane depolarization and that mitochondria sequestration by autophagy requires the active form of NIX protein. Taken together, our results indicate that hemin induces mitophagy in K562 cells, likely to allow a more efficient and faster erythroid maturation.

A15
ENDOCRINE DISRUPTION IN THE HYPOTHALAMIC-PITUITARY UNIT. EFFECTS OF BISPHENOL A.

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Bisphenol A (BPA) is a monomer of polycarbonates and a constituent of epoxy and polyestirene resins. BPA can exert its actions through estrogen receptors, acting as an agonist or antagonist; it can also antagonize the effects of the thyroid hormone and of androgens; and it can modulate enzymatic activity, among other mechanisms. *In vivo* effects have also been described in several studies. We observed that the neonatal exposure to BPA affects the hypothalamic-pituitary-gonadal axis in female Sprague-Dawley rats. It induces precocious puberty, alters gonadotropin, prolactin and sex hormone levels, and it also induces ovarian alterations similar to the ones observed in Polycystic Ovarian Syndrome (PCOS). It alters the *in vitro* respose to gonadotropin releasing hormone (GnRH), modifying GnRH-induced signaling pathways in primary pituitary cultures from rats neonatally exposed to BPA. It also alters the hypothalamic-pituitary-thyroid axis. Furthermore, it has direct effects in primary pituitary cultures from thirteen day old female rats. The results described here provide interesting data about the neuroendocrine effects of a controversial endocrine disruptor: Bisphenol A.

A16
EPIGENETIC ACTIVATION OF SOX2 ENHANCER ON EMBRYONIC NEURAL PLATE.

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During vertebrate development, the first and definitive neural marker is Sox2. The temporal and spatial control of Sox2 expression is regulated by multiple enhancers, but the N-1 and N-2 are the ones related with it early expression on the neural territory. Previous reports have described that the HP1 protein, which interacts with H3K9me3 mark, acts as a repressor until Sox2 induction. In this context, we have characterized *in vivo* the functional role of the H3K9me3 histone demethylase, JmjD2A, during chick Sox2 activation on early neural plate. JmjD2A knock down reduces the Sox2 expression. Contrarily, gain of JmjD2A function induce ectopic Sox2 expression. This effect was potentiated when we co-electroporated JmjD2A with the kinase Msk1, which can phosphorylate the H3S10 (H3S10ph) necessary for the HP1-H3K9me3 dissociation. By *BiFC* we evidenced that the adaptor protein 14-3-3, reported to bind to H3S10ph, is able to interact with JmjD2A. Finally, we have demonstrated by *ChIP* the binding of JmjD2A to the N-1 enhancer, but not to the N-2. Taking together these results suggest a series of epigenetic events necessaries for the early activation of Sox2 N-1 enhancer on neural progenitor cells.

PODIUM PRESENTATIONS

Animal Biology

A17
ROLE OF HYPOTHALAMIC PROOPIOMELANOCORTIN IN THE REGULATION OF GLYCEMIA.

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Elucidating the central nervous system mechanisms that participate in the control of glucose homeostasis could lead to new therapies for diabetes. Some of hypothalamic proopiomelanocortin (POMC) derived peptides are anorexigenic. POMC deficient patients and mice are obese and diabetic. However, it is not clear if POMC has a direct role in glucose homeostasis. To address this issue we used a mouse line that bears a reversible mutation that prevents arcuate Pomc expression (arcPomc$^{-/-}$). To determine the role of central POMC in maintaining glucose homeostasis independently of body weight we subjected arcPomc$^{-/-}$ weanling mice (that are still lean) to a glucose tolerance test. Our results show that mutant mice have reduced glucose tolerance compared to their wild type siblings (glycemia at 120´: 165 ± 10 mg/dL, vs 125 ± 7 mg/dL, arcPOMC$^{-/-}$ vs WT respectively, $p<0.05$). Notably, Pomc restoration leads to complete glucose tolerance normalization. These results indicate that hypothalamic POMC participates in the control of glucose homeostasis. Further experiments will be conducted to elucidate the involved mechanisms.

A18

DIFFERENTIAL DIGESTIVE ENZYMES ACTIVITIES RESPONSES UPON EMERSION IN CRAB *Neohelice granulata*.

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Studies on responses of various digestive enzymes upon emersion (E) in euryhaline burrowing crabs are lacking. We studied the effect of E on amylase (Amy), maltase (Mal), sucrase (Suc), lipase (Lip) and proteolytic (Prot) activities in hepatopancreas (HP) and on glucose levels in hemolymph (Glu) in N. granulata. Male crabs acclimated (10 days) in 35 ‰ salinity were immersed for 24 hs before the experiment (to) and then subjected to E for 60 min (t60). Enzyme activities and Glu were measured at to and t60. Digestive enzyme were assayed in supernatant (10000xg 15min) from HP homogenate (4ml buffer 0.1M Tris-HCl, pH 7.4xg HP^{-1}). Amy (µg maltose x min^{-1} x mg $protein^{-1}$), Mal and Sac (µg glucose x min^{-1}x mg $protein^{-1}$), Lip (µmoles pNP x min^{-1} x mg $protein^{-1}$), Prot (enzyme units×h^{-1}× mg $protein^{-1}$) were assayed by hydrolysis

of the corresponding substrate in 50mM phosphate 30ºC (Amy); 0.1 M maleate/OHNa 30ºC (Mal and Sac); 50 mM Tris/HCl 50mM 37ºC (Lip), Tris-HCl, 0.1M, 45°C (Prot). Glu (mgxmlHL-1) was determined with commercial Kit. At t60 Amy (56 %) was lower and Sac was higher (89%) that to (to= Amy: 1376; Sac:55). Mal, Lip and Prot did not change. Glu was higher (230%) that t0 (t0:86).The results suggests differential biochemical digestive and metabolic adjustments upon emersion.

A19

EFFECT OF FOOD RESTRICTION ON THE GONAD AND HEPATOPANCREAS: BIOCHEMICAL COMPOSITION OF *Cherax quadricarinatus* FEMALES.

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The objective of the present study was to evaluate the effect of food restriction on biochemical composition of the gonad and hepatopancreas of females from the parental stock, and on the histological structure of the hepatopancreas. Three females were stocked with one male in glass aquaria (n=12) with water at 26±1°C, under continuous aeration. Each aquarium was assigned to one of the following treatments: Control and Restrictive feeding (feeding at 1.5 and 0.5% of body weight, respectively). At day 105 days the animals were sacrificed and their hepatopancreas and gonads were removed. Although minor histological abnormalities were observed on the hepatopancreas of restricted females, the biochemical composition of that gland was similar between treatments. The ovaries of control females had higher lipid content, but the protein content did not differ between treatments. Based on these results, it may be possible to reduce the amount of food offered to the parental stock without affecting their nutritional state. However, this may affect the availability of reserves for subsequent spawns. PICT2012-01333, UBACYT 2011-2014 (20020100100003) y 2014-2017(20020130100186BA) and MINCYT-CAPES BR/11/21.

A20

FASTING HEPATIC RESPONSE IN MALES AND FEMALES OF *Cichlasoma dimerus*.

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The liver is a key organ to study the health and nutritional status of an animal. However, the hepatic histological changes that occur in a fasting situation are not well characterized in fish. The aim of this study was to analyze the effect of fasting on liver histology in C. dimerus. Fish pairs at the same reproductive stage (two days after spawning) were separately maintained for three weeks in small aquaria. During this period, the animals were daily fed with commercial pellets at 1.5% of their body weight or completely unfed. At the end of the three weeks period, morphological parameters, hepatosomatic indeces (HSI) and liver histology were studied. Fasted males and females C. dimerus presented lower HSI (p=0.0003) and hepatocyte area (p=0.0004) compared to fed ones. In adittion, differences were observed in hepatocytes areas between males and females of the same treatment (p=0.04). On the other hand, in unfed fish, an increase in melanomacrophages centers, an indicator of stress was observed. Finally, an increase in apoptotic cells visualized with TUNEL and active caspase-3 immunohistochemistry techniques was observed in unfed fish. In conclusion these results show that fasting induce quantifiable changes in the liver that include, among others, cell apoptosis.

A21
EFFECT OF OLIVE OIL AND PISTACHIO OIL ON THE ESTROUS CYCLE OF THE PROGENY OF DIABETIC MOTHERS.

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Changes in the intrauterine environment during pregnancy can affect fetal development. Non controlled diabetes during the pregnancy is highly correlated with hormonal disturbances in the female offspring. It is known that, the monounsaturated fatty acids inclusion in the diet, may reduce the oxidative stress. Here, the effect of: Corn oil (MZ), extra virgin olive oil (OL) and pistachio oil (PZ) intake during the first two months of life on the estrous cycle, was evaluated on SD rats born from mothers with experimental diabetes (DO; induced with streptozotocin; 30mg/Kg weight i.v.) and control mothers (CO). Dietary supplementation was realized since day 2 to day 62 of life (8µl/15g). Through 21 days, estrous cycle was evaluated at 2, 4 and 10 months of age. At 4 months abnormal cycles were observed in DO-MZ ($p<0.05$) moreover this group showed no cycles at 10 months of age. On the other hand, OL and PZ supplemented animals showed similar cycles to CO-MZ. It is possible, that OL and PZ addition to DO animals diet improves the sequence of estrous cycles, due to a reduction in the hypothalamic-pituitary-gonadal axis aging process, caused by the antioxidant properties of these oils (PICTO/2009-0158 UCCuyo)

A22
EFFECT OF CORTICOSTERONE ON ENERGY BALANCE IN *Passer domesticus*.

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In birds, blood levels of glucocorticoids, mainly corticosterone (CORT), may increase in response to stress. The objective of this study was to determine the effects in energy balance of CORT in non-migrant birds. We assayed both short- and long-term treatments, considering energy substrates in blood and digestive function. To achieve our goal we used the house sparrow (Passer domesticus) as a biological model and performed different doses of CORT solution for treatment groups. We evaluated the physiological status of the organisms under treatment with CORT, measuring blood metabolites, heterophil to lymphocyte (H/L) ratio, body weight, mass of stomach, intestine, heart and liver, and enzymatic activity levels of disaccharidases in enterocytes. We analyzed the data using an ANOVA with Tukey post-hoc test. We found that there was an increase of glucose and triglycerides levels in the short-term treatment and an increase in uric acid and glucose levels in the long-term treatment. CORT treatment decreased body mass, but this loss was no detected in the organ mass, except in the stomach. Finally, an increase of disaccharidase activities in some intestinal portions was observed. In conclusion, CORT modulated metabolic biochemical parameters in the sparrows, but its effect on digestive function was not generalized. Supported by PIP CONICET to JGC, UNSL 0612 to FDC and UNSL 0814 to ECV.

Reproduction

A23
IDENTIFICATION OF CRISP PROTEINS IN THE DOG FOR THEIR POTENTIAL USE IN IMMUNOCONTRACEPTION.

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There is a growing need to reduce the dog population levels as a means to control diseases transmitted to human (zoonosis). Based on previous results from our group supporting epididymal CRISP1 (Cysteine-Rich Secretory Protein 1) as a good immunocontraceptive target, the aim of this work has been the identification of a homologue CRISP protein in the dog that could be used for immocontraceptive development in this species. Using degenerated primers against conserved CRISP motives, we obtained a RT-PCR band of the expected size (aprox. 120 bp) from epididymal but not testicular samples. DNA sequencing of this band showed a high (>80%) homology to CRISP proteins. Western blotting results using antibodies against different CRISP proteins revealed the presence of 25 kDa band in epididymal but not testicular extracts when an anti-mouse CRISP3 antibody was used. As expected for a CRISP molecule, the epididymal band shifted its electrophoretic mobility under reducing conditions. Moreover, the protein precipitates by exposure to a 50% saturated ammonium sulfate solution as previously observed for rodent CRISP1. Altogether, these observations support the identification of a CRISP-like molecule in the dog epididymis, which is at present being further characterized for future purification and functional studies.

A24
LOCAL SPHINGOSINE 1-PHOSPHATE (S1P) ADMINISTRATION AFFECTS FOLLICULAR/LUTEAL DEVELOPMENT AND ENHANCES VASCULAR INTEGRITY IN OVARIAN HYPERSTIMULATION SYNDROME (OHSS).

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OHSS remains the most serious complication of gonadotropin treatment. The main clinical components are marked enlargement of the ovaries, with luteal and hemorrhagic cysts, and an excessive fluid shift. S1P is a lysophospholipid that acts to promote endothelial cell barrier function. Previously, we observed decreased S1P levels in ovarian follicular fluid (FF) from OHSS patients. The aim of this study was to investigate the effects of S1P administration on the follicular and luteal development, cystic structures, peri-endothelial cell area, S1P1/3 receptors, sphingosine lyase (Sly)and kinase (Sk), N-cadherin, nectin-2 and occludin levels in ovaries from an OHSS rat model. Sprague Dawley rats were injected with eCG (10 IU) followed by hCG (10 IU) (Control). The OHSS group was injected with eCG (50 IU / day) for 4 consecutive days and 24 hours later with hCG (25 IU). The group OHSS + S1P received S1P (5ul/ovary; 1mM) under the bursa of one ovary in the day of hCG inyection. The rats were sacrificed 48h post hCG. Ovaries were isolated for histological sections to detect α-actin (cell periendothelial marker) by IHQ and to isolate proteins by western blot. S1P decreased significantly the percentage of corpora lutea and cyst structures compared to OHSS group alone ($p<0.01$and $p<0.05$). No significant changes were observed in the percentage of preantral and early antral follicles. S1P treatment did not affect peri-endothelial area. S1P injection increased N-cadherin and occludin levels whereas nectin-2 levels did not change compared to OHSS group

without treatment. In OHSS group, the levels of Sly and Sk were unaffected by S1P. In addition, S1P increased levels of its own receptor, S1P1, regarding OHSS group alone. In conclusion, our findings indicate that ovarian S1P administration prevents the early onset of OHSS and decreases its severity in rats.

A25
NATURAL COMPOUNDS EVALUATED AS NEW THERAPEUTIC AGENTS FOR THE TREATMENT OF ENDOMETRIOSIS.

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Endometriosis is a disease characterized by the presence of endometrial-like tissue outside the uterine cavity. Current medical therapies available for the treatment of endometriosis have adverse effects that limit their long-term use. The objective of our study was to evaluate the effect of Wogonin (WG), an active constituent of Chinese Herbal Medicine, and two of the main antioxidant compounds found in rosemary extract: Carnosic Acid (CA) and Rosmarinic Acid (RA) in vitro and in vivo, in experimental endometriosis. Cell proliferation was evaluated by MTS assay in endometrial cell cultures. In addition, endometriosis-induced mice received daily WG intragastrically from post-surgical day (psd) 14 and continued until day 26. CA or RA was administered intraperitoneally daily from psd 14 to 28. WG 40, 80 and 160 μM; CA 10, 12.5 and 25 μg/ml and RA 25, 50 and 100 μg/ml significantly inhibited cell proliferation in T-HESC cell line and in human endometrial stromal primary cultures ($p<0.05$ vs. basal). In vivo, WG 20 mg/kg, CA 20 mg/kg and RA 3 mg/kg produced a significant reduction in the lesions size ($p<0.05$, $p<0.01$ and $p<0.05$ for WG, CA and RA respectively). In conclusion, all natural compounds evaluated exerted an inhibitory effect on endometrial growth or endometriosis development. The present findings are promising and support further investigation of these compounds as new therapeutics for endometriosis.

A26
UTERINE FUNCTION IN POLYCYSTIC OVARY SYNDROME.

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Polycystic Ovary Syndrome (PCOS) is a common endocrine system disorder among women of reproductive age. The aim of this study is to evaluate how uterine function is affected by polycystic ovary syndrome (PCOS) development. By using a PCOS murine model, pregnant Sprague Dawley rats were separated in: control (C) and prenatal hyperandrogenized (HA) rats. We evaluated inflammatory and oxidant status and the profile of peroxisome proliferator-activated receptor (PPAR) gamma of uterine tissue from the offspring from C and HA. We study the fertility at the adult stage with and without hormonal induction. Increased levels of Prostaglandin E (PGE) and the protein expression of the limiting enzyme of PGs, COX-2 were observed. These results suggest the existence of a pro-inflammatory uterine environment. No differences were obtained between the antioxidant, glutathione levels and lipid peroxidation index, suggesting an oxidant/antioxidant equilibrium. Increased levels of PPAR were found in HA. The fertility rate decreased in HA. The hormonal induction reversed the fertility rate similar found in the C group. We conclude that hyperandrogenism affects the uterine function in a murine PCOS model thus altering fertility rate.

A27
FIBRONECTIN MODULATES ENDOCANNABINOID SYSTEM IN BOVINE SPERM CAPACITATION.

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Anandamide (AEA), a major endocannabinoid, binds to cannabinoid and vanilloid receptors (CB1, CB2 and TRPV1). In addition, Fibronectin (Fn) is a glycoprotein from extracellular matrix. Both molecules affect many reproductive functions. Previously we found that AEA and Fn regulate bovine sperm capacitation through CB1 and TRPV1. Here we investigated the regulation of the endocannabinoid system during capacitation by Fn in bovine spermatozoa (SPZ). To perform sperm capacitation, SPZ were incubated in spTALP and Fn during 45 min. Sperm capacitation was evaluated by CTC and LPC-induced acrosome reaction. Fn induced changes in the location of sperm TRPV1. Also, FAAH (enzyme that hydrolyses AEA) activity was measured by radioimmunoassay. FAAH activity was modulated in SPZ incubated with Fn. To investigate nitric oxide (NO) production, SPZ were incubated with Fn and capsazepin (a TRPV1 antagonist) and DAF-FM probe (fluoresce in presence of NO). The fluorescent complex was measured by flow cytometry. Fn increased sperm NO levels and the preincubation with capsazepin reversed Fn induction. Finally, Fn up-regulated PKA substrates phosphorylation such as AEA.
The results suggest that Fn, through endocannabinoid system activation, induces events associated to sperm capacitation in bovines.

A28
Src TYROSINE KINASE LINKS PKA ACTIVATION WITH HYPERPOLARIZATION ASSOCIATED TO MOUSE SPERM CAPACITATION.

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Mammalian sperm must undergo *capacitation* before being able to fertilize, involving hyperactivation and the potential to suffer the acrosome reaction (AR). Capacitation entails ser/thr and tyr phosphorylation events and plasma membrane hyperpolarization (Em) among other processes. Previous studies showed that sperm from Src KO mouse undergo normal ser/thr and tyr phosphorylation, albeit they are infertile. The aim of this research is to gain insight into the role of Src tyr kinase in mouse sperm capacitation. Pharmacological inhibition of Src impaired the acrosome reaction (AR), without affecting sert/thr and tyr phosphorylation. Since sperm membrane hyperpolarization is both necessary and sufficient to prepare the sperm for the AR, we evaluated the role of Src in Em. Using the carbocyanine DiSC(3)5, we found a time-dependant hyperpolarization during capacitation becoming maximal and steady after 30 min. Moreover, inhibition of Src blocked hyperpolarization. It is known in other cell types that Src can be directly phosphorylated by PKA at Ser17, as well as auto-phosphorylated at tyr416. Our western blot analysis showed that whereas pSer17-Src is observed at 5 min after capacitation starts, pTyr416-Src begins after 15 min. These data are consistent with a role of Src upstream the cascade of events leading first to hyperpolarization and ultimately to the preparation to undergo the AR.

POSTER SESSIONS

Animal Biology I

A29
ROLE OF SUPRASPINOUS AND INTERSPINOUS LIGAMENTS IN THE RESTRICTION OF FLEXION MOVEMENTS OF THE CAUDAL EQUINE THORACIC SPINE.

Arzone CA, Ferraro J, Castro Molina JM, Genoud P, Rapela F, Naccarato H, Vega M, Vidal Figueredo R.

In order to analyze the role of the fibroelastic elements thoracic, we dissection the structures osteoligamentosas of nine vertebral spine, from adult horses of either sex, free of significant pathology in the tissues of interest columns was performed. The study focused on the interspinous space. Supraspinatus ligaments and interspinous were analyzed. Structure was observed in both macroscopic and appearance with 20x loupe. The supraspinatus ligament has a regular structure along the tour of the region, while the interspinous ligament anatomy shows a differential space from T10. From the latter and were readily detected in the caudal two distinct portions; ventral, thin sheet-like dorsal and one thicker, with stronger fibrillar appearance with their clearly arranged fiber bundles. Based on the observed gross anatomy of the ligament can be concluded for the presence of a change in the forces and supporting ligaments addresses, clearly related to space T10.

A30

POST-EMBRYONIC DEVELOPMENT OF *Boeckella poopoensis* MARSH, 1906 (CRUSTACEA, COPEPODA) AT TWO DIFFERENT TEMPERATURES.

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Food and temperature are the main factors control the development time of copepods. Because temperature is more important in temporary and shallow environments, where these organisms are common, the aim of this study was to determine the effect of this parameter on the post-embryonic development of *Boeckella poopoensis*, an exclusive species in the crustacean assemblage of saline lakes of South America. Life-cycle bioassays were conducted at two temperatures (15 and 22°C). Larvae were obtained from ovigerous females isolated from a monospecific culture acclimated in the laboratory. One nauplius I larva (10 replicates) was placed in each bottle (20 mL), with adequate medium and food, and observed every 24 hours until the adult stage. Both treatments differed (H=13.79; p=0.0002). In treatments at 15°C, the naupliar stage had a mean duration of 8.1 (±0.3) days and the copepodite stage 12.7 (±2.3) days, whereas in treatments at 22°C, the naupliar stage had a mean of 5.4 (±0.5) days and the copepodite stage 7.8 (±1.2) days. This means that to become adults at 15°C took almost twice as long than at 22°C. These results indicate that the increase in temperature would favor *B. poopoensis* to reach sexual maturity in a shorter period of time.

A31

INFLUENCE OF SALINITY ON THE LIFE CYCLE OF *Boeckella poopoensis* Marsh, 1906 (CRUSTACEA, COPEPODA).

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Because salinity is one of the environmental factors that directly influence the development of copepods, the aim of this study was to determine the effect of this parameter on the life cycle of *Boeckella poopoensis*, a frequent microcrustacean in neotropical saline environments. Bioassays were conducted to determine the post-embryonic development of this species at five salinities (5, 10, 20, 30 and 35 g/L). Larvae were obtained from ovigerous females isolated from a monospecific culture acclimated in the laboratory. One nauplius I larva (10 replicates) was placed in each bottle (20 mL), with adequate medium and food, and observed every 24 hours until the adult stage. Treatments with 5, 10 and 20 g/L were similar but differed from those with 30 and 35 g/L (H=25.65; p=0.0000). In the three lower salinities, the naupliar stage had a mean duration of 5.4 (± 0.3) days and the copepodite stage 7.7 (± 0.1) days. In treatments with 30 and 35 g/L, specimens needed 8 (± 1) days to reach the copepodite stage I and 23 (± 11.3) days to become adults. At higher salinities, it took more than twice as long to complete development, suggesting that the increase in salinity causes a delay in the sexual maturity of *B. poopoensis*.

A32

CHARACTERIZATION OF EMBRYONIC REPRODUCTIVE TRACT IN *Caiman latirostris.*

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Caiman latirostris exhibits temperature-dependent sex determination (TSD). Male-to-female sex reversal after in ovo estrogen/xenoestrogen exposure was demonstrated. In stage 22 of embryonic development (at the beginning of the gonadal sex differentiation), we characterized the primitive reproductive tract of embryos obtained from eggs incubated at temperature producing males (TPM) or females (TPF) or at TPM+E_2 (a dose of 17alpha-estradiol -E_2- that overrides the temperature effect was administered at stage 20). Estrogen receptor alpha (ERa) and proliferative activity were assessed by IHC and the ductal epithelium height (DEH) was determined in trichromic stained sections. TPM and TPM+ E_2 groups showed higher percentage of proliferating cells both in the primitive gonad as in the duct, compared to TPF. Moreover, ERa protein trended to a higher expression in TPM. The DEH was not different between all studied embryos. Our results demonstrate that caiman embryonic gonad and duct are highly sensitive not only to temperature effect but E_2 action allowing us to study the effect of xenoestrogen exposure on embryonic reproductive tract.

A33

INFLUENCE OF SALINITY ON THE BIOLOGY OF *Moina eugeniae* OLIVIER, 1954 (CRUSTACEA, CLADOCERA).

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Moina eugeniae is the most common endemic cladoceran in the zooplankton of saline lakes in Argentina. The aim of this study was to determine the influence of salinity on its biology. Treatments were performed with 7; 17 and 27 g/L of natural

sterilized salts. Neonates were placed in containers of 25 mL (one per flask). Every 48 hours, until its death, the medium was renewed, were fed with *Chlorella vulgaris* and the molts were measured. Survival differed (H=12.4; p=0.0021), with minimum of 18.14 ±3.5 days (7 g/L) and maximum of 29 ±2.9 days (17 g/L). The females size at first litter differed (H=23.42; p=0.0000) and varied between 0.92 ±0.09 mm and 1.19 ±0.21mm with 17 and 27 g/L. The maximum size also differed (H=20.15; p=0.0000), were larger in 17 g/L (1.97 ±0.13 mm) and smaller in 7 and 27 g/L (1.57 ±0.15 and 1.60 ±0.15). The number of litters and total offspring per female were different (H=15.48; p=0.0005 and H=20.7; p=0.0000): 3.9 (±1.3) litters and 46.3 (±11.3) neonates con 7 g/L and 8 (±1.07) litters and 135 (±13.14) neonates with 17 g/L. The higher survival, size and number of litters and offspring produced in 17 g/L, are indicative of the halophilic character of this species, although the lower values registered with the highest concentration indicate a certain stress.

A34
MORPHOMETRICS DIFFERENCES BETWEEN SEXES ON MOCKINGBIRDS (*Mimus* sp).

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Mockingbirds are monomorphic. It would be some difference in parental time spent feeding where males occupy more time in feeding chicks. The aim of this work was to search differences in morphometric measurements between sexes in three

species of this genus. Morphometric variables measured were culmen, bill height, width and length from gape, tarsus, middle toe length, tail and wing chord of: 152 adults of both sexes of *M. triurus*, 95 of *M. patagonicus* and 45 of *M. thenca* obtained from the following collections: Museo de La Plata, F. M. Lillo, Museo Argentino de Ciencias Naturales and Museo Nacional de Historia Natural (Montevideo). Comparisons of morphometric data between sexes were done using a one factor analysis of variance with sex as factor, and if Normality failed a non parametric test was used. Male mockingbirds were bigger than females in most measurements taken, except in bill width for *M. triurus* and culmen and length from gape in *M. thenca*. Significant differences between males and females were found for wing chord and tail in *M. triurus* and *M. patagonicus* and middle toe length in *M. triurus*. Our analysis failed to show significant differences between sexes on *M. thenca* measurements. Differences found on wing size might be related to behavior during courtship. Since males feed chicks, bigger wings might be related to wing-flashing behavior.

A35
EFFECT OF 100 % GLUTEN FEED DIET ON RUMINAL PARAMETERS.

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Gluten feed (GF) is an important protein-energy feed for cattle; however, the usually high sulfur (S) concentration can be detrimental to animal health due to ruminal hydrogen sulfide (H_2S) production. Liquid-gas equilibrium of ruminal H_2S is directly related to the pH. The objective was to evaluate the levels of H_2S on cattle fed with 100 % GF (Crude Protein: 23.5%, S: 0.45%). Three ruminally fistulated (1.5 cm ϴ) heifers were used. After an adaptation period of 20 days to 100 % GF diet, a micro probe was introduce to the rumen gas cap to measure H_2S and NH_3 with a portable gas detector (Eagle 2). Subsequently 20 ml of rumen fluid were extracted to, immediately measure pH, with a digital potentiometer (Denver UP-10). These measurement were done during 10 days (n=30) always 6 hours after morning feeding. Results indicate that the rumen pH was 6.08 ±0.03, H_2S concentration was of 96.55 ±3.4 ppm and NH_3 was 8.45 ±1.59 ppm. High deamination of GF protein is responsible for the high NH_3concentration and a slightly basic rumen pH keeping H_2S in solution in the rumen liquor instead of being eliminated as a gas. This condition indicates that H_2S in solution could react with bivalent metals inducing mineral deficiencies (Cu, Zn, and Fe) or with a more acidic condition of the rumen liquor there could be increased H_2S eructation with risk of inducing poisoning.

A36
LXR INCREASE GnRH EXPRESSION IN THE RAT HYPOTHALAMUS *IN VIVO*.

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Previously we described the expression of LXR receptors in the hypothalamus (HT) and its relation with the metabolism. We evaluated the action of LXR activation on the expression of factors produced in the HT in vitro and in vivo by Western Blot. We studied the expression of the leptin peptide, LHRH and GHRH (as they present LXRE sequences in their promoter region), the αMSH and NPY in male Sprague-Dawley rats. As a positive control, the cholesterol transporter ABCA1 expression was also measured. For *in vitro* assays, hypothalamic explants were exposed to two synthetic LXR agonists T0901317 (T0) or GW3965 (GW) for 2, 4, 6 and 8 h (10 uM). For *in vivo* tests, the animals were injected into the

third ventricle with either T0 or GW (1.25-2.5µg / 5 ul ICV) and after 3.5 h the HT were taken and processed. Results: No changes were observed in the expression of the factors evaluated *in vitro*. The HT explants were viable (measured by LDH) and responded to the treatment with an increased ABCA1 expression. In contrast, T0 and GW significantly increase the expression of GnRH *in vivo*. These results indicate that acute LXR activation in HT affects at least the expression of GnRH *in vivo*.

A37
BROOD PRODUCTION IN THE "RED CHERRY" SHRIMP (DECAPODA, CARIDEA).

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Neocaridina heteropoda heteropoda, also known as "red cherry" shrimp, is a promising ornamental decapod species. However, many reproductive attributes are unknown. This study evaluated brood production after the onset of female sexual maturity. Females and males were maintained at a ratio of 1:2 in plastic containers (n=19) with continuous aeration and Java moss at 27°C, during 135 days. They were daily fed with balanced food for tropical fish. Once detected, the ovigerous females were visually inspected once a day to determine the hatching date and calculate the duration of the incubation period. More than 70% of the females spawned at least three times, with an inter-spawning period averaging one month, and the eggs of 90% of the spawns were successfully hatched. The mean incubation period and number of hatched juveniles (actual fecundity) were similar in consecutive spawns, reaching mean values of 15 days and 30 juveniles *per* female and *per* spawn, respectively. Based on present results, it is concluded that female senescence may have no effect on brood production for at least the three spawning events following the onset of sexual maturity. PICT2012-01333, UBACYT2011-2014 (20020100100003) y 2014-2017(20020130100186BA) y MINCYT-CAPES BR/11/21.

A38
K^+-INDEPENDENT OUABAIN-INSENSITIVE Na^+-ATPase ACTIVITY IN HEPATOPANCREAS OF *Neohelice granulata.*

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A K^+-independent ouabain–insensitive Na^+ATPase (NA), with distinct properties, occur in several mammals tissues. In comparison information in invertebrates are scarce. We have shown the occurrence of K^+-independent, ouabain-insensitive, furosemide-sensitive Na^+-ATPase activity (NA) in muscle of the euryhaline crab *N. granulata*. Studies on NA in hepatopancreas (HP) of euryhaline crabs are lacking. The aim of this work was to study the occurrence and biochemical characteristics of NA in HP. Adult male crabs were maintained for at least 10 days at 35‰ salinity. NA was determined in HP homogenates (0.25M Sucrose/0.5mM EGTA-Tris pH 7.4). NA (µmol Pix min^{-1}x $mgprot^{-1}$) was assayed by hydrolysis of ATP in a reaction mixture containing (mM): 10 MgCl2, 0.5 EGTA, 1 ouabain, 1 Na_3N, in 20 imidazole buffer, pH 7.4, without or with of 2mM furosemide, 30ºC (Na+ curve: 0-150 mM; ATP 15 mM) (ATP curve: 0.5-50; 100 mM NaCl) (time curve: 10-30 min; 100 mM NaCl, 15 mM ATP) (furosemide curve: 0.1-2 mM; 100 mM NaCl, 15 mM ATP). NA was estimated as the difference between assays with or without 2mM furosemide. Maximal activities occurred at NaCl 100 mM, 15 mM ATP and for 20 minutes of incubation. Maximal inhibition of NA was found at 2 mM. The results show the occurrence for the first time, of NA in HP of an euryhaline crab.

A39
EFFECT OF DOPAMINE ON OUABAIN-INSENSITIVE Na^+-ATPase ACTIVITY IN MUSCLE OF *Neohelice granulata.*

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We have shown the occurrence and salinity sensibility of K^+-independent, ouabain-insensitive, furosemide-sensitive Na^+-ATPase activity (NA) in muscle of the euryhaline crab *N. granulata*. The possible regulation of this enzyme by biogenic amines (i.e. dopamine, DA) is unknown. Aim: to study the *in vitro* effect of DA on NA in muscle. Adult male crabs were maintained for 30d at 35‰ salinity. Muscle slices were incubated in the absence and in the presence of DA (10^{-5}-10^{-3}M) or DA10^{-3}M+SCH23390 (D_1 antagonist) 10^{-4}M (100mg/2ml medium mM: 400 NaCl, 13 KCl, 10 $MgCl_2$ 8.8 H_3BO_3, pH 7.6, 30ºC). NA was determined, after 0-90min of incubation, in homogenates of tissue slices (0.25M sucrose/0.5mM EGTA-Tris, pH 7.4 8ml/g). NA was measured by ATP hydrolysis (3mM, A_{700}nm) in a reaction medium (mM: 130 NaCl, 10 $MgCl_2$, 0.5 EGTA, 1 ouabain, 1Na_3N, in 20 imidazole buffer, pH 7.4), without or with of 2mM furosemide. NA was estimated as the difference between the two assays. NA was lower after 45min with DA10^{-3}M (Control:119±17, DA:54±19nmolPi/min/mg prot) (n=10, paired t-test, $p<0.05$). This effect was not blocked by SCH23390 (n=5, ANOVA, $p<0.05$). The result shows the direct effect of DA on muscle to modulate NA, apparently via other receptor than D1.

A40

LXR EXPRESSION CHANGES IN LIVER (L) AND HYPHOTALAMUS (HT) BY A HIGH FAT DIET.

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In previous assays, we evaluated the effect of different high fat diets on liver X receptors (LXR), involved in reverse cholesterol transport, in rat L and HT. In this work, the effect of a diet, with the addition of bovine fat (38%) + cholesterol (2%) + cholic acid (0.2%; FCC), and its subsequent return to normal diet (C) were analyzed. Male rats of 50 days of age, were fed with FCC diet for 10 days, and then turned to C diet the following 7 days. Assays were performed at 8, 10, 12 and 17 days. By colorimetric methods, changes respect control animals (fed only with C) were observed in serum cholesterol levels (228%, 209%; 63%; 45%) and triglycerides (8d=56%; 10d=85%; $p<0.05$). With WB, LXRα significant increases were observed in L at 10d=6% and HT at 12d= 4% ($p<0.05$). In the other hand, LXRβ increases were observed in both tissues (6-15% and 17-45%; $p<0.05$) in all days studied. Relation between subtypes (α and β) expression, could be directly involved in cholesterol regulation mechanisms in both tissues (CONICET-PIP860).

A41

SALINITY EFFECTS ON SURVIVAL AND FECUNDITY OF *Daphnia menucoensis* PAGGI, 1996 (CRUSTACEA, CLADOCERA).

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Daphnia menucoensis is an important species in saline lakes in the center of Argentina, since by its high rate of feeding modifies the ecosystems that inhabits. The aim of this work was to determine the influence of the salinity on some aspects of its biology. We performed treatments with 7; 12 y 17 g/L of sterilized natural salts. Neonates were placed in containers of 25 mL (one per flask). Every 48 hours, until its death, the medium was renewed, were fed with *Chlorella vulgaris* and the molts were measured. Survival and number of molts differed ($H=9.88$; $p=0.0075$ and $H=12.08$; $p=0.0025$), exceeded 32 days and 11 molts in the treatments with 7 g/L, but they were around 20 days and 7-8 molts in 12 y 17 g/L. The size at first litter and at death differed between treatments ($H=17.71$; $p=0.0001$ and $H=15.65$; $p=0.0004$), were larger (2.56 mm ±0.12 and 3.01 mm ±0.33 respectively) with 7 g/L, but were around 2.3-2.37 and 2.71-2.44 mm with 12 and 17 g/L. The number of litters and total offspring per female were different ($H=6.97$; $p=0.0369$ and $H=7.09$; $p=0.0295$), reached 3.5 (±2.14) and 3.07 (±2.22) litters and 22.86 (±15.51) and 17.07 (±14.09) offspring with 7 and 12 g/L, but declined to 1.18 (±0.87) litters and 6.45 (±4.3) offspring with 17 g/L. Despite being a relatively halotolerant species, we found that the stress generated by increased salinity affects survival, sizes spectrum and fertility.

Cellular and Molecular Biology I

A42

***Calendula officinalis* L. AS INHIBITOR OF LIPID PEROXIDATION OF BIOLOGICAL MEMBRANES.**

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Carotenoids act as antioxidants preventing many diseases and the importance of those not related with dietary vitamin A, in particular lutein, zeaxanthin and lycopene, has been emphasized. The extract from flowers of "marigold" (*Calendula officinalis* L., CO) contains lutein as the principal carotenoid. In this assay the *in vitro* antioxidant effect of an aqueous extract of CO on non enzymatic lipid peroxidation of microsomes and mitochondria from liver of AH/HOK Wistar rats, was studied. Microsomes and mitochondria membranes were incubated separately in an ascorbate-Fe^{+2} dependent system during 180 min at 37°C with increasing concentrations of CO total extract (50, 100, 200 and 400 µg). It was observed that the total cpm/mg of protein originated from chemiluminescence was lower in those samples with CO extract. The inhibition of lipid peroxidation by means of chemiluminescence as a lipid oxidative damage index, was dependant on the concentration of CO extract for both microsomes and mitochondria membranes. These results confirm previous observations indicating that the extracts of CO may act as antioxidants, protecting membranes from the oxidative stress. Such effects encourage the use of CO extracts in the formulation of alternative remedies (e.g. creams) in Veterinary Medicine.

A43

THE ROLE OF ME31B IN *Drosophila melanogaster* GAMETOGENESIS MEDIATED BY EIF4E.

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Development of a whole organism implies a global regulation of gene expression at several levels. At the translational level in eukaryotes, initiation is regulated by proteins which bind the eukaryotic initiation factor 4E (eIF4E). *Drosophila melanogaster* (Dm) has eight eIF4E cognates, but their specific biological role in gene regulation have not been addressed except of eIF4E1 and eIF4E8 (4E-HP). We have showed that Dm eIF4E-3 is a testis-specific protein implicated in the translation initiation control exclusively during male germline development. eIF4E-3 is required for meiotic chromosome segregation and cytokinesis, nuclear shaping and sperm individualization. The rck/p54 homolog in Dm (Me31B) is a RNA helicase which is required for mRNA silencing in cytoplasmic processing bodies. We have observed *in vitro* interaction between eIF4E3 and Me31B by yeast two hybrid assay and FRET en S2 cells. Here we show the co-localization of Me31B and eIF4E1 in Dm oocytes and the localization of Me31B and eIF4E3 in Dm testis exhibiting a similar pattern. These results suggest that Me31B exerts gene regulation mediated by eIF4E3 and eIF4E1 in Dm gametogenesis.

A44

POPULATION DIVERSITY OF THE COMT GENE IN RESISTENCIA, CHACO.

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The catechol-O-methyl transferase (COMT) metabolizes the catecholamines, controlling dopaminergic and adrenergic neurotransmission. The gene coding this enzyme presents several polymorphisms which participate in diverse metabolic phenotypes. The aim of this work was to describe the sequence variation of COMT gene in the population of Chaco province, Argentine. We analyzed 4 SNPs in DNA samples from 75 individuals from the capital city, Resistencia, through PCR-RFLP for rs740603, rs6269, rs4680, and allele-specific PCR for rs4818. The average nucleotide diversity was 45.77% +/-31.4, with heterozygosity values between 44.61% and 61.73%. The markers fit Hardy-Weinberg equilibrium (exact test of sample differentiation), and pairwise linkage disequilibrium was only found between rs6269 and rs4818 (chi square test). We compared these results with previous data from Buenos Aires province, and no significant differences were found (exact test, p=0.0313, 4 d.f.). Although heterozygosity values were not significantly different, they were higher among Chaco population than among Buenos Aires people, suggesting a possible contribution of Native variation to the genetic composition of Resistencia population. This is in accordance with previous data on neutral markers from nuclear genome.

A45

EXPRESSION OF THE AUTOANTIGEN INSULINOMA-ASSOCIATED TYROSINE PHOSPHATASE 2 (IA-2) FUSED TO THIOREDOXIN IN *E. coli.*

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IA-2, a transmembrane protein from pancreatric β cells, is an antigen in autoimmune diabetes and autoantibodies to it are early markers of the disease. The aim of this study was to express and recover properly folded IA-2 from *Escherichia coli*. The sequence coding for the intracellular domain of IA-2 ($IA\text{-}2_{ic}$) was cloned into pTrxFus vector to obtain it fused to thioredoxin ($TrxIA\text{-}2_{ic}$). *E. coli* GI724 and GI698 were transformed with $pTrxIA\text{-}2_{ic}$, cultured at 30°C and protein expression was induced with Trp 100 µg/mL at 37°C or 20°C, respectively. After induction at different times, cells lysates were obtained and TrxIA-2ic was purified by affinity chromatography from the intracellular soluble fraction. SDS-PAGE and Western Blot analysis revealed a band compatible with $TrxIA\text{-}2_{ic}$ expected molecular weight (~55.4 kDa) from both bacteria strains. Higher expression was achieved in E. coli GI724, yielding 0.72 mg of >99% pure $TrxIA\text{-}2_{ic}$/L culture. The immunochemical behaviour of $TrxIA\text{-}2_{ic}$ was assessed qualitatively by incubating 30 IA-2A(+) patients sera with [^{35}S]IA-2 (obtained by rabbit reticulocyte lysate system) in the presence of 0.2 µM TrxIA-2ic. All sera became virtually negative under antigen excess (mean Standard Deviation scores changed from 17.07 to 0.34). It was possible to obtain purified IA-2 in *E. coli* as a fusion protein. The integrity of epitopes involved in the interaction with antibodies was confirmed.

A46

BORON RELEASED FROM A BIOACTIVE GLASS MODULATES TUBULOGENESIS *IN VITRO* BY ACTIVATING p38 KINASE PROTEIN.

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As it has been established that B may perform functions in angiogenesis, the controlled and localized release of B ions from bioactive glasses (BGs) is expected to provide a promising therapeutic alternative for the regenerative medicine of

vascularized tissues, e.g. bone. We have previously shown that the B released from a BG in the SiO_2-CaO-Na_2O-P_2O_5 (45S5) system doped with 2 wt% B_2O_3 (45S5.2B) enhances *in vitro* tubulogenesis on matrigel™ and ERK1/2 and p38 phosphorylation levels. The aim of this study was to assess whether the B released from 45S5.2B could modulate *in vitro* tubulogenesis by activating ERK1/2 and/or p38 kinases. To this end, 10,000 HUVECs per well were seeded onto the surface of matrigel™ in M199 medium conditioned with U0126 (ERK1/2 inhibitor) or SB203580 (p38 inhibitor) 10 µM. After 1 h stimulation with the inhibitors, M199 was enriched with ionic dissolution products (IDPs) from 45S5.2B or B 55 µM. Non-enriched M199 and M199 with VEGF were used as controls. After 6 h, pictures were taken to quantify the number of completely closed 'tubules'. The results showed a significant decrease (*$p<0.05$) in the number of 'tubules' formed on matrigel™ when HUVECs were stimulated with IDPs from 45S5.2B or B 55 µM only in the presence of SB203580. We concluded that the B released from the bioactive glass 45S5.2B modulates *in vitro* tubulogenesis by activating p38 kinase protein but not ERK1/2 kinase protein.

Ecology, Toxicology and Behaviour I

A47
MILK LIPID COMPOSITION IS MODIFIED BY PERINATAL EXPOSURE TO BISPHENOL A.

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Previously, we demonstrated that perinatal exposure to Bisphenol A (BPA) modifies milk protein composition in lactating rats. Here, the effects of perinatal exposure to BPA on milk lipid composition were analyzed during lactation. Pregnant rats were orally exposed to 0, 0.6 or 52 µg BPA/kg/day from gestation day 9 until weaning. After puberty, F1 females were bred and, on lactation day 2 (LD2) and LD10 mammary glands were obtained. On LD10, milk samples were collected, fatty acid (FA) profile and lipid composition were established. Cryostat sections were stained with Oil Red O to quantify the area of secreted milk fat globules (MFGs) within the alveolar lumen. On LD2, MFGs in samples from both BPA groups were smaller than in control animals, whereas no changes in the growth curves were observed in BPA F2 pups. On LD10, MFGs area remained smaller in BPA0.6 F1 dams than in controls but it was significantly increased in BPA52 F1 dams. Milk lipid content and FA profile of BPA-exposed animals were associated with MFGs changes. BPA0.6 F1 dams had lower whereas BPA52 F1 dams had higher concentration of lipids than control rats. Besides, body weight gain in BPA52 F2 pups was higher than in the other pups. Our results demonstrate that milk quality, evaluated by protein and lipid content is affected by the perinatal exposure to BPA, compromising the normal growth of the offspring.

A48
MUSCULAR LESIONS IN MICE INTOXICATED WITH *Cassia occidentalis* (“cafetillo”) FROM ARGENTINA.

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Many plants have compounds capable of causing toxicity when eaten by animals. *Cassia occidentalis* (CO) is widespread around the world and is responsible of both human and animal intoxications. The toxicity of the extracts of seeds and pods of CO was investigated by oral administration to mice during 7 days. CFI mice, 20 ± 2 g, were divided into 3 groups. First group received seed extract, second received a combination of seed + pod extract, and a third group only received water (controls). At the end of the trial animals were euthanized and samples from muscular tissue were taken for both optic microscopy (OM) as well as scanning electron microscopy (SEM). OM revealed multiple foci of coagulative necrosis with neutrophilic infiltrate particulary in those animals that received seed + pod extract. SEM was in concordance with these findings. The joint action of toxic compounds from seeds and pods of CO is emphasized. These results are compatible with severe toxic miopathy associated with the consumption of the extract of de *Cassia occidentalis*. Such lesions were similar to those found by other authors regarding the intoxication with seeds of “cafetillo” in other species with severe toxic myopathy.

A49
NEREIDID POLYCHAETES AS MODEL ORGANISMS FOR DISRUPTION STUDIES BY XENOESTROGENS.

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Nereidid polychaetes are marine and estuarine worms with ancestral features of bilaterian and are considered living fossils. Recently, several evidences on development and reproduction demonstrated the presence of estrogenic signalling, enabling the use of these organisms as model organisms for endocrine disruption effects. Estrogens (estradiol E2 and estrone) were observed in coelomic fluids by GC-MS of *Alitta succinea*, and the estrogen receptor has been completely sequenced in nereidids with ligand activation. Vitellogenin synthesis increased in primary culture of female eleocytes upon exposure to E2, ethynylestradiol, EE2; nonylphenol, NP and bisphenol A. Larvae of *Platynereis dumerilii* exposed to E2 and xenoestrogens showed changes in growth and primordial germ cell (PGC) proliferation. Supernumerary PGCs were observed in larvae exposed to E2 resembling proliferative induction of PCGs in vertebrates. Chronic exposure to NP during the life cycle affected the normal male maturation, while female maturation was not affected. Impairment of male maturation occurred although no feminization processes were observed. Specific enzymatic detoxification machinery in mature females maybe involved in the tolerance to xenoestrogens. The accumulation of results on estrogenic signaling and effects, allow us to propose the use of these organisms in ecotoxicological impacts of EDCs in estuaries and coastal areas.

A50
HISTOPHATOLOGICAL STUDY OF MUSCLE REGENERATION AFTER SNAKE VENOM ENVENOMATION.

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Bothropic snake venom is characterized by a severe local myotoxicity affecting skeletal muscle. Regeneration in mice was evaluated after *Bothrops alternatus* venom injection at different doses (50 - 100 µg) during a month. Samples were processed and stained for evaluation under light microscope. Histopathological studies showed regenerated fibers at all doses tested in spite of intense hemorrhage and necrosis caused by the venom. As regards connective tissue, it was evident in those animals which received the higher doses (70 and 100 µg), respectively. Regeneration was significant, only a small degree of granulation tissue was observed, and a group of normal fibers had survived the intoxication when 50 µg of crude venom was injected. We can assume a dose-dependent myogenesis since a large number of regenerative fibers were observed in animals inoculated with the lowest dose. Regenerative ability of the damaged muscle was evident since fibers had centralized nuclei, normal staining and size proportional to the period studied. These results present the importance of local treatments supplementary to serotherapy in order to assist muscle regeneration in case of severe *B. alternatus* envenoming.

A51
PRESENCE OF PARABENS IN THE MARACANÃ RIVER (BRAZIL) AND ITS TOXICITY.

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The parabens are esters of the 4-hydroxybenzoic acid and work as preservatives. Besides, they are widely used in the pharmaceutical industry, in cosmetics and in food. Routledge et al. (1996) was the first to describe the estrogenic activity of parabens from *in vitro* assays and raised the issue of its safety and toxicity of the same. This study focus on evaluating the presence of metil_ (MP) and propylparaben (PP) in the aquatic environment. It also observes its estrogenic potencial and ecotoxicity. The estrogenic activity was determined by the *in vitro* assay YES, the acute toxicity tests were conduted with the test organisms *Daphnia similis* and *Aliivibrio fischeri* and the quantification was determined in the water of the Maracanã river (Brazil) by technique HPLC/MS. The magnitude of the response was 10^5 times less powerful than 17ß-estradiol for the MP and 10^4 for the PP in the YES assay. The EC50 values obtained for *Daphnia similis* were 29.42 mgL^{-1} and 9.94 mgL^{-1}, and for *Aliivibrio fischeri* were 3.047 mgL^{-1} and 1.946 mgL^{-1}, respectively. Therefore it was observed that PP is more toxic in all tested organisms. In the Maracanã river, concentrations were quantified as 1496 ngL^{-1} for the PP and 1426 ngL^{-1} for the MP. Then was established the estrogenic profile of pure substance for detecting synergistic effect in the aquatic environment.

A52
ESTROGENIC ACTIVITY IN SEDIMENTS FROM THE SANTA LUCÍA RIVER BASIN (URUGUAY).

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Knowledge about Endocrine disruptors (EDCs) effects on wildlife and humans is still incomplete, highlighting the need to generate new detection tools. The Yeast Estrogen Screen (YES) is an *in vitro* assay that provides information of the total estrogenicity of a sample. It has the advantage of allowing the analysis of a large number of samples, being useful as a monitoring tool in the aquatic environment. In the Santa Lucia River Basin there are different land uses that lead to the potential presence of a wide range of toxic substances. The aim of this work is to quantify the sediment estrogenicity of the Santa Lucia River through the YES assay and analyze its variation for different parts of the basin. A screening was carried out using 45 sample sites covering the entire watershed. Four surface sediment samples were taken at each point to make the YES assay and four water samples for analysis of nutrients (nitrogen, phosphorus and ammonium). Preliminary results of sediment estrogenicity will be presented. Results show that points that were closer from cities, showed higher concentrations of phosphorus. Our hypothesis is that these sites also will present the highest values of estrogenicity, because they release large amounts of substances with estrogenic potential.

A53
SUCROSE CONSUMPTION IN EARLY STAGES OF LIFE AFFECTS FEAR RELATED BEHAVIOR IN ADULTHOOD.

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Sugar consumption has increased dramatically in our society, a phenomenon that is primarily associated with increased obesity and diabetes in the population. However, whether this overconsumption has an impact on the developing CNS remains unknown. Here we studied the effects of unlimited access to sucrose (sac) in early stages of development on fear conditioning (FC) behavior in adulthood. Methods and Results: 25-50 days old (PD) rats had free access to 10% sucrose in water and water. The control group had access only to water. Rats in group sac, privileged to take this drink over the water, consuming a larger volume (+33%, $p<0.0001$). Weight differences were only observed at PD 33-40 (-30% sac vs control, $p<0.005$). After treatment all animals drank only water for another 25 days and they were tested for FC. The animals were conditioned by 5 cycles of a neutral tone (10s, 1500Hz, 85dB) combined with a foot shock (1s, 0.4mA) 20s after the end of the tone. 24 hours later, the tones were presented without shocks (15 trials, 60s intervals). The test was video-recorded and the freezing time per trial/animal was measured. The group sac presented greater freezing time compared to control ($p<0.05$) indicating a behavior disorder related to the limbic system.

A54
ISOLATION AND CYTOTOXIC ACTIVITY OF A BASIC PHOSPHOLIPASE A2 FROM *Bothrops diporus* VENOM.

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The majority of snakebites in the north-east region of Argentina are caused by *Bothrops diporus* (yarará chica). Its venom induces a complex series of local and systemic effects induced by a variety of venom components, such as phospholipases A_2 (PLA_2), thrombin-like enzymes, metalloproteinases, among others. In this work, a basic PLA_2 was isolated and its myotoxic activity *in vitro* was studied. Purification was made by a two-step procedure utilizing ion exchange and gel filtration chromatography. SDS-PAGE of the isolated enzyme showed a single typical band of ~14 kDa and PLA_2 activity was evidenced by the formation of hemolytic halos in agarose-erythrocyte egg yolk gels. Cytotoxic activity was determined on a target cell line, C2C12 (ATCC CRL-1772™). Briefly, myoblast cells were exposed to different amounts of PLA_2 (5-100 μg/ml) for 3 h, toxicity was quantitatively assayed by crystal violet method. The percentage of adherent cells in the monolayer culture was registered and its CC_{50} was calculated. Cytolisis was determined by release of the cytosolic enzyme lactate dehydrogenase (LDH). Results indicate that the isolated enzyme PLA_2 induces a dose-dependent detachment of cells, CC_{50}= 20.18μg/mL, with no evidence of membrane damage since no increase of LDH was detected. Thus, considering the CC_{50}=12.17 μg/mL previously determined for the whole venom, this toxin contributes substantially to the myotoxic activity.

A55

EFFECTS OF GLYPHOSATE-BASED HERBICIDE EXPOSURE ON REPRODUCTION AND DEVELOPMENT OF *Odontesthes bonariensis.*

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Water pollution by agrochemicals is currently one of the most critical problems for the conservation of aquatic ecosystems. Glyphosate [N-(phosphonomethyl) glycine; PMG] is the main broad-spectrum herbicide used for the control of a wide range of weeds in soybean crops. The aim of this study was to analize the impact of glyphosate-based herbicide on sperm motility and embryonic-larval development of freshwater fish *Odontesthes bonariensis*. Semen samples were activated with tap water (control) and using herbicide solutions (containing 1, 5, 10 or 50 mg $PMG.L^{-1}$), and the motility parameters were determined by Computer-Assisted Sperm Analysis (CASA) system. Embryos and larvae were incubated in an incubator chamber (temperature and photoperiod controlled) using control media or the same concentrations of glyphosate-based herbicide previously detailed. The embryonic and larval development parameters were daily analyzed. Results showed that only the highest herbicide concentration significantly affect sperm motility. The survival and hatching rate of embryos were not affected by any of the different concentrations tested. On the other hand, concentrations of 5, 10 and 50 mg $PMG.L^{-1}$ significantly increased larval mortality, confirming the high susceptibility of the larval stage to glyphosate-based herbicide exposure.

Reproduction I

A56

PCOS AND METABOLIC DISORDERS.

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Polycystic ovary syndrome (PCOS) is a common disease affecting women in their reproductive age. PCOS is diagnosed by two of th Faccinetti ese alterations:clinical or biochemical hyperandrogenism, oligo or anovulation and ovarian cysts . PCOS could be classified in two main phenotypes: hyperandrogenic (HA)(with clinical and or biochemical hyperandrogenism) and no hyperandrogenic (NHA) phenotype. We divided a group of 37 PCOS patients from Hospital Durand into HA and NHA phenotypes and studied the endocrine alterations and the relationship between these phenotypes and the presence of Metabolic Syndrome(MetS) and cardiovascular risk(CVR).The presence of MetS and CVR were independent of phenotypes.The HA showed similar levels of triglyceride, HDL and LDL cholesterol, homeostasis index, glycemia, insulin and 17 beta hidroxy steroid dehydrogenase activity as compared with NHA. HA women showed increased levels of total cholesterol and activity of P450 c17 lyase. In the HA group, hyperandrogenemia did not reflect clinical hyperandrogenism found in that patients. We conclude that hyperandrogenism may be affected by the increase on androgen production and not by the levels of the circulating androgens. We also found that the prevalence of MetS and CVR are independent of the PCOS phenotype.

A57

cAMP AND ITS METABOLITES AS BOVINE SPERM CAPACITATION INDUCERS.

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Sperm capacitation has been associated with several molecular events such as: increase in intracellular cAMP levels, kinases activation and protein phosphorylation. Our previous results show that cAMP efflux and activation of A1 adenosine (ADO) receptors are critical in the regulation of bicarbonate-induced sperm capacitation. Moreover, cAMP may act as an autocrine/paracrine factor in the extracellular compartment. In the present work we aim to deepen into the action pathway of cAMP and its metabolites (5´AMP; ADO). For that, we assayed *in vitro* capacitation in the presence of the cAMP or its metabolites and PLC and PKA inhibitors. The sperm capacitation was evaluated by CTC and LPC-induced acrosome reaction assays. Our results showed that 10 nM of cAMP or 5'AMP induce sperm capacitation, but it is necessary 10 uM ADO to accomplish the same effect. Furthermore, when spermatozoa were incubated with non-capacitating concentrations (NCC) of cAMP plus ADO, they undergo capacitation. The effect of nucleotides was reversed by A1 antagonists. Finally the effect of cAMP, ADO and both molecules in NCC was inhibited with PLC and PKA inhibitors, suggesting that these two transduction pathways may be involved in nucleotides-induced capacitation in bovines.

A58
EFFECT OF INCREASING ZINC CONCENTRATIONS DURING *IN VITRO CULTURE* OF BOVINE EMBRYOS.

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In previous studies we demonstrated that zinc (Zn) supplementation during *in vitro* maturation (IVM) improves the quality of bovine oocytes and consequently their competence for developing to blastocyst stage. However, the influence of Zn supplementation during *in vitro* culture (IVC) of preimplantational bovine embryos has not been yet investigated. The aim of this study was to evaluate the effect of Zn addition during IVC. For this purpose, cumulus oocyte-complexes were obtained from an abattoir, matured in TCM 199 medium with 10% BFS for 24 h and then *in vitro* fertilized. After IVF, presumptive cigotes were cultured during 8 Days in mSOF with: 0; 0.4; 0.8 and 1.2µg/mL Zn. A completely randomized block designs were used and statistical models included the random effects of block and the fixed effect of treatment. Cleavage and blastocyst rates were analyzed with logistic regression by using the GENMOD procedure (SAS Institute, Cary, NC) with binomial distribution. Data was expressed as percentages. There were no differences in both cleavage rates (59; 64.7; 67.5 and 70 % for 0; 0.4; 0.8 and 1.2 µg/mL Zn) and blastocyst rates (15.7; 20.8; 21.8 and 17.4 for 0, 0.4, 0.8, and 1.2 µg/mL Zn) when Zn was added to IVC medium at any concentration. In conclusion, Zn addition during IVC of bovine embryos did not modify cleavage and blastocyst rates.

A59
DIFFERENTIAL ACTIVITIES OF ISOCITRATE DEHIDROGENASE IN PORCINE COCS.

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Our aim was to determine the activity of NAD and NADP dependent isoenzymes of isocitrate dehydrogenase (IDH) in immature or *in vitro* matured cumulus-oocyte complexes (COCs). IVM was carried out during 48 h, 39ºC, 5% CO_2 in medium 199 with gonadotropins. COCs were sonicated (4 min) and centrifuged (17000xg, 20 min), being the activity determined in the supernatant. An enzymatic unit (U) was defined as the amount of enzyme necessary to form 1 µmol of NAD(P)/min. Enzymatic activity was expressed in U (U/COC) and specific activity (U/mg protein) as mean±SEM and compared by Student's t test. For the NAD isoenzyme the U was (3.56±0.56) 10^{-6} and (2.67±0.36) 10^{-6}, for immature and matured COCs, respectively. In NADP isoenzyme U was (2.81±0.64) 10^{-5} and (2.90±0.84) 10^{-5}, for immature and matured COCs, respectively. The specific activity in the NAD isoenzyme was (6.05±1.28) 10^{-4} and (1.51±0.20) 10^{-4}, for immature and matured COCs, respectively. In NADP isoenzyme the specific activity was (1.19±0.43) 10^{-2} and (0.64±0.23) 10^{-2}, for immature and matured COCs, respectively. A significant decrease ($p<0.05$) in specific activity was observed for both enzymes. The enzyme activity for NADP isoenzyme was higher ($p<0.05$) respect to IDH NAD. These results indicate that IDH enzymes are present in porcine oocytes. The higher activity of NADP isoenzyme can be related to the generation of energy by the Krebs cycle during oocyte maturation and/or regulation of intracellular redoz state.

A60
EFFECT OF A MIXTURE OF ENDOCRINE DISRUPTORS IN THE REPRODUCTIVE SYSTEM OF MALE MICE.

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Human beings are exposed daily to different chemicals that may disrupt the endocrine system. The real impact that these compounds may have on reproductive health is controversial, few studies have addressed the effect of mixtures of these endocrine disruptors (EDs). In this work we wanted to determine the effect of a mixture of 5 EDs in the male reproductive system. Pregnant mice from 0.5pcd was treated with a mixture of 30mg/Kg phtalates (DEHP, DBP, BBP), and 5mg/Kg alkylphenols (NP, OP) in the drinking water. At weaning time only male offspring were selected and the administration of EDs was continued until adulthood. We evaluate the effect of this mixture of EDs in three different doses 1X, 0.1X and 0.01X. We observed an increase in the mRNA levels of enzymes *StAR, Cyp17a1* but not in *Sp1, Hsd3β*. Morphological alterations in seminiferous tubules of treated animals were: increased number of seminiferous tubules with detachment, without lumen, without certain stages and germ cell apoptosis. We observed an increased caudal epididymis sperm concentration but not in spontaneous or progesterone induced acrosome reaction. As a new approach, we conducted a bioinformatic analysis that predicted miRNAs that regulate the network for synthesize of testosterone and estradiol. These results suggest that chronic exposure to low doses of EDs induce alterations in the reproductive system of male mice by increasing the expression of the steroidogenic pathway genes.

A61
CHARACTERIZATION OF CRISP4 KNOCKOUT MICE.

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Epididymal CRISP1 and CRISP4 proteins are present in sperm and reported to be involved in gamete fusion and sperm zona pellucida (ZP) binding, respectively. However, knockout (KO) mice for these molecules are fertile, suggesting compensatory mechanisms between homologous proteins. With the aim of developing a double KO mice, and based on the availability of CRISP1 KO mice in our laboratory, we generated and characterized a colony of CRISP4 KO mice. RT-PCR and Western blot confirmed the lack of *Crisp4* mRNA in the epididymis and of CRISP4 protein in both epididymis and sperm, as well as normal expression of CRISP1. Whereas no differences in fertility, in vivo fertilization, sperm number, motility or viability between wildtype (WT), heterozygote (HT) and KO mice were observed, HT and KO spermatozoa exhibited lower levels of progesterone-induced acrosome reaction (AR) than WT cells. Consistent with this, a lower expression of CRISP4 was found in the epididymis of HT vs WT. Exposure of fresh and capacitated sperm to different treatments showed that CRISP4 was not released by NaCl 0,6M or 5U/mL PLC-PI but was completely removed by Tritón X-100, indicating a strong association of CRISP4 with capacitated sperm. This, together with the correlation between CRISP4 presence in sperm and their ability to undergo the AR, supports a role for CRISP4 in additional stages of fertilization such as cumulus and ZP penetration. This, and CRISP1/CRISP4 KO mice fertility are at present under investigation.

A62
CHARACTERIZATION OF GONADOTROPIN INHIBITORY HORMONE IN *Cichlasoma dimerus.*

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GnIH is a novel hypothalamic RFamide neuropeptide capable of inhibiting the release of gonadotropins in birds and mammals. The aim of this work was to characterize its preprohormone sequence, tissue distribution and effect over pituitary hormones. We identified cdGnIH precursor showing 3 putative LPXRFamide peptides. Analyzing the tissue distribution of GnIH mRNA in the reproductive axis we detected a high expression in hypothalamus and testes. By immunohistochemistry we observed GnIH somas in the *nucleus posterioris periventricularis* and a great number of ir-fibers widespread in all the brain regions. Finally, we performed *in vitro* studies with synthetic cdGnIH-2 and -3 to evaluate their effects on GH, LH and FSH. Preliminary results indicate a biological effect of cdGnIH-2 on pituitaries hormone release. In conclusion, GnIH was identified in *C. dimerus* and judging the distribution pattern and *in vitro* results, GnIH could be involved in the regulation of reproductive function.

A63
CHANGES IN FOLLICULAR GROWTH OF THE OFFSPRING CAUSED BY MATERNAL OVERWEIGHT.

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Obesity and overweight are associated with increased likelihood of complications during pregnancy and childbirth. The aim of this work was to study the changes on the follicular growth of female offspring caused by maternal overweight. Adult rat were fed with either a standard laboratory diet or a high-fat palatable (cafeteria) diet ad libitum from the time of weaning to adulthood. When these animals exhibited 20% overweight compared with controls, all rats were mated and offspring from control (C) and overweight maternal (D) were used. Both diets were also used during pregnancy and lactation. Compared with C, D exhibited advanced vaginal opening (1-3 days, $p<0.05$), and had an increase in the body weight (25-35%, $p<0.001$), serum glucose (11%, $p<0.05$), ovarian weight (29%, $p<0.01$) and ovulation rate (129%, $p<0.05$). By ovarian histology, we found that D displayed lower number of both primordial (Po: 8±1, $p<0.001$) and primary (Pi: 6±1, $p<0.01$) follicles, and higher number of corpora lutea (CL: 4.5±0.3, $p<0.01$), all compared with C (Po 13±1; Pi 10±1; CL 2.8±0.4). These results indicate that only 20% of maternal overweight is able to alter the reproductive development in the offspring as early puberty, reduced follicular reserve, higher number of corpora lutea, and also probably induce metabolic disorders.

A64
MOUSE SPERM UNDERGO ACROSOMAL EXOCITOSIS IN THE UPPER SEGMENTS OF THE ISTHMUS.

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Of the millions of sperm ejaculated by natural mating, only thousands reach the isthmus of the oviduct. Among them, only a few reach the ampulla at the time of fertilization. Sperm from several species must undergo acrosomal exocytosis (AE) prior to fusing with the egg. It has been long thought that AE took place upon interaction with proteins from the zona pellucida (ZP) of the egg. However, it was recently reported that fertilizing sperm undergo AE prior to the interaction with the ZP. Thus, the physiological site for AE is still unknown. In this work, we provide a detailed description of the process of sperm migration through the oviduct, and the occurrence of AE during this journey by live imaging microscopy using double transgenic sperm having acrosomal vesicles expressing green EGFP and middle pieces expressing red Ds-Red2 (mitochondria). We observed that acrosome intact sperm were only present in the lower segments of the isthmus. However, once sperm reach the upper segments of the isthmus, around 40 – 50 % of the sperm were acrosome reacted. In addition, over 95% of the sperm underwent AE prior to entering the ampulla. These results suggest that the physiological AE might take place in the upper segments of the oviduct.

A65
NEURAL CADHERIN IS PRESENT IN BOVINE SPERM AND COC CELLS AND PARTICIPATES IN FERTILIZATION.

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Neural cadherin (N-cadherin) is a Ca^{+2}-dependent glycoprotein known to participate in cell-cell adhesion and signaling. The aim of this study was to immunodetect N-cadherin in bovine sperm and Cumulus Oocyte Complex (COC) cells and to evaluate its role in gamete interaction. The 135 kDa mature protein form was immunodetected in protein extracts from ejaculated frozen/thawed sperm, cumulus cells and immature/mature oocytes. N-cadherin was immunolocalized in the acrosomal and postacrosomal region of acrosome-intact non capacitated (83±6%) and heparin/capacitated sperm (64±5%) and in the equatorial segment of follicular fluid/acrosome-reacted sperm (73±1%) (n=21). In COC, cumulus cell projections into the *zona pellucida* (ZP) and the oolemma were immunoreactive to N-cadherin. Sperm and ZP-free oocyte preincubation with anti N-cadherin antibodies (200 µg/mL) resulted in a reduction in the % fertilized oocytes (PBS =57±6 %, anti N-cadherin=0±0 %; $P<0.01$, n=30 oocytes), and # sperm bound to oolemma (PBS =13±1, anti N-cadherin=3±1 bound sperm; $P<0.01$, n>30 ooytes). N-cadherin is expressed in bovine sperm and COC cells and would participate in events leading to gamete binding/fusion during fertilization.

A66
ACTIVITY OF KEY ENZYMES INVOLVED IN THE ENERGETIC METABOLISM OF PORCINE SPERM.

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The cryopreservation process causes damage to the sperm, affecting its fertility due to the alteration of membranes, cytoskeleton, nucleus, mitochondria and cellular metabolism. Our aim was to determine, in extracts of fresh semen or sperm frozen with or without alpha tocopherol from three different boars, the activity of key enzymes of the glycolytic pathway (phosphofructokinase, PFK) and the TCA cycle (isocitrate dehydrogenase, IDH and malate dehydrogenase, MDH. The enzyme activity was determined by spectrophotometry. Enzyme units (U) were defined in terms of each enzyme evaluated. No significant differences were observed in the U of PFK between fresh and cryopreserved samples ($p>0.05$). However, a significant decrease of the U of IDH and MDH was observed in frozen samples. This decrease was dependent of the boar analyzed and in some cases reverted by the presence of alpha tocopherol. Our results demonstrate that the cryopreservation process produce loss of certain enzymes, possibly associated with the damage on the sperm membranes and cellular components. The differences observed between the evaluated enzymes may be related to the size, cellular localization and the possible association with subcellular structures. The evaluation of the enzyme activity may be used as a marker of freezability in porcine sperm. In those boars with bad freezability, the use of alpha tocopherol during cryopreservation may preserve metabolic activity and thus sperm functionality.

A67

EXPRESSION OF GHRELIN AND ITS RECEPTOR IN BOVINE CUMULUS–OOCYTE COMPLEX.

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Ghrelin is a 28 amino acid gastric peptide. This hormone is associated with the food intake and energy homeostasis. Growing evidence indicates that ghrelin is expressed and/or operates at different levels of hypothalamo–pituitary–ovary axis. However, the presence of ghrelin and its receptor in the bovine oocyte-cumulus complex (COC) has not been described until now. The aim of this study was to localize the mRNA of ghrelin and its active receptor (GHS-R1A) in the bovine COC. Oocyte-cumulus complex were aspirated from bovine ovaries, and matured in TCM-199 medium with FSH and 10 % FBS at 39 °C in 5% CO2 and saturated humidity for 24h. Medium was supplemented with increasing concentrations of ghrelin (0pM; 20pM; 40pM; 60pM). The expression of ghrelin and its receptor were determined with PCR. The results indicated that ghrelin and its active receptor (GHSR-1a) were expressed in immature COC, and in COCs matured with 0, 20, 40 and 60 pM of ghrelin. Although these findings alone do not prove the existence of a relationship between ghrelin and fertility, they are a mandatory first step toward understanding the role of ghrelin in metabolic regulation of fertility in bovine.

A68

EXPRESSION OF KISSPEPTIN 2 AND RECEPTOR 2b DURING EARLY LARVAL STAGES IN PEJERREY (*Odontesthes bonariensis*).

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Kisspeptin is considered a gatekeeper in the regulation of gonadal maturation and puberty onset in mammals. However its role on fish reproduction is not so clear and there are few studies about the role of this system during early developmental stages. Previous studies revealed the presence of two different isoforms of *Kiss2* and *Kissr2* in some teleost species. In this context the objective of this work was to search for *Kiss2* and *Kissr2* isoforms and measure their expression levels during the first weeks after hatch (wah) in pejerrey. We could only detect splicing variants in *Kissr2b* but we designed specific primers for one exon (P1) and in an exon-exon junction (P2) for both genes (*Kiss2* and *Kissr2*). Pejerrey fry were

maintained at 17ºC, 24ºC and 29ºC during 8 wah. Five individuals were collected weekly and cDNA were obtained from heads, and then RT-QPCR was performed and normalized quantities were calculated. We found *Kiss2* and *Kissr2b* expression since week 1 (W1) to week 8 (W8). When the samples were analyzed with P1 primers we found significant increases at 17ºC for *Kiss2* at W2 and for *Kiss2rb* at W3. However, when the same samples were analyzed with P2 primers, no differences were detected. In conclusion we report a early expression of spliced variants of *Kiss2* and *Kissr2b* mRNA in pejerrey. The correct design of the primers is important to measure biologically active isoforms.

A69

HYPERANDROGENISM: APOPTOSIS & OXIDATION ON FOLLICULOGENESIS.

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Polycystic ovary syndrome (PCOS) is a heterogeneous disease responsible for infertility in women. Both ovarian apoptotic-antiapoptotic pathway and oxidant/antioxidant balance is related to infertility. Here, we propose to study whether hyperandrogenism alters ovarian follicular development by assessing apoptosis-related pathway: BCL2 (anti-apoptotic) and BAX (pro-apoptotic), lipid peroxidation (LPO) and the anti-oxidant glutathione (GSH) content. We used a PCOS model developed in rats by subcutaneous administration of dehydroepiandrosterone (DHEA). Immature Sprague Dawley rats were injected with vehicle (Control group), equine chorionic gonadotropin (eCG group) to induce folliculogenesis, or eCG plus DHEA to induce folliculogenesis and hyperandrogenism (group eCG+HA). We found that gene and protein expression of BCL2 increased whereas those corresponding to BAX decreased in groups eCG and eCG+HA versus Control. In addition, the BAX/BCL2 ratio decreased in eCG group versus eCG+HA and Control. The GSH content decreased and LPO increased in eCG+HA group versus eCG and Control. We conclude that both the ovarian apoptosis and the oxidant/antioxidant balance are altered in the PCOS rat model, and that alterations could affect normal ovarian folliculogenesis.

Animal Biology II

A70
ESOPHAGUS HISTOCHEMICAL DESCRIPTION OF *Merluccius hubbsi* LARVAE: PRELIMINARY RESULTS.

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The Argentinean hake, *Merluccius hubbsi*, is one of the most important SW Atlantic Ocean fishery resources. Several studies describe the biology of this species. However, information regarding its early ontogeny is very scarce. In fish larvae, successful development of the digestive system is crucial for survival and growth. The aim of this work was to analyze the gastrointestinal tract and its functionality during hake early developmental stages. Thus, we studied the histochemical composition and distribution of glicoconjugates (GCs) in the esophagus of hake larvae. Larvae were fixed in buffered formalin, preserved in 70% alcohol, and processed for inclusion in paraplast. Histological sections were stained with (i) PAS; (ii) AB pH 2.5; (iii) AB pH 1.0 and (iv) AB pH 0.5. Mucous cells reacted positively to the histochemical technique analyzed, indicating the presence of GCs with oxidable vicinal diols, GCs with carboxyl groups and O-sulphate esters and highly sulphated GCs. The secreted mucins could cooperate in the pre-gastric digestion, as well as in absorptive functions. Moreover, the secretion of sulphated GCs could be associated to a protective and lubricating role. This study represents the first histochemical description regarding hake larvae digestive system.

A71
PLASMA CORTICOSTERONE LEVEL AND PROTEIN ELECTROPHORESIS IN ANTARCTIC BROWN SKUA (*Stercorarius antarcticus*): EFFECTS OF BREEDING.

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During the reproductive cycle, seabirds have to cope with different energetic stress situations, and corticosterone secretion is one of the physiological means through which homeostasis is maintained. To understand better the relationship between corticosterone level and body condition we studied the hormonal behavior and plasma protein profile of the antarctic Brown Skua during breeding season. Blood samples were obtained in three different moments: *In* (incubation), *Pi* (after egg hatching) and *Pii* (during chick rearing). Plasma corticosterone was assessed by radioimmunoassay. Also, protein agarose electrophoresis was performed to assess nutritional and immune condition by analyzing albumin, α_1, α_2, β and γ globulins concentration. A rise in corticosterone level was observed during the *In* period. In addition, a significant decrease

in total protein and in albumin, β- and γ- globulin fractions were observed in the course of different periods, indicating that mobilization of energy stores via protein catabolism and immunological consequences occurred during breeding. Finally, the decline in body condition in adults during incubation together with the high levels of corticosterone describe the adaptability of skuas during periods of high energy demand.

A72
GAP 3D STRUCTURE IS CONSERVED THROUGH THE VERTEBRATE LINEAGE.

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GnRH associated peptide (GAP) is the C-terminal portion of the GnRH pre-prohormone. Although it was reported that GAP can be co-secreted with GnRH, this peptide is generally considered as a residual sequence. When we characterized the full GnRH transcripts of the teleost fish *Cichlasoma dimerus*, the putative aminoacid sequence of GAP presented high similarity with that of other teleosts. Consider these, the aim of this work was to evaluate if GAP sequences and tridimensional structures are conserved in vertebrates. GAP sequences were obtained from different databases and then a comparative analysis of the primary amino acid sequences, a phylogenetic analysis and a study of the predicted 3D structures were performed. The results show a high conservation in GAP 3D structure among vertebrates in type I and type II GnRH variants, but not in type III and IV. This conservation through the vertebrate lineage suggest that, in spite of what it is thought, GAP may have a conserved function.

A73

EPIDERMAL ORIGIN OF ALARM CHEMICAL CUES IN *Rhinella arenarum* TADPOLES.

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Larvae of many anuran taxa display strong behavioral responses to chemical cues, including alarm signals. These behaviors are common in different species, and in *Rhinella arenarum* include reducing activity, escape behaviors and area avoidance. A common process by which vertebrate prey releases alarm cues is through injury or consumption by a predator. These chemical acts as predation cues for conspecifics. In this work we investigated the tissue source of a chemical cue of predation in *Rhinella arenarum* tadpoles. We observed that tadpoles responded with antipredator behaviors when exposed to skin and tail homogenates but not to carcass homogenate. Our histological analysis of the skin and tail showed a cellular type similar to described in tadpoles of other species, the "Riecenzellen" or giant cells. These cells are round, oval or pear-shaped and their nucleus lies basally or laterally on the cell membrane. They extend to the surface of the epidermis but have no opening there and are distributed uniformly over the body surface. Apparently they are specialized for synthesis and release of the olfactory alarm pheromone. Our observations support the idea that these cells are the origin of the chemical cue, which induces antipredator behavior in conspecifics.

A74

COMPARISON OF DESICCATION TOLERANCE IN TWO GRAPSOID CRABS: *Cyrtograpsus angulatus* AND *Neohelice granulata.*

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Cyrtograpsus angulatus and *Neohelice granulata* are most common crabs in the Atlantic coast of South American, both species are present from South of Brazil to the North Patagonia in Argentina. These species are competing on the coastal wetlands for space in the intertidal zone and for food. Our goal is to determine their desiccation tolerance in lab experiments including if there are differences between sexes. The crabs were captured directly in Bahía Blanca Estuary (Argentina) (N = 60 individuals per species). The results indicated that males of *C. angulatus* had lower desiccation tolerance ($p > 0.05$) in comparison with females, while in *N. granulata*, the sexes did not differ ($p < 0.05$). With respect to the desiccation tolerance between species, *N. granulata* was more tolerant ($p > 0.01$). The latter shows that although both species share the habitat in the different wetlands of the estuary, *N. granulata* could colonize more hostile environments than *C. angulatus*.

A75

THE COLONIC GROOVE IN *Lagostomus maximus*: A MORPHOLOGICAL AND HISTOCHEMICAL STUDY.

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The ascending colon of several herbivorous mammals has a longitudinal colonic groove, which is used as a route of retrograde transport of a mixture of bacteria and mucus. The objective of this work was to perform a morphological and histochemical study of the colonic groove of *L. maximus*. Sections of ascending colon were subjected to histological techniques and histochemical procedures for glycoconjugates (GCs) identification (PAS; KOH/PA*S, PA/Bh/KOH/PAS; KOH/PA*/Bh/PAS; AB pH 2.5, 1.0 and 0.5; AT pH 5.6 and 4.2). The groove originated close to the ileocecal junction and was extended along the mesenteric side of the ascending colon. The histochemical analysis revealed significant differences between the glycosylation pattern of goblet cells present on the groove and those existing on the rest of the colonic mucosa. The groove was rich in goblet cells containing a high proportion of carboxylated and sulfated GCs. The PA/Bh/KOH/PAS technique showed an abrupt change in the histochemical profile of the goblets cells, which presented a negative reaction in the groove and a strong positive reaction in the rest of the colonic mucosa. In the area of the colon possessing the ridges, the specific goblet cells glycosylation suggest that the mucus has a role in the functioning of the groove probably related to the high density of bacteria present in this region.

A76

EFFECT OF TEMPERATURE ON MALES OF THE "RED CHERRY" SHRIMP (DECAPODA, CARIDEA).

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Neocaridina heteropoda heteropoda, also known as "red cherry" shrimp, is very popular as ornamental species. This study analyzed the effect of temperature on male growth, biochemical composition and reproductive system structure. Recently differentiated males were exposed during 90 days to one of the following temperatures: 24°, 28° and 32°C. Males were maintained in plastic containers with continuous aeration and Java moss. Results showed higher final weight and total lipid content in males exposed to 24°C (12.67 ug/mg *versus* 9,48 and 8,5 ug/mg for 28°C and 32°C, respectively) with no differences in protein content among treatments (~ 16 ug/mg). This may reflect a better efficiency in using energy reserves. No effect of temperature was detected on the histological structure of testes and vasa deferentia so it would be expected no effect of this temperature range on reproductive function. PICT2012-01333,UBACYT2011-2014 (20020100100003) y 2014-2017 (20020130100186BA),MINCYT-CAPES BR/11/21.

A77

EFFECT OF SUPPLEMENTATION WITH MICRO SILAGE OF GRAPE POMACE ON CALCIUM AND PHOSPHORUS IN LACTATING CREOLE GOATS.

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The creole goat population of La Rioja is mostly located in smallholder farming areas. The traditional production system is extensive with grazing on native forage, which presents great stational fluctuations in terms of quality and quantity. These variations of resource of feed can limit productivity being necessary the supplementation. The use of agricultural by-products is often a useful way of overcoming this problem. The objective of the present work was to evaluate the effect of supplementation with micro silage grape pomace on calcium and phosphorus in lactating creole goats under extensive management system. The study was carried out in La Rioja, in the Chaco arid region. Twenty lactating creole goats (2-3-year-old) were divided into two groups: T1 (Control) and T2 (Micro Silage Grape pomace). The grape pomace was dried naturally for a period of one day. The supplementation was offered at the beginning of each day during the lactation period (forty five day). At the beginning and end of the trial we collected blood samples to evaluate calcium and phosphorus. The results show that T2 significantly decrease the concentration of serum calcium (T1=6,58 vs T2=4,20, mg/dL, p=0,03). Knowing that the Calcium requirements in lactating goat are high is important assess this parameter in supplementation with grape pomace.

Cellular and Molecular Biology II

A78

RIBOFLAVIN TRANSPORT IN TRYPANOSOMATIDS.

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Trypanosoma cruzi, *Trypanosoma brucei* and *Leishmania mexicana* cause several diseases in humans. The current therapies are toxic and limited in efficacy, thus there is a need to identify targets to develop new treatments. Riboflavin (Rf) is an essential vitamin for all living cells. Metazoans obtain it from their diet through specific transporters. In this work we study Rf metabolism in the trypanosomatids *T. brucei*, *L. Mexicana* and, particularly, in *T. cruzi* and its life´s cycle. *In silico* analysis suggest that the three trypanosomatids are unable to synthesize Rf *de novo*, conversely their genomes encode for a putative permease which belongs to a novel family of Rf transporters. Proliferation is improved with increasing concentration of flavins and decreases in presence of Rf analogues. In *T. cruzi*, flavins also play an important role in metacyclogenesis, raising the number of trypomastigote per ml and Rf analogues impairing it. Moreover, Rf analogues have not affected infectivity but reduced the intracellular replication of amastigotes. In conclusion, flavins are essential for trypanosomatids, especially in *T. cruzi*, that incorporate it by specific transporter. This novel transporter family presents differences respect to the human, so it could be a potential target for development of new trypanocidal molecules.

A79

CYTOTOXICITY EVALUATION OF ESSENTIAL OILS OBTAINED FROM WILD AND CULTIVAR *Tagetes minuta* POPULATIONS.

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Tagetes minuta L. (Family Asteraceae) commonly known as "Suico", has cosmopolitan distribution, being abundant in Córdoba. It is widely used as antimicrobial, insecticide, in beverages and in the manufacture of perfume, as well. These facts led us to assess the in vitro cytotoxic effect of essential oils (EO) of *T. minuta*. These EO were obtained from cultivated plants enhanced genetically and were obtained three different chemotypes: Ona: ocimenona rich, OTLO: ocimenona-tagetone-limonene-ocimene rich and DHT: dihydrotagetone rich. These EO were compared with that one obtained from wild populations. In order to evaluate the cytotoxicity effect of EO the method of Neutral Red uptake on Vero cells was used. Different concentrations of each EO (by triplicate) were incubated. The cytotoxic concentration 50% (CC50) (R2> 0.9) was obtained. CC50 values obtained for each EO were 7.04 ± 1.05 ppm for wild EO, 1.33 ± 0.03, 1.9 ± 0.03 and 34.07 ± 1.33 ppm for Ona, OTLO and DHT, respectively. These results reflect the marked in vitro cytotoxicity of EO *T. minuta*, particularly the Ona and OTLO chemotypes. The differences observed would be due to chemical composition variations in the of the different EO. These results represent the basis for future EO bioactivity assays for *T. minuta* to be conducted.

A80

CHARACTERIZATION OF SECRETONEURIN VARIANTS IN PEJERREY *(Odontesthes bonariensis)*.

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Secretoneurin (SN) is a well conserved peptide from sharks to mammals, and it is the product of the cleavage of the larger precursor Secretogranin II (also called Chromogranin C). Nevertheless a receptor for SN has not been found yet; SN has been proposed to be a new hormone. Also, in some teleost fish species two SN variants have been reported and called as SNa and SNb. In this context we attempted to characterize SN variants in pejerrey and their neuroanatomical distribution to then start looking for their function. Consensus degenerated primers were designed after alignment of SNa and SNb forms in other teleost species and used on cDNAs obtained from pejerrey brains and pituitaries. Partial SNa and SNb sequences were obtained in both tissue extracts. Also pejerrey brains were treated for immunohistochemistry using a SN antiserum raised against goldfish SNa. SN-immunoreactive (SN-ir) cells were observed in pejerrey magnocellular and parvocellular cells of the preoptic nucleus with their projections ending at the neural lobe of the pituitary gland. Also, some SN-ir cells were observed in the pituitary gland, however up to the moment the nature of these cells is under study. In conclusion we report for the first time the presence of SN variants in pejerrey brain and pituitary to start studies on their physiological functions.

A81

PROKARYOTIC EXPRESSION, PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION OF ZINC TRANSPORTER 8 (ZNT8).

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ZnT8 is an islet B-cell protein identified as a novel target of humoral autoimmunity in type 1 Diabetes Mellitus. The aim of this study was to express recombinant ZnT8 useful for the development of non-radiometric immunoassays for autoantibodies to ZnT8 (ZnT8A) detection. The C-terminal of ZnT8 was cloned into pTrxFus; *E. coli* was transformed with pTrxZnT8, cultured at 30°C and induced with Trp. The chimera was purified by affinity or anion exchange chromatography. The intracellular soluble fraction (ISF) and inclusion bodies (IB) were analyzed by SDS-PAGE and Western Blot (WB). Quantitative competition assays with 5 ZnT8A+ patients sera were performed by adding TrxZnT8 (37pM to 0.7uM) to the Radioligand Binding Assay, using [^{35}S]ZnT8 (synthetized with rabbit reticulocyte lysate system). SDS-PAGE and WB showed a band of ~37.5 kDa compatible with TrxZnT8 theoretical mass. Purification of the chimera from ISF (yielding 2mg TrxZnT8/L culture) and IB was achieved. All dose-response curves showed similar protein concentration that caused 50% inhibition (41.0 to 1.4 nM). Recombinant ZnT8 was successfully expressed and purified from *E. coli* as a fusion protein with Trx. We demonstrate its proper immunochemical behaviour by displacement of [^{35}S]ZnT8 binding to ZnT8A.

A82

FIRST EVIDENCE OF THE ROLE OF PANTOTHENATE IN *Trypanosoma cruzi*.

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Chagas disease is an endemic parasitosis in Latin-America, caused by infection with the protozoan *Trypanosoma cruzi*. The current therapies are highly toxic and limited in efficacy, thus there is a need to identify targets to develop new treatments. Vitamins are essential micronutrients for all living cells; many organisms synthesize them *de novo* while others obtain them through specific transporters. The aim of this work was to study the effect of different B vitamins on *T. cruzi*. Assays performed in *T. cruzi* epimastigotes cultured in absence of these vitamins showed different responses in their proliferation rate, being pantothenate the vitamin that significantly reduced maximal density. The antiproliferative effect was recovered adding pantothenate, in a dose dependent way. Although viability was not affected, changes in pantothenate concentration affected the metacyclogenesis process. Finally, bioinformatic analysis of *T. cruzi* genome suggested it is auxotrophic for pantothenate and we found a putative transporter for this vitamin, similar to that described in fungus *Aspergillus flavus.* As pantothenate seems to play a key role in *T. cruzi* metabolism, a further characterization of this transporter would allow us to find potential target/s for new trypanocidal drugs.

A83

CELL IMAGE VELOCIMETRY ANALYSIS APPLIED TO VERO CELL COLONIES.

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Cell motility plays a key role in many biological processes. In colony expansion it contributes to determine the dynamic regime. In this contribution we show results of the motility characteristics of VERO cells colonies with large population grown in standard (S) and methyl cellulose containing medium (MC) at gell concentration. The velocity field was determines employing particle image velocimetry (PIV) and the results were compared with semi- automatic cell tracking. In S and MC medium histograms of velocity components perpendicular to the colony front are shift to positive values, Although, for S medium mean value was significantly larger than in MC one, in agreement with kinetic data. Comparing the displacements directionality and the correlation functions from PIV analysis obtained in both media, we could infer that the MC produce: (i) “pinning effects” due to both the own structure of the medium and to the appearance of enlarged cells mainly located at the colony border perturbing collective movement, (ii) the increase in the spatio-temporal heterogeneity and (iii) the increase in cell-cell cooperative displacement at the inner regions of the colonies. Similar conclusions can be obtained by cell tracking, although in this case with less statistics. This indicated that PIV analysis can be a useful tool for assessing motility data at the colony level.

Ecology, Toxicology and Behaviour II

A84

EFFECTS OF ARSENIC EXPOSURE DURING PREGNANCY ON THE REPRODUCTIVE AXIS AND FERTILITY.

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Inorganic arsenic (A) is a ground water contaminant with worldwide distribution. It has also been described as an endocrine disruptor. We have previously shown that animals exposed to A reduces body weights (BW), elevates serum levels of estradiol (E_2) and testosterone (T), and presents glucose intolerance during pregnancy. In this study, pregnant Sprague-Dawley rats were treated with sodium arsenite in drinking water: 5 (A5) or 50 (A50) ppm in distilled water or distilled water as control (C), from gestation day 1 (GD1, determined by the presence of vaginal sperm) to sacrifice (GD18) by quick decapitation. We evaluated resorption and implantation sites, the numbers of corpora lutea (CLs), ovarian (OW), placental (PW) and adrenal gland weights (AW) and ovarian content of steroid hormones. Fetal BW was recorded. No effects were found in the numbers of CLs, resorption/implantation sites or fetal BW. No differences were found on AW or PW. A50 rats have higher OW and lower ovarian E_2 content. T and P_4 content were not significantly different among groups.

Exposure to A in drinking water during pregnancy increases E_2 and T serum levels without affecting reproductive performance. Further studies are needed to clarify the impact of hormonal alterations on the development of the fetuses. (CONICET-UBA-ANPCYT).

A85
CADMIUM EFFECTS ON THE PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN RAT PLACENTAS.

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Cadmium (Cd) is a none-essential metal that targets placenta. The proliferating cell nuclear antigen (PCNA) is a nuclear protein synthesized in early G_1 and S phases of cell-cycle. Its expression is essential for DNA synthesis. In order to study the effects of Cd on placenta cell proliferation, Wistar rats were injected subcutaneously with 10 mg Cd^{+2}/kg body weight on days 4 (G_1), 7 (G_2), 10 (G_3) or 15 (G_4) of pregnancy. Control groups received an equal volume of saline. Females were euthanatised on gestation day 20. Placenta samples were processed for immunohistochemistry with an anti-PCNA antibody. Spongiotrophoblast, giant cells and placental labyrinth of 10 images (40x) per animal were monitored, and all the cells and the labeled cells were counted. The percentage of labeled cells was calculated, and results were analyzed using the Student t test. In all groups of treated animals and cell types analyzed, the percentage of labeled cells was significantly lower ($P < 0.05$) in than control dams. These results indicated that Cd had an inhibitory effect on placental cell proliferation in addition to injuries and morphometric changes found in previous studies.

A86
EXPOSURE TO BISPHENOL A ALTERS THE HYPOTHALAMIC-PITUITARY-THYROID AXIS IN FEMALE RATS.

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Bisphenol A (BPA) is a monomer of polycarbonate plastics and a constituent of epoxy and polyestirene resins used in industry. Profound in-vivo effects of BPA have been described on the hypothalamic-pituitary unit, in different animal species. We studied the effects of BPA on the hypothalamic-pituitary-thyroid axis. We used two experimental models, an in-vivo and an in-vitro model. In the in-vivo model, Sprague-Dawley (SD) female rats were exposed neonatally (from days 1 to 10 of life) to different doses of BPA (500, 50, or, in some cases, 5 μg) or vehicle as control. The in-vitro model consisted of primary pituitary cultures (PPC) from 13-day old or adult SD females. Neonatal exposure to BPA did not alter serum TSH in 13-day old females, but it did in adulthood, with TSH levels higher in animals treated with 50 μg of BPA (B50). Serum T3 and T4 levels were lower in the 500 μg-treated animals and T4 in 5 μg-treated animals. PPCs obtained from B50 adult females released more TSH than the Controls. In PPCs from normal 13-day old females, pretreatment with BPA or estradiol (1.10^-7 M) increased TSH release and lowered the response to TRH.
The results show that neonatal exposure to BPA alters the hypothalamic-pituitary-thyroid axis in adulthood, and that BPA has direct actions in the pituitary.

A87
ADVANCED *Caiman latirostris* OVIDUCTAL MATURATION AFTER POSTNATAL EXPOSURE TO XENOESTROGENS.

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Caiman latirostris oviductal maturation is a postnatal event characterized by changes in luminal epithelium and in the subepithelial stroma. We described that; adenogenesis follows gradual collagen disorganization and increased expression of smooth-muscle proteins. The aim of our study was to assess whether early postnatal exposure to 17β-estradiol (E2) or to bisphenol A (BPA) alters maturation-related parameters. Two injections (s.c), 7 days apart from each other, of E2 (1.4 and 0.014ppm), BPA (140 and 1.4ppm) or vehicle were administered to 30 day old female caimans. Seven days after the last injection, oviducts were paraffin-processed. Collagen organization was assessed by picrosirius-polarization method. Desmin and smooth-muscle α-actin (α-SMA) expression were evaluated by immunohistochemistry. All treatments increased the area occupied by poorly organized collagen. Low doses of both E2 and BPA increased the proportion of subepithelial area occupied by desmin and α-SMA. Adenogenesis was absent. Postnatal exposure to xenoestrogens advanced the oviductal maturation. These changes could impair the reproductive performance of sexually mature caimans.

A88
EFFECT OF SALINITY WATER PRODUCTION ON A VINEYARD FROM CHILECITO, LA RIOJA.

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The city of Chilecito is located in the center of the drainage basin of the Antinaco - Los Colorados Valley. This region is heavily exploited by the agricultural sector in La Rioja, being the grapevine one of the major crops. The Durazno river is

the main source of irrigation superficial water to small farmers. The salinity levels in this river are of high inter-stational variability. Depending upon the phenological stage (budburst, flowering, or véraison) water salinity has a wide range of effects on grapevine growth, development, and production. The aim of this study was to evaluate the effect of salinity of water resources used in the production of grapevine. Periodic sampling was conducted from 2011 to 2013, the water quality was evaluated every 15 days through the EC. The production of grapevine was assessed every 7 days in a farm located in the district Tilimuqui planted with the variety Torrontés Riojano, which exceeds 20 years, was analyzed. In the same berry weight were recorded. In spring, when the grapevines meet in a critical period of growth (flowering and véraison), the EC was higher in 2012 than in 2011 (0.765, 0.588 dS/m, respectively). However, the yield obtained was more abundant (634,42 g vs 504,70 g), because of more frequent watering. We can conclude that the old plantation is more susceptible to the amount of water supplied to the salt concentration that it provides him.

A89

EFFECTS OF CHLORPYRIFOS ON THE SOUND EMITTED BY TADPOLES OF *Ceratophrys ornata* (ANURA: CERATOPHRYIDAE).

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The emission of underwater sounds in anuran tadpoles has been documented in only two species from Argentina and one from Madagascar. The aim of the study was to evaluate sublethal effects of the active compound chlorpyrifos (CPF) on the sound emitted by tadpoles of *C. ornata* and its bioacoustic variables in two developmental stages of Gosner (31 and 37). Toxicity tests were performed from 0.01 to 0.7 mgCPF/L, with one larvae per chamber and 20 replicates. Audio recordings were digitalized and analyzed using Adobe Audition 1.5. Analyzed variables were: duration of the sound (s), number of pulses and interpulses, dominant frequency (Hz). Results showed significant differences ($p<0.05$) between variables and all treatments, being duration of the sound of exposed tadpoles significantly shorter than control group. This is the first evidence of effects of chlorpyrifos on the sound emitted by larvae of *C. ornata*.

A90

IN VIVO EFFECT OF ENDOCRINE DISRUPTORS (ETHANOL AND ENDOSULFAN) ON CHROMATIN DECONDENSATION IN RODENT SPECIES.

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Chromatin decondensation is the first step towards syngamy after the spermatozoon penetrates the oocyte. It involves protamine disulfide bond reduction as well as protamine - oocyte histone exchange with the aid of a negatively charged

molecule, s. Ethanol and endosulfan are known for their endocrine disrupting effects on gonads. We have previously reported that endosulfan, increases mouse sperm chromatin decondensation *in vitro*. Here we studied the *in vivo* effect of both ethanol and endosulfan on male mice and rats, respectively. Male mice (60-90 days old) were fed with 15% ethanol in drinking water, for 15 days, and then euthanized. Male rats were injected with 0.6 mg/kg body weight of endosulfan in mineral oil at day 1, 3, 5 and 7 after birth and euthanized at day 60. Spermatozoa were obtained from the cauda epididymis and decondensation analyzed in the presence of glutathion and heparine/dermatan sulfate. While mouse spermatozoa decondensed after a 60 minute incubation, rat spermatozoa needed 17 hours in order to achieve a similar degree of decondensation. Ethanol significantly increased (48 ± 3 % control, n=8 versus 57 ± 4 % treated, n=10, $p<0.05$) chromatin decondensation. Conversely, endosulfan treatment completely abolished sperm chromatin decondensation in rats (36 ± 12 % in controls). These results suggest that different endocrine disruptors could be differentially affecting each animal species.

A91

ESTROGENIC ACTIVITY IN SURFACE WATERS OF RIO DE JANEIRO, BRAZIL.

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The need to elucidate the potential effects of micropollutants in the environment, has led to an increasing development of assays to detect them. The Yeast Estrogen Screen (YES) is an *in vitro* assay that provides information of the total estrogenicity of a sample, whose main advantage is its sensitive to low concentrations, besides allowing the analysis of large numbers of samples. The aim of this study is to evaluate the YES assay for the determination of estrogenic activity of environmental samples, and assess the quality of surface waters of Rio de Janeiro, Brazil. Surface water from three different points, Guandu River, Morto River and the Arroio Fundo Channel were collected. The highest values of

estrogenicity were found in the Arroio Fundo Channel, where there is a large release of raw sewage. The YES assay was sucessufuly implemented and this study clearly demonstrated its utility to detect estrogenicity in environmental samples.

A92

IN VITRO EFFECT OF ATRAZINE ON THE OVARIAN ENDOCRINE REGULATION OF 17-OH PROGESTERONE IN _Neohelice granulata._

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We evaluated the *in vitro* effects of the herbicide atrazine, a well-known endocrine disruptor, on hormonal regulation of reproduction in the adult female crab *Neohelice granulata,* during the pre-reproductive period. Animals were anesthetized, and the ovary was dissected in four sections (similar weight and size). Ovarian pieces were incubated in plates containing M199 medium. An aliquot of 5mg/L formulated atrazine was added to the treatment wells, and were also incubated with 17-OH progesterone (17PG): 0.15, 1.5, and 15µM. Besides, an aliquot of tritiated leucine (^{3}H-Leu) was added in order to measure *de novo* ovarian protein synthesis. Our results indicate that ovarian sections incubated with 17PG alone (at concentrations 0.15 and 1.5µM) increased ovarian protein synthesis, as measured by ^{3}H-Leu incorporation. In addition, co-incubation of ovary with both 17PG and atrazine resulted in a significantly ($p<0.05$) higher increase of ^{3}H-Leu incorporation, compared to 17PG alone. The ^{3}H-Leu incorporation in ovaries incubated with atrazine alone did not differ from that of the control group. These preliminary results indicate a possible enhancer effect of the herbicide atrazine on the 17PG ovarian endocrine regulation.

A93

CYTOTOXICITY OF BALTERGIN ON ENDOTHELIAL CELLS.

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The most important events associated to the development of local tissue damage in viperid snakebites depend on a limited number of components. Metalloproteinases type P-III are multiple domain enzymes whose principal toxic effects are due to disruption of the hemostatic system. Hemorrhage, a relevant local manifestation in envenomation by *Bothrops* genus, may result from a direct action of these metalloproteinases upon extracellular matrix components. In this work, the mechanism of damage was investigated on a target cell line, thus cytotoxic activity on endothelial cells induced by baltergin, a hemorrhagic metalloproteinase isolated from *Bothrops alternatus* venom, was evaluated. Briefly, cells (tEnd cell line) were exposed to different concentrations of baltergin (50-200 µg/ml) for 3 h at 37ºC-5% CO_2, toxicity was quantitatively assayed by crystal violet method. The percentage of cell detachment was registered and its DC_{50} was calculated. Cytolisis was determined by release of the cytosolic enzyme lactate dehydrogenase (LDH). Acridine orange–ethidium bromide double staining was performed to evaluate morphological alterations. Results indicate that the metalloproteinase induces a dose-dependent detachment of cells, DC_{50}= 173.17µg/mL, with no cell lysis (LDH not detected). Fluorescence analysis showed typical features of apoptosis, chromatin condensation and nuclear fragmentation, cell shrinkage and membrane

blebbing. Therefore, baltergin trigger cell detaching from the surrounding extracellular matrix and possibly induce a particular type of apoptosis denominated "anoikis".

Reproduction II

A94

ALTERATION OF LIVER LIPID METABOLISM BY PRENATAL HIPERANDROGENISM.

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Polycystic Ovary Syndrome (PCOS) is associated to Metabolic Syndrome (MetS), which could cause liver injury and even lead to non-alcoholic fatty liver disease (NAFLD) that correlates to altered lipid metabolism. In our laboratory we have developed a murine PCOS model by prenatal hyperandrogenism in which we demonstrated elevated risk of MetS and an incipient liver injury. Our objective is to evaluate the trygliceride (TG) content of liver and the state of the peroxisome proliferator-activated receptor gamma (PPARɣ) and its co-activator (PGC1-α) both involved in lipid metabolism. Pregnant Sprague Dawley rats were separated in two groups:control (C) and prenatal hiperandrogenizated (PH) with testosterone. In puberal offspring, we evaluated TG in liver tissue by a commercial kit and the gene expression of PPARɣ and PGC1-α.We found no significant differences between treatments in liver TG content. The levels of PPARɣ in the PH and C group

presented no significant differences, whereas PGC1- α was lower in the PH group compared to C. We conclude that the molecular pathway controlling hepatic lipid metabolism, mediated by these molecules, is affected in the PH group.

A95
EFFECT OF COPPER ON BULL SPERMATOZOA VIABILITY AND SPERM–ZONA PELLUCIDA BINDING.

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Copper (Cu) deficiency is linked to a variety of clinical signs, including impaired reproductive performance. The present study was focused on the effect of Cu on sperm viability and sperm–zona pellucida binding (ZP-b). Motile spermatozoa from bull frozen semen were separated by a discontinuous Percoll gradient. Sperm were incubated in TALP medium containing 0 (control); 2; 4; and 6 µg/mL Cu at 39°C in 5% CO2 in air. Sperm viability was evaluated at 0 h and 6 h of incubation with eosin-nigrosin stain. The ZP-b assay was carried out by sperm co-incubation with immature denuded oocytes for 2 h. Then the oocytes were washed and stained with Hoechst 33342. The number of spermatozoa bound to oocytes was recorded with an epifluorescence microscope. After 6 h of incubation, sperm viability did not differ with 0; 2 and 6 µg/mL Cu, but were lower with 4 µg/mL Cu ($P < 0.05$). The presence of 4 µg/mL Cu during gamete co-incubation increased the number of sperm bound to zona pellucida. In conclusion, 4 µg/mL Cu concentrations enhanced fertility parameters of bull spermatozoa.

A96
APOPTOSIS IN LONG TERM DIABETIC MICE GONADS.

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Alteration in follicular structure, masculine germ cells and an increase of apoptosis are frequently in diabetic mice. The objective was to analyze the balance between the apoptosis pathways in female and masculine gonads in diabetic mice. 50 BALB/c female and masculine mice were used. Mice received five injections of streptozotocin during 5 consecutive days. Animals were selected as diabetic when glucose level was > 200 mg/dl. Five diabetic and four control animals were sacrificed between days 15 and 80 post-treatment. Gonads were removed and fixed in PFA for immunodetection. Bax and Bcl-2 expression was constant at all time-points, with no differences between control and diabetic mice. Extrinsic pathway markers (Fas, Fas-L and t-Bid) showed strong cytoplasmic detection in oocyte and granulosa cells and masculine germ cells of diabetic mice. Active caspase 3 was positive in granulosa cells of atretic antral follicles and masculine germ cells. Apoptosis is enhanced in the ovary and testicle of diabetic-induced mice.

A97
GONADOTROPINS AND INSULIN EFFECT ON GLUCOSE UPTAKE IN PORCINE CUMULUS-OOCYTE COMPLEXES.

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GLUT4 is an insulin-dependent glucose transporter. FSH and LH stimulate glycolysis in porcine cumulus-oocyte complexes (COCs). We evaluated the influence of gonadotropins and insulin on glucose uptake in porcine COCs during *in vitro* maturation (IVM). COCs were obtained from ovarian follicles of slaughtered gilts and cultured 44 h in medium 199. *In vitro* fertilization (IVF) was performed in mTBM. Glucose concentration in IVM medium was determined by spectrophotometry and GLUT4 presence was determined by immunocytochemistry. Gonadotropins alone or combined with insulin increased glucose consumption, GLUT4 presence and meiotic maturation rate ($p<0.05$), but insulin alone had no effect. Pronuclear formation increased by gonadotropins or insulin or their combination ($p<0.05$). Immunofluorescence revealed GLUT4 in the porcine COC for the first time. The mark increased in cumulus cells and in the oocyte after maturation with insulin and in cumulus cells after maturation with gonadotropins. Gonadotropins and insulin have different effects on glucose uptake. The first ones stimulate glucose consumption through GLUT4, while insulin only induces GLUT4 expression.

A98
LPA REGULATES FIRST TRIMESTER TROPHOBLAST FUNCTIONS.

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Lysophosphatidic acid (LPA) is a small and bioactive phospholipid that plays an important role during the window of implantation. Previously, we observed that LPA, through LPA3 receptor, acts like a pro-implantatory molecule in the uterus. The aim of the present study was to investigate the effect of LPA in the fetal side of the maternal-fetal interphase. Thus, we studied LPA role in first trimester trophoblast vascularization and proliferation. First, we detected the expression of the mRNA and the protein of LPA3 receptor, as well as lyso-PLD, the main enzyme that synthesizes LPA, in first trimester trophoblast cell line, HTR-8/SVneo. Also, LPA3 was localized in the cytoplasm and plasma membrane of HTR-8/SVneo cells. Then, HTR-8/SVneo cells were incubated with LPA to assay tube formation (5, 10 and 20 μM, 3 and 6 hs) and proliferation (50 μM, 48 hs). After 3 hs of incubation, LPA stimulated capillary tube formation at the three concentrations tested ($p<0.05$ vs control). However, LPA did not influence HTR-8/SVneo cells proliferation. Our results suggest that LPA may play different roles on trophoblast functions and promotes vascularization, which is a crucial process during the first trimester of gestation.

A99
CALCIUM INFLUX IS REQUIRED FOR PHOSPHATIDYLSERINE EXPOSURE DURING MOUSE EGG ACTIVATION.

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Intracellular Ca^{2+} increase after fertilization is essential to initiate egg activation. We have recently found that this Ca^{2+} rise results in a transient exposure of phosphatidylserine (PS) in fertilized eggs that is not associated with apoptosis. The aim of this work was to evaluate the source of Ca^{2+} involved in PS exposure in activated eggs. The incubation of eggs with different known parthenogenetic egg activators showed that whereas $SrCl_2$ and ethanol induced PS exposure, Ca^{2+} ionophore A23187 did not. Given that, differently from $SrCl_2$ and ethanol, the Ca^{2+} increase produced by A23187 is originated from intracellular sources, we next evaluated whether extracellular Ca^{2+} influx was necessary to induce the translocation of PS. For this purpose, eggs were incubated with either 2-APB, a membrane Ca^{2+} channel agonist, or thimerosal that induces Ca^{2+} oscillations by releasing this ion from the endoplasmic reticulum, and the presence of externalized PS was evaluated. In parallel, two parameters of egg activation (i.e. cortical granule exocytosis and resumption of meiosis) were also evaluated. Whereas 2-APB and thimerosal produced high percentages of egg activation, only 2-APB was able to induce PS exposure. Altogether, these results indicate that Ca^{2+} influx from extracellular medium is required for the mobilization of PS during egg activation.

A100
LIVER INJURY IN A PCOS MURINE MODEL.

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Polycystic ovary syndrome (PCOS) is strongly associated with Metabolic Syndrome (MetS) that induces liver injury known as non-alcoholic fatty liver disease (NAFLD). This liver pathology is characterized by a deregulation of lipid content and an inflammatory state. PCOS etiology remains unknown but it is hypothesized that an androgen excess during gestation causes fetal programming that affects postnatal life. In our laboratory we have developed a PCOS murine model by prenatal hyperandrogenism (PH) in which we demonstrated an elevated risk of MetS development and an incipient liver injury. Our objective is to evaluate the inflammatory state and regulation of the Prostaglandin E2(PGE2) system, also involved in liver lipolysis. Pregnant Sprague Dawley rats were separated in two groups:control (C) and PH with testosterone. We evaluated, in puberal offspring, liver lipid content by histological staining, protein levels of the limiting enzyme in PGE2 synthesis, cyclooxygenase 2 (COX-2) by Western blotting and PGE2 levels by radioinmmuno assay. We found no differences on lipid liver content between C and PH groups but COX-2 and PGE2 levels decreased in PH group. We conclude that there is a depletion of the pro-inflammatory system mediated by PGE2 and COX-2 that could be repressing the development of steatohepatitis.

A101

IDENTIFICATION AND EFFECT OF A OVIDUCTAL GLYCOPROTEIN IN *Bufo arenarum* SPERM.

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Previous studies showed that the oviduct has a continuous secretory activity throughout the reproductive cycle, mainly of the 74 KD glycoprotein (gp74). This protein is present in the structural matrix of the jelly coats that surround gametes, has high diffusion capability to fecundation medium and plays an essential role in fertilization. Until now, there is not data about their specific role in gamete interaction. The objectives of this work were to determine the effect of gp74 in the acrosomal reaction (AR) and to identify its interaction with sperm. It was demonstrated that gp74 produce characteristic changes in the oocytes surface, similar to those induced by acrosomal lysines which are physiologically released during the AR. The addition of Ca^{2+} enhances this effect on oocyte surface, however it is not visible in the absence of sperm. Nor lytic effect was observed when insemination was performed with sperm previously treated with gp74 in the presence or absence of Ca^{2+}. The biotin-labeled gp74 and incubated with FITC-extravidin reveal fluorescence in the sperm head. The results show, for the first time, that a glycoprotein secreted in anuran oviduct and present in the jelly coats interacts with sperm promoting the release of acrosomal contents.

A102

DETECTION OF ALTERNATIVE SPLICING EVENTS IN THE KISSPEPTIN RECEPTORS IN PEJERREY (*Odontesthes bonariensis*).

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In mammals, the kisspeptin system is composed by the kisspeptin neuropeptide ligand and its receptor, *KISSR*. This system is primarily involved in the neuroendocrine regulation of puberty and sexual maturation. Recent studies have demonstrated the existence of a teleost-specific whole genomic duplication. Then, several fish species presented two *kissr* genes (*Kiss1rb* and *Kiss2rb*). In this study, we report the presence of two kisspeptin receptors in the pejerrey, *Odontesthes bonariensis*: pj*Kiss1rb* and pj*Kiss2rb*. The genomic structure of pj*Kiss1rb* comprises seven exons and six introns. On the other hand pj*Kiss2b* comprises five exons and four introns. In the case of pj*Kiss2rb,* two distinct transcripts were detected having a difference of 100 bp in length. The longer transcript contained an insert of 98 nucleotides caused by the retention of third intron. The sequence of this transcript has two premature stop codons. Future studies will aim to understand the importance of alternative transcription in the regulation of kisspeptin receptor expression.

A103

HYALURONIC ACID (HA) AND CD44 IN ANGIOGENESIS AND ENDOMETRIOSIS (EDT).

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HA-CD44 interaction has been described in physiologic and pathologic conditions. Adherence, proliferation and angiogenesis are amongst the processes they promote. To study this interaction we used different models in which we evaluated angiogenesis and EDT development. EDT is a benign disease that relies on angiogenesis. To study the effect of the HA-synthesis inhibitor 4-methylumbelliferone (4-MU) on angiogenesis we obtained aortic rings from Sprague Dawley

male rats, cultured them for 6 days with 4-MU and then measured sprout growth. We also performed an *in vivo* assay using the skinfold chamber model (SCM) into which endometrial fragments were transplanted and monitored by means of intravital fluorescent microscopy at different time points for: graft size, vascularized area (VA) and functional capillary density (FCD); cell proliferation, apoptosis and an endothelial marker expression were evaluated; mice were daily treated ip with 4-MU for 15 days. In addition, we induced EDT in $CD44^{-/-}$ (KO) and wild type (wt) mice by singeneic transplant of endometrial fragments from KO and wt mice. Animals were left untreated for one month. Lesion growth, VEGF content in peritoneal fluid and von Willebrand factor (vW) expression were evaluated. 4-MU inhibited sprout growth *in vitro*. It also affected the VA, FCD and CD31 expression while proliferation and apoptosis were unaffected in the SCM. Strikingly, bigger lesions developed in $CD44^{-/-}$ mice either with KO or wt transplanted tissue, but they were less adhered. No differences in vW or VEGF were observed. More studies will be designed to elucidate the mechanisms involved in EDT; nevertheless, these results are promising in the identification of a new target for angiogenesis.

A104
EXPRESSION OF GIP RECEPTOR IN THE BOVINE CUMULUS OOCYTE COMPLEX.

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The glucose-dependent insulinotropic polypeptide (GIP) is a hormone released by the duodenum. In ruminants, GIP concentration increases when the energy balance changes from negative to positive. In cattle, GIP decreases the rate of lipolysis in subcutaneous adipose tissue and plays an important role in the regulation of the energy use (energy partition). So far, there are not studies linking GIP with reproductive function. Until now, it has not been demonstrated the presence of GIP receptor in bovine reproductive tissues. The aim of this study was to investigate the mRNA expression of GIP receptor in bovine cumulus oocyte complex (COC). Oocyte-cumulus complex were aspirated from bovine ovaries. The presence of GIP receptor in the bovine COC was evaluated by PCR. We demonstrated that GIP receptor was expressed in the immature COCs bovine.These data strongly suggest that GIP may have a potential regulatory action in the control of oocyte maturation.

A105
A PROANGIOGENIC FACTOR IMPROVES GAMMA-SECRETASE INHIBITOR ANTITUMORAL EFFECT IN OVARIAN CANCER XENOGRAFTS.

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Notch and PDGF systems are involved in angiogenic process in physiological and pathological conditions. Here, we developed tumours in nude mice injecting an epithelial ovarian tumour cell line. SKOV3 cells (1.10^6cells in 100 µl) were inoculated subcutaneously into one flank of 6-10 week-old female nude mice. When the tumours were palpable, the mice were divided in three groups that received 1. Control, 2. DAPT (5mg/kg gamma-secretasa inhibitor), and 3. DAPT+PDGFB (0,1mg/kg). The treatments were administered during four consecutive days (day 1-4). At day 8, the animals were sacrificed and we determined: a. mice and tumour weight, b. tumour area, c. pericyte area and d. phosphor-AKT and PCNA (cell proliferation marker). Mice weight did not change between treatments. Tumour weight significantly decreased when PDGFB was co-administered with DAPT compared to Control group, but no differences were found between Control and DAPT treatments. Tumour area decreased with DAPT but not statistically different respect to Control. Interestingly, the co-treatment completely abolished the tumour growth, being the difference highly significant on days 7 and 8 post treatment. Similarly, the periendothelial area increased with PDGFB and DAPT administration. PCNA levels were significantly decreased in DAPT+PDGF group, but phosphorylated AKT did not change between groups. We conclude that PDGFB improves the antitumoral effect of gamma secretasa inhibitor, in part, recruiting periendothelial cells, stabilizing tumour vasculture, and thus, allowing the inhibitor to better reach the tumour and exert its antiproliferative effect.

A106
PARTICIPATION OF LDH IN CAPACITATION AND ACROSOME REACTION IN PORCINE SPERM.

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The aim of this study was to determine the activity of lactate dehydrogenase (LDH; 1.1.1.27) and evaluate its participation in capacitation and acrosome reaction (AR) in porcine spermatozoa. The activity of LDH was determined spectrophotometrically at 340 nm, during 3 minutes, at 37°C. Enzyme unit (U) was defined as the amount of LDH that catalyzes the oxidation of 1 µmol of NADH/min. Capacitation and AR were determined, in the presence or absence of oxamate (competitive inhibitor of LDH; 10, 25 and 50 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. Enzyme activity and capacitation and AR percentages were analysed by ANOVA and Bonferroni test. The activity of LDH was 3,40±1,21 $U/10^{10}$ sperrmatozoa and the specific activity was 5,02±1,66 U per mg protein. Capacitation and AR were significantly diminished by the addition of 50 mM and 25mM of oxamate, respectively without affecting sperm viability. Our results demonstrate the activity of LDH and its participation in capacitation and AR in porcine spermatozoa, indicating the importance of the fermentative pathway in these processes.

A107
CHARACTERIZATION OF INTRACELLULAR CALCIUM INCREASE IN RESPONSE TO PROGESTERONE IN MOUSE SPERM.

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Sperm acrosome reaction (AR) is an essential step in the mammalian fertilization process. AR requires an increase of intracellular calcium ($[Ca^{2+}]i$). Progesterone (P4) produced by cumulus cells has been recently proposed as AR inductor in several species including human sperm. Few studies reported that P4 promotes an $[Ca^{2+}]i$ increase, however some of their results are contradictory. Our goal is to study the $[Ca^{2+}]i$ increase promoted by P4 using single sperm imaging in fluo-3AM loaded mice sperm. P4 stimulation either with 40 or 100µM promotes a $[Ca^{2+}]i$ increase in a statistically significant percentage of sperm ($p<0.05$). Furthermore five different patterns of $[Ca^{2+}]i$ increase in response to P4 were observed: gradual increase, oscillatory, late transitory, immediate transitory and sustained. The latter two are the most frequent patterns (31 and 30%, respectively). Interestingly we also observed that the $[Ca^{2+}]i$ increase promoted by P4 starts at different regions of the sperm, such as the middle piece or the sperm head. In conclusion, P4 significantly stimulates the percentage of sperm that respond by raising their [Ca2+]i following predominantly two patterns of response.

A108
COPPER DURING *IN VITRO* MATURATION OF BOVINE OOCYTES: ROLE OF CUMULUS CELLS.

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Our previous studies have demonstrated that copper (Cu) improves in vitro maturation (IVM) of bovine oocytes. Cumulus cells (CC) have an important role in the relationship between the oocyte and the microenvironment. The aim of this study was to investigate the role of CC during IVM in the presence of Cu. Cumulus oocyte-complexes (COC) were matured during 24 h with or without 0.6µg/mL Cu sulphate in three maturation systems: intact COC; denuded oocytes with cumulus cells monolayer (DO + CC); and denuded oocytes (DO). Oocytes were fertilized in vitro and presumptive zygotes were cultured in vitro for 8 days. Cleavage and blastocyst percentages were analyzed with a 2x3 factorial arrangement. There were no differences in cleavage rate between COC and DO+CC. The lowest cleavage rates were obtained in DO ($p< 0.05$). In addition, blastocyst rates were significantly higher in COC than in DO + CC and DO ($p< 0.05$). Independently of the presence of cumulus cells (COC, DO + CC or DO), the blastocyst rates were higher when 0.6 µg/ml Cu was added to IVM medium ($p< 0.05$). In conclusion, Cu increased the blastocyst rates regardless of the presence of CC during IVM.

A109
EVIDENCE FOR THE EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTORS IN THE HUMAN TESTIS

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Fibroblast Growth Factor Receptors (FGFRs) have been widely characterized in several tissues; however, there is scarce evidence for their presence and function in the reproductive tissue and gametes. The objective of the present study was to describe FGFR1, 2, 3 and 4 expression in the human testis and in testicular sperm. Using RT-PCR and specific primers, all FGFR transcripts were detected in the human testis, as well as in MCF7 cells used as control. In Western immunoblotting studies, specific bands of the expected molecular weight for the FGFRs were found in testicular protein extracts. The localization of FGFRs in the seminiferous epithelium was analyzed by immunohistochemistry: FGFR1 was found in the nuclei of Sertoli cells, spermatogonia and spermatids, FGFR2 was detected in the nuclei of Sertoli cells, FGFR3 was found in spermatogonia plasma membrane and cytoplasm as well as in spermatid acrosome, and FGFR4 was mainly localized in the nuclei of Sertoli and all germ cells. FGFRs were also immunodetected in the acrosomal region and/or the flagellum of sperm recovered from the testis. In conclusion, our study showed that FGFRs are expressed in the human testis, and localized in Sertoli and germ cells, suggesting that they would participate in spermatogenesis and/or in testicular function.

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A110
ANALYSIS OF DEATH BY APOPTOSIS IN EMBRYONIC AND NEONATAL OVARIES OF *Columba livia* (AVES: COLUMBIFORMES)

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Programmed Cell Death (MCP) is a common step in the germ line during development. The knowledge of these events is essential for the interpretation of processes involved in the MCP of gametes. However, in birds embryonic and neonatal level, there are only references to model species as Gallus gallus domesticus and Coturnix coturnix. The aim of this study was to examine the MCP process linked to the differentiation of oogonia in embryos and hatchlings of Columba livia. Analysis was performed on the expression of Bcl family proteins (Bax, Bcl2). For detecting Bcl2 activity, was used as the primary antibody rabbit polyclonal anti Bcl2 anti mouse origin, and to Bax, the primary antibody anti Bax monoclonal mouse anti-human origin, rat and mouse. Apoptosis was evident in the functional ovary both in marrow and in oocytes, increasing post-hatching stage. These results highlight the death of bone marrow cells and ovarian oocytes during the embryonic stage and post-natal. They serve as a basis for future studies related to the quantification from apoptotic indices at each stage of development.

A111
SCREENING OF TRANSFORMANT CLONES OF THE YEAST *Pichia pastoris* WITH HIGHER PRODUCTION OF RECOMBINANT BOVINE CHYMOSIN

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Bovine chymosin is the preferred proteolytic enzyme for cheese making because it is able to specifically cleave the k-caseins, which causes coagulation of milk. Previously, we have expressed this enzyme in the methylotrophic yeast *Pichia pastoris* under the control of AOX1 promoter which is strongly induced by methanol. This expression system has important advantages such as the efficient production of heterologous proteins with post-translational modifications. Objectives: Determine by the clotting-activity test, transformant clones of *P. pastoris* that produce greater quantities of recombinant bovine chymosin in YPD medium (glucose as carbon source). Conduct a second screening of the selected clones in order to detect transformants which produce high levels of chymosin in basal salt medium (BSM) with glycerol as carbon source. Evaluate the growth of one transformant clone in BSM containing biodiesel-derived crude glycerol. Analyze the stability of the recombinant chymosin under different temperatures (5°C, 20°C and 37°C). Results: From the analysis of 200 transformant clones of *P. pastoris*, we have identified 15 clones that exhibit an increased production of recombinant bovine chymosin in YPD medium. From the second screening in BSM, we determined that five clones showed higher milk-clotting activity. An optimal growth in BSM with biodiesel-derived glycerol was reached, obtaining similar kinetic parameters to those achieved with analytical glycerol. Stability analysis of the recombinant chymosin showed that the coagulant activity remained stable when incubation was performed at 5°C for 90 days.

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A112
BOVINE OOCYTE CO-CULTURED WITH GRANULOSA CELLS. IMPROVEMENT IN *IN VITRO* MATURATION (IVM)

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In vitro maturation of oocytes is a critical step in the performance of *in vitro* embryo production. There are controversies on whether the co-culture with granulosa cells promotes or blocks the oocyte meiotic resumption. The aim of this study was to evaluate the effect of granulosa cells (GCs) on bovine oocyte nuclear maturation. GCs were isolated from follicular fluid obtained from puncturing ovaries with corpus luteum. They were later seeded to confluent and were cryopreserved until required. They were finally thawed and seeded (132 cells/mm^2) in maturation medium (TCM-199, 10% FBS), 24 hours before IVM. Cumulus oocyte complexes (COCs) were punctured from follicles of slaughterhouse ovaries. Oocytes were matured *in vitro* for 22 hours with and without GCs, denuded and stained with Hoechst. Oocytes arrested at metaphase II stage and the presence of the first polar body indicated nuclear maturation. The co-culture significantly ($p \square 0.05$) increased the rate of IVM (83 %, n = 143), as against the control (66 %, n = 83). This method could be used to optimize the competition for the subsequent development of oocytes.

A113

IMMUNOCYTOCHEMICAL EVALUATION OF APOPTOSIS IN SWINE COCs

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The oocyte quality assessment has become greatly relevant due to the increasing demand of biotechnology techniques for the *in vitro* embryos production. Moreover, there are considerable controversies about how apoptosis is associated with oocyte quality. The aim of this study was to evaluate the apoptosis status by immunocytochemical techniques (ICQ) in thin sections of immature *cumulus oocyte complexes* (COCs) previously embedded in historesin and their relation to the morphology. The COCs were aspirated from 3-8 mm follicles from sows`ovaries collected at the abattoir, and under a stereomicroscope were classified according to their morphology in 6 categories, were fixed (4% paraformaldehyde), dehydrated (alcohols) and embedded in hydroxyethylmethacrylate (resin). After the polymerization were cut (1-2 microns) using an ultramicrotome and after removing the resin were performed ICQ (active caspase-3, Bax, Bcl-2). The integrated optical density (DOI) was measured in photomicrographs obtained digitally (DC-180) and analyzed with the soft IM50 (Leica Co.). There was a relation between the presence of apoptosis and the COCs morphology but it is not consistent with the categories into which COCs were classified. However, it was evidenced less intensity staining in cumulus cells and oocytes of good quality COCs. The morphology does not allow for a selection of COCs with no apoptosis. Certain degree of apoptosis would be auspicious on the developmental potential of oocytes.

AUTHOR INDEX