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"To code or not to code: role of non-coding RNAs in physiology and pathology"

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CONFERENCES

A1
TO CODE OR NOT TO CODE: A TRANSCRIPTIONAL QUESTION

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In recent years, noncoding RNAs have emerged as major components of the eukaryotic transcriptome. Long non-protein coding RNAs (lncRNAs) represent an emerging class of riboregulators, which act either directly in a long form or are processed to shorter miRNA and siRNAs. Plant and animals use long and small ncRNAs for post-transcriptional mRNA and epigenetic regulations. Genome-wide RNA sequencing in root apexes identified many Arabidopsis lncRNAs, such as si/miRNA precursors, antisense or intergenic lncRNAs. Their expression pattern suggested a link with the adaptation of root growth and development to the environment. Interestingly, certain 24nt siRNAs derived from the lncRNA APOLO were present in the promoter region of PID, an important regulatory gene controlling phytohormonal responses. The APOLO lncRNA can modulate the dynamic of a chromatin loop encompassing the PID promoter and, consequently, the epigenetic status of this neighbouring locus. Another lncRNA, the ASCO lncRNA, interacted with RNA-binding proteins named NSRs (for Nuclear Speckle RNA-binding proteins) which regulate alternative splicing patterns of several mRNAs. This lncRNA may hijack the splicing machinery by "mimicking" introns and revealed new relationships between lncRNA action and alternative splicing during root organogenesis. Finally, we used a combination of Translating Ribosome Affinity Purification (TRAP) and sequencing of ribosome footprints (RF) to monitor ribosome position and numbers across transcripts and analyse translatome responses of Arabidopsis roots undergoing phosphate (Pi) deprivation. We observed the association of hundreds of known and putative novel long non-coding RNAs (lncRNA) with Arabidopsis ribosomes footprints but also well characterized lncRNAs. Small ORFs prediction, RF distribution and proteomic analysis revealed that lncRNAs represent a reservoir of several hundreds of translated small ORF in Arabidopsis roots. Targeted analysis of a small ORF embedded in the ta-siRNA precursor TAS3 revealed its role in ta-siRNA biogenesis as well as the syntenic conservation of tasiORF positioning inside TAS3 lncRNAs throughout the green lineage. Translatome analysis also identified a new class of lncRNA which are ribosome-associated outside of predicted sORF regions (ra-lncRNA). Hence, ra-lncRNAs may reveal new lncRNA-dependent translational regulations controlling gene expression. Globally, our results support the notion that ncRNAs (both long and small) through their interaction with specific regulatory genes (such as splicing factors or chromatin regulators) may modulate root developmental plasticity. The evolution of the non-coding genome can be integrated into the mechanisms adapting plants to different environments. A general role of lncRNA in evolution and developmental plasticity will be discussed.

A2
NON-CODING RNA FRAGMENTS IN THE EXTRACELLULAR SPACE AND THEIR INVOLVEMENT IN INTERCELLULAR COMMUNICATION

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Interested in the role of miRNAs in cancer pathogenesis, our group transiently moved to RNAi-negative parasitic protozoa in an effort to uncover miRNA-like small RNAs in these organisms. Failing to find miRNAs in T.cruzi, we did find, however, a vast population of tRNA halves derived from specific substrates which were cut at the anticodon loop. By the same time, tRNA halves and other tRNA-derived fragments (tRFs) were described in many organisms as a novel family of small RNAs with regulatory functions which are still being addressed. Surprisingly, we also found that tRNA halves from T.cruzi were secreted by the parasite in membrane shed vesicles which could deliver their cargo to susceptible mammalian cells, implying that tRNA halves could mediate cross-kingdom intercellular communication in a host-pathogen model. In 2012, a team of Chinese scientists described the uptake of rice miRNAs in humans and mice. The uptake of diet-derived plant miRNAs was reported to provoke silencing of a LDL receptor associated protein in the liver, with consequent changes in plasma LDL levels. Thus, RNA-mediated cross-kingdom intercellular communication was extended to a human/diet model. Initially excited by this paradigmatic discovery (although many groups failed to reproduce the same results), we mined human small RNA datasets and found plant and other exogenous RNAs in many human tissues and cells, including the brain and gametes. However, we found conclusive evidence that the original report was affected by lab-derived contamination with plant RNA, a problem which is ubiquitous and extended although usually underestimated. Last, we asked whether tRNA-derived fragments and other small RNAs were also being secreted to the extracellular space by human cancer cell lines. We performed small RNA sequencing in different extracellular fractions of malignant and non-malignant breast cells, finding that extracellular tRNA halves are mainly not associated with extracellular vesicles, and showed patterns of selective secretion (in contrast to miRNAs). We are currently characterizing extracellular transport and function of tRNA-derived fragments, which constitute the most abundant small RNA population in the extracellular space

Pre-CONGRESS COURSE &SYMPOSIA

PRE-CONGRESS COURSE

A3
LONG NON-CODING RNAs IN THE CONTROL OF EPIGENETICS AND GENOME TOPOLOGY

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During the last years, long noncoding RNAs (lncRNAs) have been linked to a wide range of mechanisms impacting gene expression regulation, including chromatin remodeling, the modulation of alternative splicing and miRNAs activity, as well as the accumulation and translation of mRNAs. In plants, lncRNAs can be transcribed by RNA Pol II, or alternatively by Pol IV and V, participating in RNA-directed DNA Methylation (RdDM). In this talk, we will analyze the case of the *APOLO* lncRNA, from *Arabidopsis thaliana*. *APOLO* is transcribed by Pol II, as well as Pol IV and V, and is involved in the regulation of a chromatin loop encompassing the promoter region of its neighboring gene, *PID*. In response to the phytohormones auxins, the loop is open and *APOLO* and *PID* are divergently transcribed. Then, *APOLO* recruits Polycomb and DNA remodeler proteins, reestablishing the epigenetic landscape of the locus and conforming again the chromatin loop. As a result, *PID* and *APOLO* transcription is finally repressed, fine-tuning their dynamic behavior.

A4
SMALL RNAs, RNA QUALITY CONTROL IN THE PLANT VIRUS INTERACTION.

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To establish successful infections, plant pathogens cause profound alterations in the host's physiology by disturbing endogenous processes that in turns contribute to the development of disease symptoms. These effects are the main cause of yield losses in crops. It has been shown that viral infection produces a major genomic reprogramming that could explain the observed phenotypes. The alteration of the expression of some microRNAs produces phenotypes similar to the viral symptoms, it also has been demonstrated that the viral infections produce great changes in the accumulation profile of diverse small RNAs (sRNAs) of the plant that propose an important regulatory role of sRNAs in the transcriptomic reprogramming associated to virus infection In this class, we will give a general overview of the role of sRNAs in plant viral infection processes and the production of disease symptoms. We will discuss the interrelationship of the immune defense system called silencing and the viral suppressor proteins and their impact on the biogenesis of microRNAs. The relation of the alteration of microRNAs and the alterations of the development that produce the symptomatology. A general idea of the quality control system of mRNAs (RNA Decay) and its impact on viral infection and its consequences in the reduction of the level of immunity mediated by this alteration will be given. Finally, we will address the impact of alteration of sRNAs mediated by viral infection on the epigenome and its consequences on plant virus interaction.

SYMPOSIUM OF BIOLOGY SOCIETIES

A5
miRNAa IN AIRWAY DISEASES

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miRNAs are endogenous ~23 nt RNAs that play important gene-regulatory roles by pairing to the mRNAs of protein-coding genes to direct their post-transcriptional repression. These small molecules are involved in a wide range of physiological responses, including development, differentiation and homeostasis. Recent findings point out that miRNAs may also be involved in modulating corticosteroid sensitivity by regulating the expression of the active glucocorticoid receptor (hGR□□. Understanding the molecular mechanisms underlying resistance to corticosteroid is an essential requirement for the development of new therapeutic approaches. In tuberculosis, previews experiments performed in the lab showed that patients develop an imbalanced immune-endocrine response which may explain the increased inflammation and tissue destruction that occur during

the course of the infection. Our studies comparing miRNA expression patterns in patients with active pulmonary tuberculosis and tuberculous pleurisy, a benign and self-limited form of the disease, showed that several miRNA are differentially expressed in infected individuals and identified miR-30c as a specific marker of pulmonary manifestations of tuberculosis potentially involved in modulating glucocorticoid sensitivity in these patients. Synthetic glucocorticoids are also employed as anti-inflammatory drugs for the treatment of many chronic inflammatory and immune diseases, being the most effective therapy for asthma yet relatively ineffective in chronic obstructive pulmonary disease. Additional studies are being performed in patients tending to identify miRNAs involved in modulating corticosteroid sensitivity in these airway diseases in order to find common regulatory features that could constitute new therapeutic targets with possible pharmacological applications.

A6
ANDROGENS AND CARDIOPULMONARY RISK. MARKERS OF MORBIDITY

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Sex differences in diverse organ biology and the influence of sex hormones in modulating health and disease, are increasingly relevant in many clinical areas. Testosterone deficiency (TD) is a relatively common condition, with a considerable prevalence in 40–69 years old men. Decline in testosterone, shown to occur with aging, may negatively affect health and quality of life. Professional guidelines recommend testosterone replacement therapy (TRT) in patients with signs and symptoms of hypogonadism and documented evidence of low testosterone (T) levels. Furthermore, there are a predominance of respiratory dysfunction patients in the ages between 76 to 85 years old, as well as elderly patients with respiratory diseases had more comorbidity. Both situations have conduced to analyzed cardiopulmonary systems altogether.

Normal lungs have androgen receptors, and the number of these receptors varies with age, sex and hormonal conditions. Our data, in adult rat demonstrates that lung phospholipids are increased with testosterone deprivation. Phospholipids composition of microsomes and extracellular surfactant in the male rat lung is highly dependent on androgenic stimulation, as far as lung damage during testosterone absence. In order to elucidate the possible causes of pulmonary diseases with decrease or eventual deprivation of testosterone, we study the effect of androgen on oxidative stress and cytoprotective markers. Taken together, all the results suggest that androgen absence induce oxidative stress and lipid peroxidation, synchronically with changes in cytoprotective markers expression, in lung. This would lead to weak lung stroma, susceptible to undergo several pulmonary diseases. On the other hand, new results showed that oxidative stress caused by testosterone deficiency also affects aorta artery. All situations suggest that cardiopulmonary system should be regarded as a whole system, which can be modifying by gonadal steroid imbalances. Finally, new injury biological markers and level of testosterone have improved the management of the patient's clinical status.

A7
ROLE OF NONCODING RNA IN VASCULAR REMODELING

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Vascular remodeling consists in the excessive accumulation of ded ifferentiated smooth muscle cells (SMC) in the intima of arteries and arterioles. This process is central to a number of vascular diseases such as arteriosclerosis, pulmonary hypertension and chronic obstructive pulmonary diseases. In contrast to other cells, differentiated SMC retain high plasticity in response to environmental cues. After an injury, differentiated/contractile SMC undergo phenotypic modulation to a proliferative/dedifferentiated phenotype, which is tightly regulated by different molecular pathways. In the last decade, it has become evident that noncoding RNAs (ncRNAs) control cell biology in physiological and pathological states. MicroRNAs (miRNAs) are small RNAs that post-transcriptionally inhibit expression of target genes. We analyzed the expression profile of miRNAs in highly remodeled pulmonary arteries compared to control arteries. We found that a number of miRNAs are dysregulated in SMC during pulmonary vascular remodeling. This alteration is linked to defects in SMC homeostasis by controlling genes related to cell cycle. Another less studied small RNAs are the YRNAs. Very little is known about these molecules and their mechanism of action. We have analyzed the function of YRNAs during in vitro SMC differentiation and found that hY3 regulates SMC phenotypic switch. In the presentation, a short summary of our results will be discussed.

A8

Lactobacillus casei: A POTENTIAL AGENT MODULATOR OF IMMUNO-COAGULATIVE RESPONSE

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Inflammation and coagulation influence each other in a bidirectionalprocess. Inflammatory challenges of a certain severity are capable of producing a hemostatic imbalance, activating coagulation and inhibiting regulatory mechanisms. Lactobacillus casei, a probiotic lactic acid bacteria, can exert their beneficial effect on the host through their immunomodulatory activity. Our group demonstrated that oral administration of Lactobacillus casei CRL 431 (Lc) was effective to regulate coagulation activation and fibrinolysis inhibition caused by an inflammatory process in experimental murine models of pneumopathy and acute inflammation induced by lipopolysaccharide. Furthermore, repletion of malnourished mice with supplemental Lc given by oral route was able to beneficially modulate the inflammation-coagulation relationship during the pneumococcal infection. This conference synthesizes the underlying mechanisms of the interaction between inflammation and coagulation, and highlinghting our findings on Lactobacillus casei capacity to regulate the immune-coagulative response favoring the rapid recovery of the haemostatic balance. These Lc effects might be helpful to propose improved new therapies for patients with severe systemic injuries and septic shock.

YOUNG SCIENTISTS SYMPOSIUM

A9

DEVELOPMENT OF A NEW VACCINE ADJUVANT, STUDY OF ITS MECHANISM OF ACTION AND USES THEREOF

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In our laboratory we are working with a Brucella abortus protein called U-Omp19. We demonstrated that U-Omp19 is a broad spectrum protease inhibitor. U-Omp19 inhibited the aspartic protease pepsin, serin proteases (pancreatic elastase, trypsin and α-chymotrypsin), and cystein proteases (cathepsin L, B and S). Stability studies showed that U-Omp19 retained its full protease inhibitor activity when previously exposed to a broad pH (2-8) or temperature (25-100°C) range. U-Omp19 behaves as an important component of vaccine formulations against infectious diseases. When co-delivered orally with an antigen (Ag), U-Omp19: i) can bypass the harsh environment of the gastrointestinal tract by inhibiting stomach and intestine proteases and consequently increases the half-life of the co-delivered Ag at immune inductive sites: Peyer's Patches and mesenteric lymph nodes while ii) it induces the recruitment and activation of antigen presenting cells (APCs) and increases the amount of intracellular Ag inside APCs. Besides, U-Omp19 reduces the amount of digested Ag withinAPCs at inductive sites increasing Ag cross presentation. Therefore, mucosal as well as systemic Ag-specific immune responses, antibodies, Th1, Th17 and $CD8^+$ T cells are enhanced when U-Omp19 is co-delivered with the Ag orally. Finally, this bacterial protease inhibitor in oral vaccine formulations confers mucosal protection against LT or CT-induced diarrhea, and reduces bacterial or parasite loads after oral challenge with virulent *Salmonella*, enterohemorrhagic *Escherichia coli* O157:H7or *Toxoplasma gondii*.

A10

IMPLEMENTING PERSONALISED MEDICINE IN MELANOMA PATIENTS

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BRAF is mutated in about 50% of human melanomas and treatment with BRAF or MEK inhibitors have resulted in increased progression-free and overall survival in melanoma patients. However, the majority of patients relapse after a relatively short period of disease control. Furthermore, after treatment with targeted therapy, most patients derive little benefit from immune checkpoint inhibitors. Resistance to targeted agents is driven by several mechanisms, so selecting second line therapies is challenging. Current advice includes the option to continue treatment beyond progression, but it is unclear how to select the patients that will benefit from this, so detecting disease progression early and elucidating the mechanisms of resistance to therapy will help optimise the clinical care of these patients. Treatment options are also needed for the ~50% of melanoma patients who are BRAF wild-type. We used whole exome sequencing (WES) to provide insight into the mechanisms of resistance to BRAF

inhibition and identifynew therapeutic strategies for BRAF wild-type melanomas. We present the case of a patient that was wild-type for V600 BRAF, but carried HRAS and Rb1 mutations, allowing us to predict that the patient's tumour would be sensitive to the combination of a MEK inhibitor plus paclitaxel and we validated this therapy in a xenograft derived from the patient (PDX). Thus we show that genome analysis can be used to develop novel hypothesis-driven therapeutic strategies for patients and we show that these treatments can be validated in the patients' PDXs. Finally, we describe the use of circulating tumour DNA (ctDNA) as a predictive biomarker of response to therapy and as a powerful approach to reveal and then monitor mechanisms of resistance. In summary, we are implementing a powerful combination of techniques for personalised medicine to improve clinical management of BRAF wild-type and BRAF mutant melanoma patients.

EXPERT SCIENTISTS SYMPOSIUM

A11
SMALL, CIRCULAR AND ESSENTIAL: CONTROL OF NEURONAL MATURATION BY microRNAs AND CIRCULAR RNAs

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MicroRNAs (miRNAs) are conserved noncoding RNAs that function as posttranscriptional regulators of gene expression. MiR-9 is one of the most abundant miRNAs in the brain. Although the function of miR-9 has been well characterized in neural progenitors, its role in dendritic and synaptic development remains largely unknown. In order to target miR-9 in vivo, we developed a transgenic miRNA sponge mouse line allowing conditional inactivation of the miR-9 family in a spatio-temporal-controlled manner. Using this novel approach, we found that miR-9 controls dendritic growth and synaptic transmission in vivo. Furthermore, we demonstrate that miR-9-mediated downregulation of the transcriptional repressor REST is essential for proper dendritic growth. Circular RNAs (circRNAs) have emerged as a large class of animal RNAs with complex tissue- and stage-dependent expression patterns. Most of them are derived from protein-coding genes and can be distinguished from their linear counterparts by their remarkable continuous closed loop structure. We have recently revealed, by comprehensive sequencing and analysis of ribosomal-depleted RNA from 29 different types or stages of neural cells and tissues, a high and specific expression of circRNAs in the brain. In addition, we show that circRNAs are upregulated during neuronal differentiation and that many circRNAs exhibit an independent dynamic compared to linear transcripts derived from the same gene. Interestingly, our study strongly suggests that circRNAs are not equally distributed in the neuronal compartments, but are highly enriched in the synapses. The highest expressed circRNAs, both in the whole brain and in synaptic fractions, are being currently investigated for functional characterization.

A12
MicroRNAs REGULATION OF BREAST CANCER GROWTH, METASTASIS, AND RESPONSE TO THERAPY

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We revealed a novel mechanism driving ErbB-2-positive breast cancer (BC) growth, where ErbB-2 stimulation of Erk1/2 and PI3K/AKT cascades induces c-Myc transcriptional activation. Activated c-Myc then binds to the microRNA-16 (miR-16) promoter and represses miR-16 expression. This results in increased levels of two newly identified miR-16 targets, FUBP1 and CCNJ, both of which promote BC proliferation. Furthermore, we found that nuclear actions of ErbB-2 up-regulate the expression of the oncogenic miR-21, which mediates ErbB-2-induced BC metastasis.

SHORT COMMUNICATIONS

REPRODUCTION I

A13
EMBRYONIC GONADS OF HORMONAL SEX DETERMINED *Caiman latirostris*FEMALES DIFFER FROM THOSE OF TEMPERATURE SEX DETERMINED EXHIBITING AN UNBALANCE BETWEEN PROLIFERATION AND APOPTOSIS

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Ovarian development in *Caiman latirostris* embryos can be triggered by any of these two factors: temperature (TSD) and estrogenic action known as hormone-dependent sex determination (HSD). To obtain a healthy population of oocytes in the adulthood, during the female gonadal differentiation process, germinal cells exhibit proliferation, meiosis and apoptosis. In this context, a fine tuning of the proliferation/apoptosis balance is expected. In stage 22, 24 and 27 of embryonic development (embryonic gonad differentiation period) of TSD and HSD females, we assessed by immunohistochemistry the expression of Proliferating Cell Nuclear Antigen (PCNA) and TAp63 isoform (p53 family member). In mammals, TAp63 participate in the oocyte DNA damage response check point, one of the early events in the apoptotic-mediated selection process. Regarding cell proliferation, we observed higher expression of PCNA in the stage 22 of the HSD group. Also, we detected specific TAp63 immunoreactivity in caiman embryos germ cells (previously identified by VASA), being higher in the gonads of HSD females in all embryonic stages. In our knowledge, this is the first report describing the expression of TAp63 during the gonad development of a reptile. Increased expression of TAp63 suggests increased DNA damage of germ cells in the gonad of HSD females thus increased germ cell apoptotic death could be expected. Proliferation/apoptosis unbalance could produce significant alterations of the ovarian oocytes reserve during adulthood.

A14
EARLY EVENTS OF THE APOPTOTIC PROCESS IN CHILLED BOAR SPERMATOZOA

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Boar sperm is highly susceptible to temperatures below 15°C which makes them very sensitive to freezing. Classical methods of semen assessment have low power in predicting fertility. Low rates of sperm with apoptotic changes lead to better results in artificial insemination. The aim of this study was to assess early events of the apoptotic changes as indicators of damage in boar sperm cooled to 5°C. Eight sperm pools from ejaculates of three boars were used. Routine assessments of sperm quality -sperm motility (SM), plasmatic and acrosomal (AMI) membrane integrity, plasmatic membrane functionality (HOS) and early apoptotic changes: translocation of phosphatidylserine phospholipid (PS) and mitochondrial membrane potential (Ψm) were carried out at 37ºC and at the end of the stabilization plateaus (17ºC and 5ºC). Kruskal Wallis test was applied ($\alpha \leq 0.05$). Significant differences between 37ºC and 5ºC (SM:84.4±1.1%; 50±3.1%; HOS:69.9±1.5%; 53.2±3.3% AMI:94.6±0.5%; 86.5±2.8%), an increase in the number of spermatozoa with low Ψm at 5ºC (36.9±3.8%; 58.1±7.2%) and a significant increase of necrotic cells (25.3±5%; 38.5±5.68%) were observed, indicating that the sperm cooling to 5ºC affected the boar sperm viability. However, there were no increases in sperm subpopulation with PS translocation through the plasmatic membrane. Based on our results, damages in the cooled semen samples could be mediated by other mechanisms different from apoptosis.

A15
CHARACTERIZATION OF SPHEROIDAL EXPLANT CULTURE FROM OVIDUCTAL PORCINE CELLS

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Oviductal epithelial cells cultured in monolayers or in suspension (spheroidal vesicles) were developed to improve the performance of the in vitro embryo production in porcine. It would help to understand the interactions that are physiologically established in vivo between the oviduct and the embryo. The aim of this study was to obtain and characterize explant cultures as spheroidal vesicles (SV) of porcine oviductal cells. Culturing was carried out with sow oviducts which ovaries had corpus luteum. The cell suspension was obtained by external soft pressure with slide glass, passed through vortex and decanted in an

incubator. The cell suspension at a concentration of 3 x 10^5 per coverslips was strewn in culture dishes with DMEM supplemented with FBS, gentamicin and fungizone. As a result, a mixed monolayer culture and SV were obtained. Characterization was assessed by fresh observation, hematoxylin stain (H) and immunohistochemistry (IHC) anti E-cadherin, to determine purity of the culture. The monolayer oviductal cells showed a radially defined orientation, with lipid cytoplasmic vacuoles negative to H stain. Besides, It could also be observed that SV had their own mobility due to cilia, which were lost between 48h and 72h post strew. After that, the SV clung and in some cases were associated to the monolayer. The E-cadherin positive mark indicated the epithelial origin of the cell culture. It can be conclude that the culture model characterized in this work lays the foundation and is potential to be used, in future, as co-culture systems with porcine embryos.

A16
MACROSCOPIC AND MICROSCOPIC CHARACTERISTICS OF THE STALLION TESTIS AND EPIDIDYMIS AROUND PUBERTY

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During puberty, several changes take place in the mammalian testis and epididymis. Despite its relevance, few reports are available with detailed information on the stallion testis and epididymis around puberty. The aim of this study was to perform a systematic analysis of 18 peripubertal and 55 postpubertal stallion testes and epididymides to determine a set of macroscopic (anatomic and morphometric) and microscopic (histomorphometric and cellular) characteristics. Macroscopic: Postpubertal testes showed dark parenchyma pigmentation (78%) while peripubertal testes showed clear parenchyma pigmentation (89%; $p<0.0001$). Postpubertal testes and epididymides showed higher ($p<0.0001$) weight, volume and size than peripubertal tissues. Volume differences were also found when comparing peripubertal and postpubertal caput, corpus and cauda epididymis ($p<0.05$). Microscopic: Only seminiferous tubules of postpubertal testes showed all germinal cell stages. Postpubertal corpus and cauda had higher ($p<0.005$) lumen diameter and perimeter than peripubertal epididymal segments. Also, postpubertal caput and corpus had higher ($p<0.005$) epithelium height and sterocilia length than peripubertal epididymal segments. Finally, postpubertal epididymides had higher ($p<0.005$) muscle layer thickness and cell layer number in all segments compared to peripubertal tissues Altogether, our findings revealed a higher anatomical and histological development of postpubertal than of peripubertal reproductive tissues.

A17
SEASONAL EFFECTS ON EPIGENETIC MACHINERY GENE EXPRESSION IN BOVINE OOCYTE

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Santiago del Estero became in one of the regions most important livestock production in Argentina. In this region predominate very high temperatures which often exceed 40°C in the summer. Mammalian oocytes acquire their meiotic competence and fertilization potential in a stepwise manner. As a result, they are potentially exposed to various environmental stressors during follicular development. During oogenesis, epigenetic modifications are established which are required for normal embryonic progression. DNA methylation is the archetypal epigenetic mark. In the oocyte, an overall increase in global DNA methylation occur during the growth phase. In this study, we examined the association between seasons and expression of genes playing an essential role as part of epigenetic machinery in immature bovine oocyte. We analyzed the expression levels of DNA methyltransferases DNMT1, DNMT3a and DNMT3b; demethylase TET3 and STELLA, a maternal factor that protect the genome from active DNA demethylation that occurs soon after fertilization. Also, we examinated the expression of PRDX1 involved in oxidative stress response, heat shock protein 70 (HSP70), BMP15 and GDF9. Bovine ovaries were obtained from a local abattoir from Braford cows during the summer (jan-feb) and winter (jun-jul) seasons. Cumulus–oocyte complexes (COCs) were aspirated from 3 to 8 mm follicles. The oocytes were pipetted vigorously to ensure that they were fully denuded. To determine cross-contamination we carried out a semi-quantitative PCR using marker genes for oocyte: ZAR, granulosa cells: CYP 19A1 and thecal cells: CYP 17A1. Real-time PCRs were carried out with GAPDH and 18S rRNA as reference genes. STELLA expression was lower in oocyte from cold season versus the hot season counterparts. Results show a tendency of lower DNMT3a mRNA level in winter. As long as TET3, DNMT1, DNMT3b and HSP70 expression had no significative differences. We found that GDF9 and BMP15 mRNA expression decrease in cold season versus hot season and PRDX show a tendency of lower expression in cold season. Taken together, these results suggest a deleterious seasonal effect on gene expression of epigenetic machinery and oocyte developmental competence.

A18
OXIDATIVE AND MITOCHONDRIAL ACTIVITIES IN VITRIFIED PORCINE OOCYTES

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To date, porcine oocyte vitrification presents suboptimal results, which could be due to changes in their redox state. Therefore, the aim of this study was to evaluate oxidative and mitochondrial activities on in vitro matured porcine oocytes after vitrification-warming. Cumulus-oocyte complexes were collected by aspiration of antral follicles and matured in medium 199 + porcine follicular fluid + gentamicin sulfate + FSH + LH under mineral oil at 39ºC for 48h in 5% CO_2 atmosphere. Then, oocytes were denuded and vitrified by Cryotech© method. Matured non-vitrified porcine oocytes were used as a control group. Oxidative and mitochondrial activities were determined by RedoxSensor Red CC-1/MitoTracker Green FM dual stain at 0, 3 and 21h after warming. For Mitotracker Green FM stain, both vitrified and fresh mature oocytes showed a decrease in luminosity at 21h ($p<0.05$), but vitrified oocytes presented higher values when compared with fresh oocytes at each time point ($p<0.05$). With RedoxSensor Red CC-1, fresh mature oocytes presented a significant decrease in luminosity at 3 and 21h compared with 0h ($p<0.05$), while in vitrified oocytes luminosity did not change during the evaluated times. Comparing vitrified versus control group, there was only a significant increase at 3h in the vitrified group ($p<0.05$). In conclusion, in vitro matured porcine oocytes presented higher oxidative and mitochondrial activities after being submitted to the vitrification-warming processes.

A19
COMPENSATORY ENDOCYTOSIS OCCURS AFTER CORTICAL REACTION DURING MOUSE EGG ACTIVATION

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The fertilizing sperm triggers in the egg a series of events collectively referred to as "egg activation". Among them, massive exocytosis of cortical granules occurs a few minutes after fertilization and it is involved in the block to polyspermy. It has been described that after exocytotic activity cells maintain their surface area by triggering compensatory endocytosis (CE) mechanisms, with the retrieval of excess surface membrane. In particular, CE occurring in sea urchin eggs is essential to maintain embryonic cell surface homeostasis during early development. Therefore, the aim of this work was to evaluate the occurrence of CE as a consequence of the cortical exocytosis in mammalian eggs. For this purpose, we developed pulse-chase experiments employing rhodamine-coupled Lens Culinaris Agglutinin (LCA), which binds to cortical granule exudate. First, zona-free mouse eggs were activated by different agents (i.e., 10mM Sr^{2+} and 10uM Ca^{2+} ionophore A23187), and incubated at 4°C for 30' in the presence of LCA (pulse). Then, cells were incubated for 30' at 4°C or 37°C to allow the internalization of membrane-attached LCA (chase) and analyzed by confocal microscopy. Results showed a massive labeling internalization in Sr^{2+}-activated eggs when chasing at 37ºC, that was not detected after activation with A23187, highlighting once again the mechanistic differences between activation methods. Moreover, when the assay was performed in the presence of different CE inhibitors (25nM staurosporine or 1uM cyclosporin A), a significant decrease in the internalized labeling of Sr^{2+}-activated eggs was observed. Finally, fluorescence internalization was also observed in in vitro fertilized eggs. Altogether, these results demonstrate for the first time the occurrence of a CE mechanism after massive cortical granule exocytosis, as a consequence of egg activation.

A20
PRETREATMENT OF RAM SPERM WITH CHOLESTEROL AND DESMOSTEROL FOR CRYOPRESERVATION INDUCES CHANGES IN BIOPHYSICAL PROPERTIES AND IMPROVES COLD SHOCK RESPONSE

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Due to its lipid composition, ram sperm is particularly sensitive to freezing hazards. Incubation with cholesterol loaded Mehyl-β-Cyclodextrin (MβCD) enhances cryotolerance of sperm in several species. The use of desmosterol, the immediate precursor of cholesterol, in cryopreservation of ram sperm has not been evaluated. The objective of this study was to investigate the effect of adding different concentrations of cholesterol and desmosterol to ram sperm membranes on: i) sterol incorporation and membrane biophysical properties, and ii) sperm membrane integrity and hipoosmotic swelling test (HOSt) response during cooling, freezing and thawing. We determined that treatment of sperm with 2.5, 10 and 25mM MβCD-cholesterol or -desmosterol increases sterol content measured by an enzymatic method and BODIPY-cholesterol labeling. The latter allowed us to localize incorporated sterols in the cells, thus showing that cholesterol was incorporated mainly in the acrosome region and that desmosterol was distributed both in the acrosome and in the midpiece. Biophysical studies revealed that cholesterol incorporated into sperm membranes increases lipid order at temperatures ranging from 4-38ºC, as determined by fluorescence

spectroscopy (Laurdan). MβCD treatment with both sterols improved cold shock response in terms of membrane integrity (eosin nigrosin staining) and HOSt. After thawing, the percentage of sperm with intact membrane was higher in the control and in 2.5 mM MβCD-sterol with respect to the other concentrations evaluated. No differences were found between control and treated sperm in HOSt. We conclude that increasing sterol membrane content at 2.5 mM MβCD complexed either with cholesterol or desmosterol induces a higher membrane lipid order and increases ram sperm membrane integrity and osmotic tolerance after refrigeration. Whether these effects impact on additional sperm functional parameters in cryopreservation remains to be established.

A21

METABOLIC PROFILE OF MATURED BOVINE OOCYTES VITRIFIED WITH CRYOTECH®

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The objective was to evaluate reactive oxygen species (ROS) production and redox state in vitrified matured bovine oocytes. Cumulus-oocyte complexes were obtained by aspiration of antral ovarian follicles and matured in 199 medium with bovine fetal serum, FSH and LH at 39°C, 5% CO_2 in humidified air for 22h. Matured oocytes were denuded and a group was vitrified through the minimum volume vitrification method Cryotech®. Then vitrified oocytes were subjected to warning. Later we measured the autofluorescence intensity of NAD(P)H at three different times (0, 3, 21h; n= 180 oocytes). To evaluate ROS production we incubated bovine oocytes (n= 180 oocytes) in 2'-7'-dichloro-dihydro-diacetate fluorescein (DCHFDA), determining the ratio between DCHFDA fluorescence and average diacetate fluorescein brightness. As regards the oocyte redox state the control group presented a significant increase at 21 compared with 0 and 3h ($p<0.05$), while in vitrified oocytes autofluorescence did not change during the evaluated times and no significant differences were detected between both groups at any time point. On the other hand, ROS production presented a significant decrease at 3 and 21 compared to 0h in both groups ($p<0.05$), but no significant differences were obtained between the control and the study group at each time evaluated. To sum up, the minimum volume vitrification method Cryotech® procedure does not modify the metabolic profile of bovine oocytes. Further studies are necessary to evaluate the competence of vitrified oocytes in this species.

A22

IN VITRO CAPACITATION AND ACROSOME REACTION IN FRESH AND CRYOPRESERVED PORCINE SPERMATOZOA. PARTICIPATION OF LACTATE DEHYDROGENASE

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The aim of this study was to determine the activity of lactate dehydrogenase (LDH; 1.1.1.27) and study its participation in the processes of the in vitro capacitation and acrosome reaction (AR) in fresh and cryopreserved porcine spermatozoa. Fresh and cryopreserved spermatozoa were incubated in TBM medium with bicarbonate and follicular fluid as capacitation and acrosome reaction inducers, respectively in the presence of different concentrations of sodium oxamate (competitive inhibitor of LDH). Capacitation and AR percentages were determined by the CTC technique and trypan blue combined with DIC, respectively. Sperm viability and motility were evaluated by the eosin/nigrosin technique and optic microscopy in thermal stage, respectively. The LDH activity was determined spectrophotometrically at 600 nm, during 2 minutes, at 37°C and the enzyme unit (U) was defined as the amount of LDH that oxidizes 1 µmol of NADH/minute. The results were analysed by ANOVA and Bonferroni test. The addition of the competitive inhibitor of the enzyme significantly diminished capacitation and AR without affecting sperm viability, at different concentrations in fresh (50mM for capacitation and 25mM for AR) or cryopreserved sperm (1 mM for both processes).These concentrations also inhibited the activity of LDH in fresh (76,8 ± 6,1 and 86,9 ± 7,5 % of inhibition for 25mM and 50mM, respectively) and cryopreserved spermatozoa (73,0 ± 16,4 % of inhibition for 1 mM). Our results demonstrated the differential participation of the enzyme lactate dehydrogenase in the processes that are involved in the acquisition of the fertilizing ability in fresh and cryopreserved spermatozoa. This information can be useful for the formulation of incubation media to use in biotechnological reproduction techniques.

ANIMAL BIOLOGY

A23
BEHAVIOR AND HABITAT USE BY *Puma concolor* IN A PERI-URBAN LANDSCAPE: PRELIMINARY RESULTS

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The mountain lion (Puma concolor) is the American felid with widest distribution. In Argentina it is present throughout the national territory except in Tierra del Fuego province. The expansion of the farming frontier, development of urban centers, fragmentation of the original habitats available is exposing the feline to increasingly encounter with humans. In the last months of 2016 it was recorded cougar presence closed to urban areas of Buenos Aires province, one in Mar del Plata city and the other to Pehuen Có village. The aim of this project is to investigate the territorial behavior and habitat use of a cougar population in the proximity of Pehuen Có. Between December 2015 and September 2016 a survey of indirect (n=49) and direct (n=4) evidences of P. concolor was carried out. Most records belong to adults. 43% of them were tracks, 23% scrapes, 15% feces, 11% scratches on trees and finally the 8% were sightings. The environment most used by cougars adults were pine forests surrounding the village (<1000 m). This information represents a novel evidence of the presence, behavior and use of the environment by the cougar in peri-urban habitats. The closeness of the species to the town is a challenge to preserve these landscapes. The development of an education program and management plans could prevent future negative incidents.

A24
TROPHIC SPECTRUM OF THE NEOTROPIC CORMORANT (*Phalacrocorax brasilianus*) IN SAN MIGUEL DEL MONTE LAGOON

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The Neotropic Cormorant Phalacrocorax brasilianus inhabits freshwater and marine environments from the Southern USA to the Horn Cape. It is an ichthyophagous species which shows a great ecological plasticity. We analyzed the Neotropic Cormorant diet at the San Miguel del Monte Lagoon, Buenos Aires province. We studied 24 stomach contents, identifying fish species through otoliths and bone remains. The 8 % of the stomachs were empty, and a total of 240 otoliths were found in the other 22 ones. The most frequent species was the Sabalito Cyphocharax voga present in 67 % of stomachs followed by the Vieja del agua Hypostomus commersoni and the Mojarra Astyanax eigenmanniorum. We also found other 9 species with representatives of other 4 orders (Clupeiformes, Siluriformes, Atheriniformes, Cyprinodontiformes, and Perciformes). In an ichthyological survey carried out in this lagoon the most represented species was C. voga. This results show that Neotropic Cormorant forage on fish species that are most abundant in Del Monte lagoon. Our study shows the large trophic spectrum of the Neotropic Cormorant, and also shows that it does not prey significantly on fish species of commercial importance. The Mojarra A. eigenmaniorum, Madrecita Jenynsia multidentata, and Chanchita Australoheros facetus as Neotropic Cormorant prey items are mentioned for the first time.

A25
ESTIMATION OF PARASITE-PREY ASSOCIATION IN PELLETS OF TWO PATAGONIAN CORMORANT SPECIES

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Helminth parasites of marine birds usually have indirect life cycles and are mostly acquired through the diet. Usually, Anisakidae use fish as intermediate before reaching piscivorous birds. Objectives were to identify prey items, Anisakidae found in pellets from both the Imperial Cormorant Phalacrocorax atriceps and Red-legged Cormorant P. gaimardi; and to establish parasite-prey association between prey items and anisakids. A total of 82 pellets of P. atriceps and 72 of P. gaimardi from Punta León colony, Chubut, and Isla Elena, Ría Deseado, Santa Cruz, respectively. The parasite-prey association was estimated using Fisher`s Test, and Spearman's Correlation. The Imperial cormorant's diet included 22 fish, 11 crustacean, 6 mollusk, and 3 polychaete species. Red-legged Cormorant diet consisted of 6 fish, 2 cephalopods, 1 polychaete, and 1 crustacean species. Pseudoterranova was the

most prevalent and intense (P=65, MI=4) in P. atriceps. On P. gaimardi, Hysterothylacium (P=60) was the most prevalent and Contracaecum the most intense (MI= 4). The highest significant parasite-prey associations were those of Contracaecum L3 and the Fuegian Sprat Sprattus fueguensis, and the squid Loligo gahi in P. gaimardi. On P. atriceps, Contracaecum was associated significantly with Paralichthys sp., Engraulis anchoita, Gammaridea, Raneya brasiliensis, Patagonotothen sp., Ostracoda, and finally Percophis sp. These results suggest that pellets can be postulated as good indicator of parasite-prey association between cormorant prey items and anisakid species, since all information they can provide about cormorant feeding habits and parasite burdens and diversity.

A26
ENZYMATIC STABILITY OF CRUDE GASTRIC EXTRACT FROM PACÚ (*Piaractus mesopotamicus*)

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Pacú (Piaractus mesopotamicus) production represents 40% of northeast fish yield. It is of interest to valorize the current disposal of fish processing through the use of viscera waste, source of enzymes such as Pepsin. The objective of this work was to obtain a pepsin-enriched extract from gastric mucosa and analyzed its enzymatic stability. Samples were provided by "La Elina" establishment. Preparation of crude extract was made by mechanical and sonic digestion of gastric mucosa and pepsin activity was estimated by acid hemoglobin method. Enzymatic stability was evaluated under different conditions of pH (1-8), temperature (0-80ºC, 2h exposure) and time (1-120 h, at room temperature). Results indicated that the effect of pH was evident with values above 5, decreasing enzymatic activity as pH increases. After two hours of exposure, optimal temperature was defined at 25ºC, when the extract was incubated at 45ºC activity starts to decline and finally showed to be null at 60ºC and 80ºC. Regard to stability in time, enzymatic stability was not affected even after 5 days at room temperature. In conclusion, this information will be primordial for ulterior purification process of pepsin and the obtainment of a high quality and stable enzyme. Moreover, efficient utilization of by-products has direct impact on the economy and environmental pollution.

A27
NUTRICIONAL VALUE OF SPECIES OF TUCUMAN SCRUBLAND AND THEIR USE AS FOOD TO GOAT LIVESTOCK

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In extensive and small production systems we find a variety of food resources but sometimes the use of them is inappropriate.The small producer uses the forage resource that provides the scrubland, as nutritional supplement or as main base to feed their animals. Objective: To assess the nutritional contribution of Tucumán scrubland, species used as forage resource by small goats farmers . Materials and Methods: Two goats establishments were surveyed to compare the species used in the feeding of goats in the area of Taco Ralo, during the fall and winter with low rainfall Sampling in a three species in each farm. Analytical methods to determine crude protein (CP)by A.O.A.C. method and neutral detergent fiber (NDF) by ANKOM equipment The digestibility values (D) and metabolic energy (ME) were estimated. Results: Palm (Bactris gasipaesKunth): % CP = 4,51σ ± 0.57% ± 0.16 NDF = 46,84σ,% D = 55,86σ ± 2.39, 0.37 ME = 192,71 Mcal/g σ ±0,37. Quebracho (Schinopsismarginata)% CP = 3,74σ ± 0.81% ± 0.28 NDF = 46,41σ,% D = 35,47σ ± 0.095, ME = 128,05σ ± 0,34.Malva (Malvasylvestris)% CP = 3,67σ ± 0 , 58,% NDF = 54,44σ ± 0.58% ± 0.18 D = 39,1σ, EM = 141,15σ ± 0,65.GattonPanic (PanicumMaximun)% CP = 12,9σ ± 0.048% NDF = 67,69σ ± 0.17,% D = 0.02 46,43σ ±, EM = 167,63σ ± 0,09.Sorgo (Sorghumspp) .CP = 5,07σ ± 0.79,% NDF = 61, 39σ ± 0.76% ± 1.81 D = 38,82σ, EM = 140,13σ ± 6,53.Ataco (Amaranthus quitensis)% CP = 4,28σ ± 0.9, 49.58% NDF = σ ± 0.70% ± 0.36 D = 37,40σ, EM = 135,02σ ± 1,17.In the first establishment the contribution of protein and energy is it is insufficient to the animals requirements. In the second establishment based Gatton panic and supplement sorghum and ataco, the diets covers the the animals requirements. The Scrubland is an important forage resource especially if we use as nutritional supplement.

A28
FILAMENTOUS FUNGI IN WATER AND ORGANIC MATTER PRESENT IN FISH FARMING TANKS

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Fungal species were ubiquitous, filamentous fungi and yeasts interacted with natural organic matter and particles. Aspergillus fumigatus,Aspergillus nidulans, Aspergills niger, Cladosporium cladosporioides, Cladosporium spp., Fusarium oxysporum, Mucor alternans, Penicillium chrysogenum, Penicillium thomii, Penicillium spp., Trichoderma harzianum and Verticillium spp. were isolated and identified in water, organic matter and sediments from fish farming tanks (Chascomús, Prov. BA, Argentina)

with Odontesthes bonariensis juveniles. Physiological, morphological and molecular characterization were impletented to identified the fungal species. A. nidulans and A. niger were the most frequent fungi, 38%, and were also observed in the superficial tissues of 15 juvenils. A significant increment of these fungi as pellets were obtained along the samplig period, 75 days. Although physical and chemicals conditions did not change, the filamentous fungi increased when Saprolegnia ferax was observed as pathogen in 30% O. bonariensis juveniles. The damage and the number of infected individuals increased a 12% at the 45th. day. Filamentous fungal infection in fish was considered secondary to other factors as consequences of water quality, poor conditions, trauma, rough handling, aggression, bacterial disease.

A29
SERUM LIPOPROTEINS, LIPID CLASSES AND FATTY ACID CHARACTERIZATION IN BROWN SKUA (*Stercorarius antarcticus*) DURING BREEDING

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In migratory birds, circulating lipids provide most of the energy during breeding. This energy source is mobilized through the blood by lipoproteins to be used by other tissues. After migration, food sources with high-energy fats may be important to birds preparing for breeding. In this work we studied the circulating lipid amount, classes and transport in adult Brown skuas serum during the breeding cycle. Blood samples were obtained in three different moments: In (incubation), Pi (after egg hatching) and Pii (during chick rearing). Lipoprotein levels (VLDL, LDL and HDL) were determined by agarose (2%) electrophoresis. Lipids were extracted by Bligh & Dyer method and characterized by Thin Layer Cromatography (TLC) and total fatty acids (FA) were analyzed by gas chromatography. Differences in lipoproteins levels were observed, with higher values of VLDL during In and higher levels of HDL in Pii stage. Whereas no differences in total lipid amount were observed, differences in total FA relative amounts through the reproductive stages were found. Moreover, a slight difference in saturated (18:0 and 22:0), monounsaturated (18:1(n9)) and polyunsaturated (18:2(n6)) FA levels was observed. Altoghether, the differences observed may be related to the nutritional status as well as to the foraging behaviour of Brown skua during breeding.

A30
EFFECTS OF SALINITY ON BIOLOGICAL ASPECTS OF *Daphnia menucoensis paggi*, 1996 (CRUSTACEA, CLADOCERA) IN BIOASSAYS AT 15°C

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In central region and northern Patagonia there are many saline lakes. In this is frequent *Daphnia menucoensis*, an important cladoceran since by its high feed rate modifies ecosystems that inhabits. The objective was to determine the influence of salinity on aspects of its biology at 15°C and compared with previous results at 22°C. Three treatments are performed with 7, 12 y 17 g/L of salts. Neonates were placed in 25 ml containers (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 the number of molts differed (H=23.42; p=0.0000 and H=22.8; p=0.0000), they exceeded 33 days and 7 molts in 12 g/L treatments, but they were around 17-18 days and 4.7-5.4 molts in 7 and 17 g/L treatments. Neither female can reproduce at 17 g/L and the maximum size of the specimens differed (H=22.12; p=0.0000), they were larger (3.14 mm ±0.11) with 12 g/L and they measured 2.54 mm (±0.33) and 2.4 mm (±0.15) with 7 and 17 g/L respectively. The number of litters and total offspring per female were different (H=20.65; p=0.0000 and H=19.84; p=0.0000), reached 0.47 (±0.5) and 2.5 (±0.67) litters and 1.9 (±2.49) and 27.7 (±9.28) offspring with 7 and 12 g/L. While in bioassays at 22°C the highest survival, size, number of molts, litters and offspring were registered in treatments with 7 g/L, at 15°C these biological parameters were optimal with 12 g/L, which is consistent with field observations that indicate these values as relatively optimal for the development of this species.

A31
FISH DAMAGES IN RELATION WITH MYCONANOPARTICLES

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Metals had become objects of study for fish, due to increasing awareness of antimicrobial silver nanoparticles (NPs); however thay had different toxicological effects on medaka embryos. Japanese medaka is a standard organism for toxicity studies in aquatic habitat. While it is important to start with biological models, future work should monitor nanotoxicity in wild susceptible fishes. Its toxicity depended from the form and the NPs-coating, with chemicals (citrate, gum arabic, polyvinylpyrrolidone) or fungal mycelium. After NPs exposure to *Cyprinus carpio*, the gill cells produced mucus that aggregated NPs, increasing the

effective levels and the damages. Although silver-NPs were coated with chemicals, the shape with sharp edges broke cell membranes. Fish dissection slices examined by hyper-spectral dark field imaging to locate silver nanoparticles, determined that gills were the main entry point, and cells with morphological deformations were observed. However, when fungi coated NPs, no aggregation was obtained. It could be assessed that myconanoparticles showed less toxicity to fish in aquatic habitats.

A32
THE USE OF SWEET POTATO CROP RESIDUES AS A FEED SUPPLEMENT FOR FATTENING PIGS

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The use of crop waste to feed animals has spread as food practice in certain productions, such as swine production, given the high cost of food. Gastona area in the province of Tucuman, the agricultural production is based on sugar cane and sweet potatoes. Given the high carbohydrate content, there was a fattening pig feed based on a commercial feed with the addition of sweet potato crop. Objective: Make sustainable food for fattening pigs using sweet potato crop residues. Material and method: We worked with 10 Creole race pigs of a entrepreneurship carry out of the school Gastona Sud Agrotechnics ,distant 90 km from San Miguel de Tucuman. Food components are analyzed. AOAC crude protein, digestibility and metabolic energy were estimated from acid detergent fiber. Results: Corn (Zea mays)%CP= 6,9 σ±0,9,% D=85,9σ± 0,10,ME=310 Mcal/g σ±0,39 .Sweet potato(Ipomea batata))%CP= 4,34 σ±0,47,% D=81,26σ± 0,38,ME=293 Mcal/g σ±1,39.Fattening food: %CP= 3,63 σ±0,30,% D=76,97σ± 1,85,ME=277 Mcal/g σ±6,68. At the start of the test animals have a weight of 20 kg, reaching a weight of 80 kg, after two months and 25 days for fattening. Upon completion of testing, animals were slaughtered and the fat content in meat was analyzed, resulting = 78, 9% of total lipids. Conclusion: Given the high% fat in meat animals it is necessary0 to prove different percentage of sweet potato added to the fattening basal diet and also it should continue investigating about the action of the antimetabolite present in sweet potatoes when feeding, animals in the stages of growth- fattening.

PHYSIOPATHOLOGY I

A33
PHYTOCHEMICAL CHARACTERIZATION OF A METHANOLIC EXTRACT OF CESTRUM PARQUI L´HERIT AND ITS EFFECT OF THE PEROXIDATION OF RAT LIVER MICROSOMES. IS IT PRO-OXIDATIVE OR ANTIOXIDATIVE?

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Cestrum parqui L´Herit is a poisonous plant of the Solanaceae family that affects large animals when they consume the leaves. The toxic compound is an atractyloside that inhibits the ADP/ATP carrier leading to ATP depletion that affects cells, especially hepatocytes, being coagulative necrosis the main lesion. Besides the toxic compound other active principles are present in the plant. It has been state that natives from southern Chile use the aereal parts of the C. parqui to treat skin diseases. Aim of this study was to characterize the phytochemistry of the plant as well as to investigate the effect of C. parqui methanolic extract on the peroxidation of microsome membranes of hepatocytes. Rat liver microsomes where incubated with a methanolic extract of the plant in an in vitro non-enzymatic ascorbic acid-Fe^{+2} system in order to determine the oxidative effect on membranes and to quantify peroxidation level in standardized conditions. After the phytochemical analysis of the methanolic extract, which demonstrated the presence of phenolic compounds, different concentrations were used (0.05, 0.10, 0.20, 0.40 mg) to determine the effect of peroxidation on membranes, which was determined by means of chemiluminiscence. Membranes without extract and with only ascorbic acid were used as controls. Surprisingly, the methanolic extract of C. parqui exhibited antioxidative properties. It has been demonstrated that many chemicals show toxicity in an increasing concentration-dependent manner as well as route of entry. In the case of C. parqui, cattle ingest a lot of leaves during the winter season due to hunger and because of the atractylosides the plant cause hepatotoxicity. On the contrary, when the plant is used on the skin it shows antibacterial effects that may be related to the phenolic compounds that have antioxidative properties.

A34
IN-VITRO EFFECTS OF BISPHENOL A IN IMMATURE GnRH NEURONS

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Bisphenol A, (BPA), a component of polycarbonate plastics, epoxy resins and polystyrene found in many common products, is an endocrine disruptor that alters several functions in different species, including rats, mice and humans. Previously we described the effects of BPA on the hypothalamic-pituitary-gonadal axis of female rats. In this study we analyzed the in vitro effects of BPA in immature GnRH neurons, GN11 cells, developed by Susan Wray, USA. We studied cell proliferation using a Non-Radioactive Cell Proliferation Assay, MTS (Promega, WI, USA) in response to BPA ($1x10^{-9}$ and$1x10^{-7}$M) and estradiol (E_2, $1x10^{-9}$and$1x10^{-7}$M). Results were recorded as Abs490/Abs490 (Control), presented as Mean±SE and analyzed by ANOVA with a Fisher posttest (Statistica, StatSoft, OK, USA). Twenty-four h treatment with BPA and E_2 increased cell proliferation relative to control (Control: 1±0.2, BPA $1x10^{-7}$M: 1.5±0.2, BPA $1x10^{-9}$M: 1.9±0.4, E_2 $1x10^{-7}$: 1.9±0.4, E_2 $1x10^{-9}$: 1.7±0.3,n=5, $p<0.05$). To our knowledge, our results show for the first time a direct effect of BPA on GN11 cell proliferation. More studies are underway to further dissect the mechanisms involved. Supported by CONICET, ANPCyT and UBA.

A35
NITRIC OXIDE SYNTHASE (NOS) EXPRESSION IN THE HIPPOCAMPUS FROM THE PROGENY OF EXPERIMENTAL DIABETIC MOTHERS. EFFECT OF THE INTAKE OF VEGETABLE OILS

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Uncontrolled maternal hyperglycemia during pregnancy produces alterations in the developing nervous system of the offspring. In previous studies, we showed that pistachio oil (PS) increases exploratory activity in the open field test. The aim of this study was to: 1) Evaluate the expression of hippocampal NOS in adult rats (SD, male/female, 8 months old) born from control mothers (CO) or mothers with experimental diabetes (streptozotocin, 30 mg /Kg iv; DO) and 2) Study the effect of early dietary supplementation with corn oil (MZ), extra virgin olive oil (OL) and PS. The oils were administered from day 2 to 62 of age (8µl /15g). NOS expression was analyzed by western blot. In males NOS level was significantly higher in the DO than in CO (ANOVA, $p<0.001$) with or without oil supplementation. CO highest expression was observed with PS ($p<0.001$), whereas in DO it was found with OL ($p<0.01$), no significant differences between CO-PS and DO-PS ($p = 0.871$) was found. The results were similar in females, higher level of NOS in DO than CO ($p<0.001$) and OL and PS increased NOS expression in CO and DO ($p<0.01$) respectively. These studies indicate that early supplementation with oils rich in ω3 to DO does not decrease the hippocampal expression of NOS. In addition, it seems to be some sex dependent responses to the treatments. (CONICET-PIP0243; PICTO/UCCuyo2009-0158; CICITCA UNSJ-IDeA1400.0107/2012).

A36
DISTURBANCE OF PLACENTAL AND FETAL RAT DEVELOPMENT CAUSED BY CAFETERIA DIET.

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Obesity is increasingly common in Western societies and is associated with fetal and placental growth restriction. The mechanism underlying the fetal and placental growth restriction is unknown. In addition, estrogens, through estrogen receptors (ERs) affect placental and fetal development. The aim was evaluate the cafeteria diet (CAF) effects on: 1) reproductive performance, 2) fetal and placental growth on gestational day 21 (GD21), 3) weight of pups at birth and 4) expression of ER alpha (ERa) mRNA in rat placentas on DG21. Twenty-one-day-old female Wistar rats were fed after weaning with a standard rodent chow diet (control) or cafeteria diet (CAF) with highly palatable energy dense foods. On postnatal day 90, rats were mated to evaluate the reproductive performance, placental and fetal weight, and placental-fetal weight ratio. Total RNA from the frozen placenta samples (GD21) was extracted and subjected to real-time RT-PCR analysis of ERa transcripts. The CAF diet did not alter reproductive performance and fetal weights, however CAF group showed a decrease placental weight, placental-fetal weight ratio, and weight of pups at birth. Furthermore, the ERα mRNA expression was lower in CAF group. These results suggest that CAF diet alters the fetal and placental development with a down regulation of ERα mRNA expression in placental tissues.

A37
A CHRONIC EXPOSURE TO A MIXTURE OF ENDOCRINE DISRUPTORS (PHTHALATES AND ALKYLPHENOLS) INCREASES MIR-200B AND AFFECTS THE FEMALE REPRODUCTIVE CYCLE AND FERTILITY IN MICE

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Endocrine disruptors (EDs) such as phthalates (DEHP, DBP and BBP) and alkylphenols (NP and OP) are present in food and everyday products, therefore, humans are daily exposed to small amounts of EDs. These compounds have, in fact, been found in human follicular fluid and are associated with infertility. However, the combined effect of EDs on the female reproductive cycle is unknown. In our laboratory, we have shown that exposure to a mixture of EDs alters microRNAs expression levels in the gonad, even perhaps affecting the germ line. Our goal was to determine the effect of a chronic exposure to two low doses of a mixture of EDs on the fertility of female mice. To do this, females were exposed, since conception until adulthood, to 1 and 10 mg / kg / d of a mixture of DEHP, DBP, BBP, NP and OP or vehicle (in drinking water) and the onset of puberty and estrous cycle were evaluated. In the ovary, preantral and antral follicles numbers were assessed by histology, mRNA and protein levels of StAR and CYP19A1 were measured by qPCR and western blot, respectively and pre-miR-200b/let7f and miR-200b levels were determined. Furthermore, plasma levels of progesterone (P4), testosterone (T) and estradiol (E2) by radioimmunoassay and the fertility rate were evaluated. Results show that a chronic exposure to the doses disrupted the estrous cycle, decreasing plasma levels of E2 and P4, as well as the relative weight of ovaries when compared with the vehicle, however, only the dose of 1 mg / kg / d delayed the onset of puberty. This dose also increased the number of preantral follicles, yet, both doses reduced the number of antral follicles when compared to the vehicle. On the other hand, a decrease in mRNA and protein levels of StAR and CYP19A1 and an increase of pre-miR-200b and miR-200b levels were shown when comparing both treatments with the vehicle. No differences were observed with respect to pre-mir-let7f. Finally, the fertility rate decreased in both treatments when compared with the vehicle. In conclusion, our results suggest that a chronic exposure to the doses is targeting the population of ovarian follicles inducing a reduction in steroidogenesis, which affects the female reproductive cycle and lowers the fertility rates possibly through a mechanism dependent of miR-200b.

A38
SEASONAL STUDY AND EFFECTS OF CASTRATION AND MELATONIN ADMINISTRATION ON THE SUSTENTACULAR CELLS OF THE ADRENAL MEDULLA OF MALE VISCACHA

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The parenchyma of the adrenal medulla is constituted by chromaffin cells, ganglion cells and sustentacular cells. Although the function of chromaffins cells is well known, the role of sustentacular cells remains unclarified. The aim of this work is to analyze the morphology and distribution of sustentacular cells in relation to season and under experimental conditions (melatonin administration and castration) in the male viscacha. The adrenal glands were processed for immunohistochemistry using an antibody against S-100 protein. Image analysis software was used to measure the percentage of immunopositive area (%IA). Sustentacular cells exhibited oval and elongated nuclei, scarce cytoplasm and long citoplasmic processes. These cells showed a regular distribution within the parenchyma, with higher expression of S-100 protein near large medullary blood vessels. Significant differences ($p<0.05$) in the %IA were observed according to winter (4.01±0.35) and summer (3.51±0.15) values. In castrated animals, the %IA (6.05±0.35) was significantly higher in relation to intact animals (3.95±0.40). Likewise, in melatonin-treated animals the %IA (3.62±0.23) was significantly higher compared to control animals (2.65±0.26).These results suggest that sustentacular cells are sensitive to both, environmental factors and hormonal stimuli (androgens and melatonin). Thus, it is likely that these cells participate in the adrenal medulla activity regulation processes.

A39
ER DEPENDENT AND INDEPENDENT EFFECT OF ISOPROTERENOL ON MAMMARY GLAND MORPHOGENESIS

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Previous results from our lab have shown that β-adrenergic stimulation with isoproterenol (ISO) induces differentiation of breast cells in vitro and estrogen receptor (ER) dependent branching of mammary gland in vivo. The aim of this study was to analyze the molecular mechanisms through which β-adrenergic stimulation regulates mammary morphogenesis. ISO was found to significantly increase ERα expression in murine mammary glands ($p<0.01$). This was also observed in ovariectomized- (OVX) and fulvestrant- (FULV) treated mice ($p<0.05$). ISO also increased Ephrin-B1 expression in the mammary gland of control

animals but was abolished in FULV-treated mice, suggesting an ER-dependent effect. On the other hand, ISO had an effect on the architecture of mammary ducts of both OVX- and FULV-treated mice, restoring the number of cells per duct and lumen area. In order to elucidate the mechanism behind this ER-independent effect, the expression of FGF family of growth factors was studied. Glands of ISO-treated mice significantly increased FGFR2, FGF10 and FGF2 cell expression in control as well as in FULV-treated animals. These findings suggest that ISO effects on lumen architecture and on FGF family expression are independent of ER. Reinforcing these results is the finding that incubation of non-tumor MCF-10A mammary cells with ISO induced ERα, FGF2, FGF10, FGFR2 and Ephrine-B1 expression measured by western blot ($p<0.05$). In MCF-10A 3D cell culture, FULV treatment partially reversed the formation of complex structures induced by ISO but did not affect lumen development. Hence, β-adrenergic stimulation regulates ER- dependent and independent mechanisms involved in mammary gland morphogenesis.

A40
PEROXIDATION OF CELLULAR MEMBRANES OBTAINED FROM RAT LIVER: SILYMARIN AS ANTIOXIDANT

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Silymarin (SM) found in “Cardo Asnal” seeds is chemically composed of 4 structurally similar flavolignans. It acts as an antioxidant in liver cells, protects against damage caused by free radicals, increases the capacity for regeneration, produce new cells and removes toxins from the body. SM is considered the main liver protective compound of the "cardo asnal”. SM (silymarine phosphatide 16%) used in this work was kindly suppled by Vetanco Laboratories S.A. The aim of this study was to analyze the antioxidant role of SM on peroxidation of microsomal and mitochondrial membranes obtained from Wistar rat liver AH / HOK. Membranes were incubated in an in vitro system ascorbate $–Fe^{2+}$ dependent, for 180 min. at 37°C in the presence of increasing amounts of SM (12.5 µg, 25 µg and 50 µg) per mg of protein. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Was observed that the total cpm/mg protein originated from light emission: chemiluminescence, was lower in rat liver miscrosomes and mitochondria obtained from SM group than in the control group (without SM). The percentages of inhibition of peroxidation by effect of silymarin were concentration dependent, in microsomes 12%, 32% and 60% and in mitochondria, 35% 45% and 59% respectively. These results shown that silymarine may act as an antioxidant protecting rat liver microsomes and mitochondria from oxidative damage.

PHYSIOPATHOLOGY II

A41
DIFFERENTIATION THERAPY IN ACUTE MYELOID LEUKEMIA. H2 HISTAMINE RECEPTOR AND MRP4/ABCC4AS MOLECULAR TARGETS

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Acute myeloid leukemia (AML) is a heterogeneous clonal disorder where early hematopoietic cells fail to differentiate and do not undergo programmed cell death or apoptosis. Over the last few decades, the concept of differentiation therapy aroused considerable interest. Previously we reported that increment in intracellular cAMP levels by the modulation of different proteins involved in its metabolism, namely the histamine H2 receptor (H2R) (cAMP production), phosphodiesterases (PDE) (cAMP degradation); and MRP4 (cAMP efflux) play an important role in leukemic cell differentiation. Here, we evaluated in human promonocytic leukemia U937 cells the regulation of MRP4 expression by H2R, and the impact of extracellular cAMP on leukemic cell proliferation. We show that H2R stimulation induced an increase in MRP4 mRNA and protein expression. Likewise, U937 cells stably overexpressing H2R (B10 clone) revealed higher levels of MRP4 than U937 cells, which correlate with higher cAMP intracellular levels. As well, the increment of MRP4 levels induced by H2R stimulation in B10 cells was significantly higher than in U937 cells. To determine the effect of extracellular cAMP on U937 cell proliferation, [^{3}H]Thymidine assays were performed in the presence of different cAMP concentration. Our results show a concentration-dependent increment in cell proliferation, indicating that extracellular cAMP levels play an important role in U937 cell proliferation. Altogether, the results provide new data about cAMP role in leukemic cell proliferation and support that polypharmacological differentiation strategy with H2R agonist and MRP4 inhibitors would be beneficial to avoid possible resistance.

A42
NOTCH AND WNT/BETA-CATENIN PATHWAYS: CROSSTALK IN OVARIAN CANCER CELL PROLIFERATION

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Notch and Wnt/β-catenin are highly conserved pathways which regulate proliferation, apoptosis and differentiation. While Notch system has widely been demonstrated to be involved in cancer development and dissemination, Wnt/β-catenin pathway has been poorly studied in these tumors. Besides, there is little evidence that suggests a crosstalk between them. We analyzed the effect of inhibiting these two pathways and their interaction in a human ovarian adenocarcinoma cell line (IGROV-1) and a human granulosa-like tumor cell line (KGN). Cells were incubated in the presence of a Wnt inhibitor (XAV939: 1, 10, 20 and 50 μM), a Notch inhibitor (DAPT: 15, 20 μM) or both. We evaluated the involvement of Wnt/β-catenin pathway and a crosstalk with Notch system in cellular proliferation. Our results show a significant decrease in proliferation when IGROV-1 cells were incubated in the presence of XAV939 (10, 20 and 50 μM) or DAPT (15, 20 μM). There was also a significant decrease in β-Catenin and Cyclin D1 levels together with an increase of total Axin when cells were treated with XAV939. KGN cells also showed a significant decrease in proliferation after incubation with XAV939 (50μM). Most importantly, when IGROV-1 and KGN cells were incubated in the presence of both inhibitors, there was a synergistic decrease in proliferation suggesting a novel crosstalk between these pathways in ovarian cancer cell lines.

A43
ENDOMETRIOSIS IMPAIRS OVARIAN RESERVE AND OVULATION IN A RAT MODEL

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One of the main symptoms of endometriosis is infertility. Several studies have shown a decrease in ovarian reserve (OR) related to the presence of ovarian endometriomas. Other works state that this reduction is due to the surgery performed to remove them. However little is known about the effect of peritoneal endometriosis on OR. Antimüllerian hormone (AMH) levels are used as OR biomarker. Kit ligand (KL) is involved in primordial, primary and preovulatory follicle development. Growth differentiation factor-9 (GDF-9) plays a role in follicle growth since primary stage. The objective of this study was to evaluate the effect of peritoneal endometriosis on follicles, ovulated oocytes and ovarian levels of AMH, KL and GDF-9 in a rat model. Endometriosis was surgically induced in Sprague Dawley rats by autotransplantation of uterine horn pieces to the bowel mesothelium. Sham animals were used as controls. Rats were sacrificed one month after surgery. Follicles were counted in ovary sections and oocytes were isolated from the oviductal ampulla. AMH, KL and GDF-9 expression were assessed by western blot. Rats with endometriosis showed a reduced number of primordial, primary and preantral follicles ($p<0.05$). AMH decreased in endometriosis ($p<0.05$). No significant differences were observed in the number of preovulatory follicles between groups but the number of ovulated oocytes was decreased in endometriosis ($p<0.05$). KL is diminished in endometriosis rat ovaries ($p<0.05$). There were no significant differences in GDF-9 levels between groups. We conclude that one month of peritoneal endometriosis induction is sufficient to diminish OR and the number of ovulated oocytes in a rat model.

A44
OBESITY CAUSES ABERRANT UTERINE EMBRYO DISTRIBUTION AND MACROSOMIA AT TERM

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The prevalence of obesity is increasing worldwide. It is related to several reproductive disorders but the molecular mechanisms linking them remain unclear.Using cafeteria diet-induced obesity as animal model, we found thatobesity causes aberrant uterine fetal distribution and macrosomia at term (gestation day (gd) 18.5) when comparedto controls. To elucidate whether thisalteration is consequence of fetal re absorptions or due to alterations in the foregoing embryo spacing, we analyzed embryo distribution just after implantation time (on gd5.5).We also found asymmetric uterine embryo distribution on gd 5.5, indicating that obesity alters the uterine embryo distribution prior to implantation. Then, to analyze embryo distribution just before implantation, uterus from rats at gd 4.5 were flushed and embryos were collected. The total number of embryos detected in obese rats was lower than in controls ($p<0.001$). When each uterine horn was analyzed separately, it was found that this reduction was due to a lower number of embryos present in one of the horns($p<0.01$), while the number of embryos in the other horn was similar to controls. Since adrenergic receptor beta2 (b2AR) is involved in the uterine embryo distribution, we analyzed its gene expression by qPCR. We found that the b2ARexpression was up regulated ($p<0.05$) in uterus from obese rats at gd 4.5 when compared to controls. When we evaluated the b2ARexpression in the pre-conception period we found that its gene ($p<0.001$) and protein ($p<0.01$) expressions were down regulated in obese uteri when compare to controls. These alterations may be one of the mechanisms underlying the aberrant uterine embryo distribution observed in obese animals. Since it is known that embryo implantation at wrong place can

cause adverse effects on pregnancy outcomethe study of this mechanismcould be useful to prevent pregnancy loss in obese patients.

A45
EXPRESSION AND FUNCTION OF HUMANIN PEPTIDES IN OVARIAN CELLS

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Humanin (HN) and Rattin (HNr, rat homologous peptide) have cytoprotective action in several cell types such as neurons, spermatogonias and Leydig cells. However, little is known about the expression and action of these peptides in the ovary. We aimed to explore the expression and function of HN peptides in the ovary from prepuberal rats, cycling adult rats and in a human granulosa-like tumor cell line (KGN). We investigated the expression of HNr in ovarian sections from untreated prepuberal rats (rich in preantral follicles), or treated with DES (rich in early antral follicles) or PMSG (rich in preovulatory follicles). Immunohistochemical (IHC) staining showed HNr expression in granulose and theca cells, and also in oocytes. The HNr expression pattern was similar among follicles of the same type in untreated or treated prepuberal rats. In PMSG-treated rats, HNr was mainly expressed in theca cells. In ovarian sections from cycling rats the pattern of HNr expression was similar to that of treated prepuberal rats and HNr was also expressed in luteal cells. In addition, KGN cells expressed HN. To study the role of HNr, we performed the TUNEL assay together with IHC for HNr in ovarian sections. HNr+ cells were TUNEL-negative in PMSG-treated rats. We analyzed the effect of HN on viability of KGN cells by MTT assay. HN increased the viability of KGN cells (C: 0.24 ± 0.02, HN 0.25 µM: 0.40 ± 0.02, HN 0.5 µM: 0.36 ± 0.02, HN 1µM: 0.41 ± 0.01, $p < 0.01$ vs control). Our results show that HNr is present in all follicular cells, including oocytes, and also show strong intensity in luteal cells. Considering that HNr is absent in apoptotic ovarian cells and that HN increases the viability of KGN cells, our results suggest that HNr/HN may play a cytoprotective role in ovarian cells.

A46
CHOLESTEROL CHARACTERIZATION OF HUMAN FOLLICULAR FLUIDS FROM PATIENTS AT RISK OF DEVELOPING OVARIAN HYPERSTIMULATION SYNDROME (OhSS)

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OHSS is a complication of ovarian stimulation with gonadotropins following human chorionic gonadotropin (hCG) administration. Enlarged ovaries, increased vascular permeability and excessive production of steroid hormones and vasoactive compounds are main features of this syndrome, though its exact pathophysiology remains unknown. The aim of this study was to characterize the cholesterol and lipoprotein profile of human follicular fluids (hFFs) from patients at risk of developing OHSS. For hFF collection, aspirates were obtained from patients 25–39 years old undergoing oocyte retrieval at the Reproductive Medicine Center Pregna and were classified into control group or OHSS group. Cholesterol concentration was obtained by an enzymatic assay, apolipoprotein A-I (ApoAI) levels were measured by western blot and lipoprotein cholesterol profiles were assessed by fast-performance liquid chromatography (FPLC). Total concentration of cholesterol was higher in OHSS hFF than in control hFF, while ApoAI levels were significantly lower in the OHSS group. Finally, VDLD, LDL and HDL elution fractions from FPLC chromatograms were analysed. The HDL-cholesterol peak was significantly higher and also slightly shifted to the left in OHSS hFF compared to control, indicative of larger particles containing more cholesterol. In conclusion, cholesterol metabolism seems to be altered in OHSS, providing useful information for novel approaches to this pathology.

A47
EFFECT OF PHOTOTHERAPY WITH LOW LEVEL LASER ON FEMALE REPRODUCTIVE SYSTEM

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LLL therapy (LLLT) is the application of light to promote tissue repair, reduce inflammation or induce analgesia. This work proposes the following photobiomodulator strategy to improve female fertility by local application of LLLT. The objectives were: a) To evaluate the in vivo effect of LLLT in ovaries of adult mouse on follicular dynamics, angiogenesis, and fertilization rate and embryo development and b) To analyze the in vitro effect of LBI in a culture of rat granulosa (CG) on cell proliferation (Ki67) and expression of VEGF. For objective a, F1 mice (BalbC x C57 / BL6) (8 weeks) were used and LLLT (4, 8 and 16

Joule) was applied. For objective b, a culture of rat granulosa cells (GC) (Sprague-Dawley, 21-23 days) was performed and LLLT (2-8 Joule) was applied. The results showed that in adult mice the LLLT (4 and 8 J) increased % of primary and preantral follicles ($p<0.01$), and decreased % atretic follicles ($p<0.05$) compared to control. LLLT decreased endothelial area and increased periendothelial area compared untreated group ($p<0.01$). Besides, LLLT drastically improved the fertilization capacity of oocytes compared to the control group ($p<0.05$). LLLT induced cell proliferation (8 Joule) and expression of VEGF 121 isoform (4 Joule) in CG compared to untreated group ($p<0.05$). Therefore, the biomodulator effect LLLT enhances folliculogenesis in the early stages and could be mediated in part by modulation of vascular development and follicular atresia.

REPRODUCTION II

A48
CHARACTERIZATION OF cAMP EFFLUX SYSTEM IN MICE SPERM

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To fertilise the oocyte, mammal ejaculated spermatozoa must undergo a series of biochemical and structural changes known as capacitation. This process correlates with HCO_3^- and Ca2+ influx, activation of soluble adenylyl cyclase (sAC), cAMP production, PKA activation, and increase in tyrosine phosphorylation (pTyr). Although cAMP levels are mainly regulated by its synthesis and degradation, in other tissues, extrusion through multidrug resistance protein 4 (MRP4) transporter is also involved in this modulation. In our laboratory we found that cAMP efflux by MRP4 is critical for bovine sperm capacitation. Moreover, supplementation of incubation media with non-permeable cAMP triggers signalling events associated with this process. In this work we aim to characterize cAMP efflux system and evaluate its possible role in mice sperm physiology. Results indicated that mice sperm possess MRP4 and detectable levels of cAMP were measured in the incubation media. In vitro capacitation was performed in the presence of MRPs inhibitors. Even though there was no inhibition of pTyr, an accumulation of PKA phosphorylated substrates was observed. MRP4 is localized in the tail of mice sperm at control conditions; but under capacitating conditions, a significant reduction (50%) of the label was detected. Altogether, these results suggest that cAMP exclusion is present and may play a role in mice sperm function.

A49
EXPRESSION OF FIBROBLAST GROWTH FACTOR 2 (FGF2) IN THE FEMALE REPRODUCTIVE TRACT AND ITS ROLE IN THE REGULATION OF SPERM PHYSIOLOGY

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Fibroblast Growth Factor 2 (FGF2) and its receptors (FGFRs) have been described in different tissues, where they regulate cellular proliferation, differentiation, motility and apoptosis. However, there is not enough evidence of their expression in the reproductive tract and gametes and their involvement in the maintenance of sperm physiology. The objectives of the present study were: 1) to determine the presence of FGF2 in the murine female reproductive tract, and 2) to assess the role of the FGF2/FGFRs system in the regulation of sperm function. The expression of FGF2 isoforms (18, 20.5 and 21 kDa) was observed in protein extracts from the uterus and oviduct of adult mice by SDS-PAGE followed by Western immunoblotting (WIB) using anti FGF2 antibody. Immunofluorescence studies showed FGF2 localization in the epithelial cells and lumen of the uterus, isthmus and ampulla at different stages of the estrous cycle, as well as in the cumulus-oocyte complex. In sperm, the presence of the 4 FGFRs was determined by WIB and flow cytometry, and they were localized in the flagellum and acrosomal region by immunocytochemistry. Incubation with recombinant FGF2 led to an increase in the percentage of motile sperm in comparison with the control, but it did not affect sperm protein tyrosine phosphorylation or the occurrence of acrosomal exocytosis ($n\geq4$). In conclusion, this study shows that FGF2 is expressed in tissues of the female reproductive tract, that the FGFRs are present in the sperm cells, and that this system would be involved in the regulation of sperm motility.

A50
CELL PROLIFERATION IN PITUITARY OF NON-PREGNANT AND PREGNANT VISCACHAS

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Proliferating cell nuclear antigen (PCNA) has been used to identify replicating cells in different tissues. Immunocytochemical staining patterns of PCNA have permitted the recognition of specific cell-cycle stages. In other species, it has been demonstrated that the number of pituitary cells increase during pregnancy and lactation. In viscacha, pregnancy lasts approximately 154 days, and three stages can be described: early (EP), mid (MP)-and late pregnancy (LP). The aim of this work was to analyze the expression of PCNA in pituitary pars distalis (PD) and pars tuberalis (PT) of non-pregnant (NP) and pregnant viscachas. Sixteen pituitaries (n=4, per group) were processed for light microscopy. PCNA immunoreactive (ir) cells were detected by immunocytochemistry and quantified by image analysis. In PT only nuclear labeling for PCNA was observed. The percentage of PCNA-ir cells increased during pregnancy compared with NP animals (NP: 0.46 ± 0.04% vs. EP: 1.13 ± 0.24%; MP: 0.90 ± 0.10%; and LP: 1.14 ± 0.09%; $p<0.05$). In PD, the immunolabeling pattern was mainly nuclear. However, some pituitary cells have cytoplasmic and nuclear labeling. The percentage of PCNA-ir cells did not differ significantly among different groups of animals (NP: 0.75 ± 0.06%; EP: 0.76 ± 0.07%; PM: 1.03 ± 0.16%; LP: 1.09 ± 0.19%; $p>0.05$). In addition, the percentage of PCNA-ir cells was higher in PD compared with PT of NP animals. These results demonstrate and quantify the cell proliferation in pituitary PD and PT of female viscachas. The differences in the pattern of immunostaining for PCNA suggest that these cells are in different stages of cell-cycle. The increase of cell proliferation values during pregnancy might be relate to the increase of some pituitary cell populations, and with highest progesterone and androstenedione serum levels, as previously reported during the pregnancy of this rodent.

A51
EARLY POSTNATAL EXPOSURE TO BPA ALTERS ANDROGEN RECEPTOR EXPRESSION IN THE BROAD-SNOUTED CAIMAN (*Caiman latirostris*) OVIDUCT

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The female reproductive tract, named oviduct in oviparous species, is target of endogenous steroid sex hormones and hormonally active pollutants. Androgens are thought to play a direct role in oviduct growth, differentiation, and secretory functions and sperm storage through receptor binding. The aims of this study were to establish androgen receptor (AR) dynamics in Caiman latirostris oviduct from neonatal to pre-pubertal juvenile and its responsiveness to early post-natal xenoestrogen exposure. C. latirostris females raised in control conditions were euthanized at neonatal, early and late postnatal or juvenile stages. Early postnatal were injected (sc) twice, 7 days apart, with 17-beta estradiol (E2) (0.014 or 1.4ppm) or BPA (1.4 or 140 ppm) and euthanized 7 days after last injection. Immunohistochemical localization of AR was performed on oviduct sections using a polyclonal Ab. Cytoplasmic AR expression characterized the earliest developmental stages. Sustained increase in nuclear AR as developmental stages advanced was observed. Nuclear epithelial AR significantly increased at the juvenile stage. Early postnatal exposure to the lowest dose of E2 or BPA up regulated nuclear AR to juvenile levels. These results demonstrate that the early post-natal caiman oviduct is labile to xenoestrogen exposure suggesting precocious development and differentiation that could impair oviduct physiology later in life.

A52
EFFECT OF HCG OR GnRH POST INSEMINATION ON PROGESTERONE CONCENTRATION IN SHEEP

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The aim of the study was to evaluate the effect of the administration of gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) at day 4 post timed artificial insemination (TAI) on the formation of accessory corpora lutea (ACCCL) and on the production of serum progesterone (P_4) concentration. A total of 27 pregnant adult Merino ewes were assigned randomly to three groups on day 4 post TAI: 1. GnRH group (n= 8) was i.m. injected 4 µg of GnRH analogue (Buserelin. Receptal®, Intervet, Argentine), 2. hCG group (n= 8) was i.m. administered 300 IU of hCG (Gonacor®, Ferring, Argentine) and 3. Control group (n= 11) was i.m. applied 1 ml of saline solution. Laparoscopic observation of the ovaries at day 4 and 10 post TAI was performed to determine the presence of ovulatory CL and ACCCL, respectively. Serum P4 concentration was assessed by chemiluminescence on days 4, 7, 10, 13, 17 and 21 post TAI. The hCG group showed higher mean concentrations of P4 on days 7, 10, 13 and 17 post TAI (4.1±2.1, 10.5±4.6, 9.4±3.2, 7.4±2.4 ng/ml) compared with the GnRH group (2.3±1.1, 5.6±2.5, 5.6±2.6, 5.7±1.9 ng/ml) and the Control group (2.5±1.3, 5.3±2.0, 5.2±2.0, 4.8±2.1 ng/ml; $P<0.05$), while no differences were observed between these two latter groups. Mean P4 concentrations showed no differences on days 4 and 21

between the three groups (P>0.05). The presence of ACCCL was observed in all ewes treated with hCG or GnRH, whereas no ewes with ACCCL were observed in the Control group (P<0.05). Although GnRH and hCG treatments induced ACCCL, only ewes treated with hCG considerably increased P4 concentrations during 10 days. Differences in the pharmacodynamics of these two hormones might induce ACCCL with different steroidogenic capacity.

A53
AUTOLOGOUS SEMINAL PLASMA PREVENTS SPERM CAPACITATION OF EQUINE SPERM

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The seminal plasma (SP) has been reported to modulate sperm function(s) in several species. The aim of this study was to evaluate the effect of autologous SP on stallion sperm in vitro capacitation. Semen from fertile stallions (n=4; 3 replicates each) was subjected to Androcoll-E^{TM} colloid centrifugation. Selected sperm were incubated under capacitating conditions for 3 h in MW-BSA medium alone or with 2.5, 5 and 10% SP. Sperm capacitation was evaluated by a) chlortetracycline (CTC) staining (B-pattern), b) immunocytochemical detection of tyrosine phosphorylated proteins (PTyrP) following HOST (HOST/PTyrP) (correlation between CTC-B pattern and HOST+/PTyrP+, r=0.81; p<0.05). c) sperm acrosome reaction in response to Progesterone (Prg) treatment assessed by staining with FITC-conjugated peanut agglutinin and propidium iodide (FITC-PNA/PI) and the hypoosmotic swelling test (HOST) combined with Coomassie brilliant blue staining (HOST/CBB) (correlation between FITC-PNA+/PI- and HOST+/CBB-, r=0.78; p<0.05). Sperm incubation under conditions to promote capacitation led to an increase (p<0.05) in all parameters evaluated. Similar results were obtained in sperm incubated with 2.5% SP. Contrasting, these changes were not observed in sperm suspensions exposed to 5% and 10% SP. In conclusion, SP was found to modulate equine sperm capacitation when added at 5-10% to capacitation media. SP supplementation in artificial insemination and/or cryopreservation protocols may be beneficial to prevent premature capacitation.

A54
EMBRYO CARDIO-PLACENTAL ANOMALY IN MURINE ORGANOGENESIS FOLLOWING MATERNAL ALCOHOL CONSUMPTION. EFFECTS ON THE VGEF/RECEPTOR SYSTEM

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Maternal alcohol consumption causes the fetal alcohol syndrome, characterized by various congenital diseases. Previously, we saw delayed embryo growth and increased dysmorphogenesis after perigestational 10% alcohol treatment up to murine organogenesis. Since embryo-fetal cardiogenesis is one of the targets of maternal alcohol intake and cardiac abnormalities may be closely related to altered labyrinthine vascular development due to imbalances in the VEGF/receptors system, the objectives were to analyze, the organogenic embryo cardiac histopathology (H&E), the labyrinthine histo-morphology and growth (H&E, nuclear Hoechst fluorescent staining, Image Pro Plus), the proliferation (PCNA immunohistochemistry (IHC), the apoptotic index (TUNEL) and VEGF, KDR and Flt-1 expressions (IHC and western blot (WB) in the embryo-trophoblast tissues. Ethanol 10%/drinking water was administered to murine CF-1 females for 15 days before and until day 10 of gestation (TF) (Control females (CF) without ethanol). TF had elevated % of embryos with irregular-discontinuous endocardium (p<0.01) and disorganized myocardium (p<0.01, vs CF). The PCNA-positive cell Nr/area tissue of ventricular myocardium was lower than in CF (p<0.05), which correlated with reduced VEGF expression (p<0.05). However, the embryonic immunoexpression and levels of KDR and Flt-1 receptors increased (p<0.05, vs CF). The trophoblastic zone of TF had histological alterations, increased trophoblast giant cells with irregular nuclei, and reduced labyrinthine growth with increased trophoblastic apoptosis. In TF-derived placenta, the VEGF immunostaining was lower but Flt-1 was higher vs CF. Murine perigestational alcohol exposure leads to embryo cardiopathy and abnormal labyrinthine growth associated with impairment of the angiogenic VEGF/receptors system.

A55

PARTICIPATION OF SUCCINATE DEHYDROGENASE IN CAPACITATION AND ACROSOME REACTION IN CRYOPRESERVED PORCINE SPERMATOZOA

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The aim of this study was to determine the activity of succinate dehydrogenase (SDH; 1.3.5.1) and evaluate its participation in capacitation and acrosome reaction (AR) in cryopreserved porcine spermatozoa. This enzyme is involved in the production of the energy required for these sperm metabolic processes and its activity was previously determined in fresh porcine sperm. The activity of SDH was determined spectrophotometrically at 600 nm, during 2 minutes, at 37°C. Enzyme unit (U) was defined as the amount of SDH that catalyzes the reduction of 1 µmol of DCPIP/min. Capacitation and AR were determined, in the presence or absence of sodium malonate (competitive inhibitor of SDH; 1, 5 and 10 mM), by CTC technique and trypan blue combined with DIC, respectively. Sperm viability was evaluated by the eosin-nigrosin technique and motility was evaluated by optic microscopy, with a thermal stage. The results are expressed as means±SEM and were analysed by ANOVA and Bonferroni test. The activity of SDH was 0,7±0,1 U/10^{10} spermatozoa. The addition of the enzyme inhibitor (1mM) significantly ($p<0,05$) diminished capacitation levels (12±3) respect to the control (20±1). Sperm motility was significantly ($p<0,05$) diminished by 10mM sodium malonate respect to the control group, while sperm viability was not affected by any of the concentrations used. The addition of the inhibitor (1mM) significantly ($p<0,05$) diminished AR (9±2) respect to the control group (19±3). During AR, sperm motility and viability were not affected by any of the inhibitor´s concentrations used. Our results demonstrate the activity of SDH and its participation in the mechanisms involved in capacitation and AR in cryopreserved porcine spermatozoa.

A56

EFFECT OF THE COCULTURE OF PORCINE LUTEAL CELLS WITH PORCINE CUMULUS OOCYTE COMPLEXES ON THE IN VITRO NUCLEAR MATURATION RATE

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Nuclear and cytoplasmic maturation has not been well described in porcine until the moment. The establishment and maintenance of a suitable in vitro microenvironment plays an essential role in maturation and subsequent fertilization, whereby the utilization of a standardized coculture would recreate in a better way the in vivo microenvironment. The choice of porcine corpus luteum (PCL) cells in monolayer for coculture with cumulus oocyte complexes (COC) is based on the production of progesterone of this cells, a hormone that has an antiapoptotic and antioxidant effect. The aim of this study was to evaluate the effect of the coculture with PCL cells on the in vitro nuclear maturation rates of porcine oocytes. Slaughterhouse ovaries were used for the PCL culture and for COC aspiration. COC were matured in vitro for 44 h in drops of 100 µL with supplemented TCM 199 without hMG (control without hormones), with hMG (control with hormones) and over a monolayer of PCL culture passage 1 in a cell concentration of 2 X 10^4 cel/mL using the same media without the addition of hormones. Nuclear maturation rates were assessed by Hoechst 33342 stain (displaying the metaphase plate) and it was considered as significant a $p < 0.05$. It was observed a significant difference between the coculture treatment and the control without hormones (coculture 72% n = 104 y control 51.5% n = 66), and no significant differences were observed between coculture treatment and control with hormones (79% n = 64). We conclude that nuclear maturation in coculture is similar than nuclear maturation with hormones. So we could replace maturation with the addition of hormones using this coculture system, however other parameters must be evaluated such as cytoplasmic maturation to confirm these results.

A57

REACTIVE OXYGEN SPECIES PRODUCTION IN VITRIFIED PORCINE OOCYTES

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In order to evaluate reactive oxygen species (ROS) production in porcine oocytes matured in vitro and subjected to the process of vitrification-warming, Cumulus-oocyte complexes were obtained by aspiration of antral ovarian follicles and matured in medium 199 with porcine follicular fluid, FSH and LH, and incubated at 39°C, 5% CO_2 in humidified air for 48h. Matured oocytes (n = 75) were denuded and a group was cryopreserved using the minimum volume vitrification method Cryotech® .Then oocytes were subjected to warning. ROS were measured at 0, 3 and 21h by the incubation of oocytes in PBS-PVA and DCHF-DA, while a sample was incubated to evaluate esterase activity in PBS-PVA and FDA. Fluorescence levels of DCHF-DA depends on intracellular esterase activity, so the ratio between the brightness obtained from DCHF-DA for each oocyte and the average brightness by FDA of each treatment was considered as a measure of ROS production. In fresh oocytes ROS production was higher at 0 compared with 3 and 21h ($p<0.05$), while in vitrified oocytes no significant differences were found between 0, 3 y 21 h. Analysing the effect of vitrification, ROS levels were higher in vitrified than in fresh oocytes at each time point (Fresh: 0h

694.4±63.92; 3h 281.47±60.82; 21h 235.88±16.78 vs. Vitrified: 0h 1066.8±88.64; 3h 1079.8±64.42; 21h 1400.9±126.6 arbitrary units/oocyte) ($p<0.05$). In conclusion, the vitrification-warming process induces an increase in ROS levels in porcine oocytes.

CELLULAR AND MOLECULAR BIOLOGY

A58

REGULATORY miRNAs INVOLVED IN THE *Phaseolus vulgaris - Rhizobium etli* SYMBIOSIS

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Phaseolus vulgaris establishes a symbiotic interaction with Rhizobium etli, resulting in the formation of root nitrogen-fixing nodules. Interestingly, plants from the Mesoamerican center of genetic diversification are more efficiently and preferentially nodulated by strains that are predominant in soils from the same geographical region. Our group has identified several genes that are involved in this preferential interaction. More recently, we have focused on the study of small non-coding RNAs (sRNAs), which are keys regulators of post-transcriptional gene expression, particularly in the establishment and regulation of nodulation. To get insight into sRNAs whose levels are modified during this preferential symbiosis, we have constructed and sequenced Illumina libraries of sRNAs from roots inoculated with either a more or a less efficient strain. More than 28 million of reads per library were obtained, filtered and mapped to the P. vulgaris genome and analyzed using the Workbench tools. We focused on the identification of microRNAs (miRNAs) that change their abundance in the more efficient interaction as compared to the less efficient one. One of them, miR390b, is a miRNA evolutionary conserved in the plant kingdom with important roles in leaf development and root architecture. Overexpression of the miR390b precursor in P. vulgaris hairy roots negatively affected nodule formation in the more efficient, but not in the less efficient symbiotic interaction. Interestingly, we have also identified two new miRNAs that are processed from the untranslated region of protein coding genes and are accumulated at higher levels in the more efficient interaction. Further characterization of these new miRNAs by functional genetic will reveal whether they play an important role in the establishment of the efficient symbiosis.

A59

MicroRNAs IN PARASITIC CESTODES: DISCOVERY, FUNCTIONAL ANALYSIS AND ROLE IN HOST-PARASITE COMMUNICATION

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Tapeworms (Cestoda) are etiological agents of neglected diseases such as hydatidosis and neurocysticercosis, caused by the larval stages of Echinococcus and Taenia solium, respectively. Understanding the mechanisms that regulate their particular characteristics of development and survival in the host may allow identifying new therapeutic targets. MicroRNAs are small silencing RNAs that impact eukaryotic development and are receiving growing attention as novel therapeutic and diagnosis targets. Our team identified miRNAs in cestodes for the first time and reported differential expression among developmental stages and species with diverse developmental and morphological characteristics by means of smallRNAseq and big data analysis at the Bioinformatic Node at IMPaM. We performed prediction of miRNA targets by an integrated bioinformatics pipeline that included 3'UTR annotation and RNAseq data analysis. In this way, 941 potential miRNA target sites distributed in 724 3'UTRs were found in Echinococcus canadensis. Most of them were found to be conserved among species of the genus Echinococcus, adding confidence to the predictions obtained. Functional analysis of miRNA targets showed that MAPK and WNT signaling pathways were among the most represented, suggesting miRNA roles in parasite growth and development. In order to know if miRNAs are secreted by cestode parasites and could represent a way of parasite-host communication, we searched for extracellular vesicles (EVs) in 2 model cestode parasites: Taenia crassiceps and Mesocestoides corti. As a result, we demonstrated the in vitro secretion of membranous structures compatible with EVs by transmission electron microscopy and identified, by proteomics approach, expected eukaryotic EV markers and also, among others, proteins used for immunodiagnosis of cestode infection as well as host immunoglobulins. Finally, we proved by capillary electrophoresis that cestode EVs carry small RNAs and then microRNAs were detected by RT-(q)PCR. In conclusion, miRNAs have emerged as the main smallRNA silencing molecules in cestodes being several of them absent in the host or highly divergent with respect to host orthologs. In addition, important pathways were predicted to be targeted by miRNAs. Also, these small RNA regulators were shown to be secreted in EVs, and could thus be involved in host-parasite communication. These results suggest that miRNAs are candidates for novel diagnosis and therapeutic interventions against parasitic neglected diseases and pave the way for further functional studies.

A60

MicroRNAs IN *Taenia solium*: CHARACTERIZATION AND FUTURE APPLICATIONS

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Over the last decade, there has been increasing interest in small RNAs from parasitic cestodes. microRNAs (miRNAs) have emerged as a major class of regulatory genes present in most metazoans and are important for a diverse range of biological functions. It is, therefore, highly significant to study the mechanism of miRNA function in these parasites. Prediction and identification of miRNA target genes is the basis for the functional study of miRNAs. We have employed high throughput small RNA sequencing to characterize at genome level the small RNAomes of *Taenia solium* and miRNA expression profile of the model cestode *Taenia crassiceps*cysticercus stage. Using bioinformatics tools and the bioinformatic IMPaM node, different targets that interact with the previously identified microRNAs were predicted. We found that miRNAs are the most abundant type of small RNAs in cysticerci of *T. crassiceps*. The percentage of miRNAs in this larvae form (~83%) exceeds the previously reported in other cestodes. piRNAs were not detected in this approach. Our results showed that some miRNAs were expressed with high predominance, mainly tcra-miR-10-5p, tcra-miR-71-5p, tcra-bantam-5p and tcra-let-7-5p. This is in agreement with observations made in other cestodes like *Echinococcus* spp and other platyhelminths. The comprehensive miRNA repertoire, using deep sequencing technology, includes conserved miRNAs in cestodes as well as novel miRNAs in *T.solium* genome and *T. crassiceps* cysticercus. Furthermore, we used two algorithms (miRDeep and miRNASOM) to confirm the novel miRNAs precursors and some miRNAs with particularly long hairpins. Six potential miRNAs that were predicted to fold into a stem-loop precursor structure where further detected by Northern blot to confirm their size and sequence. This experimental validation allowed us to detect, for the first time in cestodes, bands corresponding to miRNA precursors (~70 nt). miRanda software was used to predicted target genes. The initial miRanda output list of targets was manually curated to generate a final high confidence set of targets with i) strict complementarity in the seed region, ii) multiple sites and iii) conserved sites in ortholog genes of several cestodes. Among selected targets, genes involved in programmed cell death and several transcription factors were identified suggesting that miRNAs could be involved in growth and development of these highly proliferating parasites. These results provide the basis for understanding one of the mechanisms that regulate the particular characteristics of growth and development of *Taenia* parasites and may allow identifying new therapeutic targets for the neglected diseases they cause.

A61

DYNAMIC REGULATION OF THE *Medicago truncatula*TRANSLATOME MEDIATED BY LONG NON-CODING RNAS DURING ROOT NODULE SYMBIOSIS

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The development of transcriptomic techniques has led to the use of steady-state levels of mRNAs as a criterion to select and study genes in the context of a biological process. This approach has excluded levels of post-transcriptional regulation, such as the rapid response through the translational activation of mRNAs. We have shown that genes involved in the root nodule symbiosis are regulated at the level of their association with the translation machinery. Here, we used Translating Ribosome Affinity Purification (TRAP) combined with RNA-sequencing to characterize the populations of mRNAs and non-coding RNAs associated to polysomes (referred to as the translatome). The characterization of dynamic changes in the translatome of M. truncatula at early stages of the root nodule symbiosis led us to the identification of mRNAs that significantly change their levels of association with polysomes in response to rhizobia. Some of these mRNAs play essential roles in nodulation (e.g., NIN, NF-YA, pectate lyase, SINA and NCR peptides). We have also identified a group of mRNAs that are either up- or down-regulated at the translational level, which encode proteins that participate in epigenetic and post-transcriptional regulation (a Superkiller-like protein, a DNA methylase, a CCR4-NOT subunit and a La-related protein). In addition, we identified a significant number of lncRNAs that change their association with polysomes in response to rhizobial infection. These lncRNAs might act either repressing or activating the translation of other mRNAs or they might encode functional small peptides (as described in mammals). These changes in the translatome might contribute to the reprogramming of root cells during early stages of the symbiotic interaction.

A62

LINKING THE MIR390/TAS3 AND THE NOD SIGNALING PATHWAYS DURING THE NITROGEN-FIXING SYMBIOSIS

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MicroRNAs (miRNAs) act as post-transcriptional regulators of gene expression during development or in response to environmental stimuli. Under low nitrogen conditions, legume roots establish symbiotic associations with soil bacteria known as rhizobium that results in the development of a new organ, the nodule, within which rhizobia fix the atmospheric nitrogen into

reduced forms that are readily assimilated by the plant. Previous analysis in M. truncatula revealed that levels of miR390 are reduced at early stages of the symbiotic interaction. miR390 targets the non-coding transcript TAS3 and triggers the production of the trans-acting small interference RNAs (tasiRNAs). In turn, these tasiRNAs control the stability of transcripts encoding the Auxin Response Factors 2, 3 and 4 (ARF2/3 /4). Overexpression of miR390 (OX390) negatively affected nodule organogenesis and rhizobial infection. On the other hand, expression of a target mimicry of the miR390 (MIM390), which significantly reduced tasiARFs production, enhanced nodulation and altered the morphology and distribution of nodules. Activation of the miR390/TAS3 pathway prevented the induction of the Nodulation Signaling Pathway 1(NSP1) and NSP2 genes in response to rhizobial infection, whereas inactivation of this pathway results in increased levels of NSP1/2, NIN and ERN1, even in the absence of rhizobia. These results suggest that the miR390/TAS3 pathway regulates, either directly or indirectly, the expression of symbiotic genes that are essential for nodulation. Ongoing RNA-seq and ChIP-seq analysis will allow us to identify the putative direct targets of this pathway under symbiotic conditions.

A63
PRELIMINARY CHARACTERIZATION OF THE ECDYSONE RECEPTOR IN THE CHAGAS DISEASE VECTOR *Rhodnius prolixus*

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The transition during growth in each instar, is regulated by ecdysteroids, particullary the 20-hydroxyecdysone (20E). 20E binds to its receptor, a heterodimeric combination of two transcription factors: the ecdysone receptor (EcR) and the retinoid X receptor (RXR)-homologue ultraspiracle (USP). By the BLAST algorithm the homologous sequence corresponding to Rhodnius prolixus (Hemiptera: Reduviidae) was obtained. For cloning and genomic characterization of the putative receptor (RpEcR), specific primers were designed and a 480 bp conserved sequence and a double stranded RNA (dsRNA) was constructed for interference assays. A commercial kit (MegaScript) was used for the synthesis of the probe. A group of IV instar larvae (1 ug of dsRpEcR / insect) was injected 48 hours before feeding. As a control, other group of nymphs were injected with a YPF dsRNA. Furthermore two groups of virgin females were injected with the specific and control probe. RpEcR expression levels were measured by RT-qPCR. While control larvae molted normally, the individuals injected with dsRNA RpEcR (interfered) failed to molt. Analysis of dissection of interfered nymphs that did not complete the molt, showed the new cuticle, suggesting partial inhibition of receptor expression. The levels of RpEcR expression in treated nymphs were significantly lower. The first results in adult females did not show visible differences in developing ovaries suggesting that RpEcR is not involved in vitellogenesis.

A64
PEROXIDATION OF EQUINE ERYTHROCYTE MEMBRANES: LUTEIN AS ANTIOXIDANT. A PRELIMINARY STUDY

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Circulating erythrocytes are regularly exposed to high oxygen concentrations, as well as to the presence of intra and extracellular free radicals, thus being highly vulnerable to peroxidation processes. Chemiluminescence (CL) started with t-butyl hydroperoxide (t-BHP) in suspension of lysed red cells is a technique that allows to assess oxidative stress in these cells. Lutein is known for its antioxidant effect. It is chemically a dihydric derivative of α-carotene and belongs to the group of xanthophylls. The aim of this study was to analyze the antioxidant role of lutein on peroxidation of equine erythrocytes membranes. Suspension of lysed erythrocytes with hypotonic phosphate buffer (previously washed in isotonic buffer), were maintained at 4°C for 48 h with or without the addition of increased quantities (5, 15 and 25 μg) of lutein (Lutein 20 mg. Piping Rock Laboratories, USA). Later, they were incubated at a final concentration of 0.25 mg/ml total hemoglobin in an in vitro system for 60 min at 37°C in the presence of 2 mM of t-BHP. Identical aliquots of the preparation were incubated without lutein (controls). Peroxidation was measured by monitoring light emission with a liquid scintillation analyzer Packard 1900 TR. Chemiluminescence was determined every 10 min and recorded as count per minute (cpm). It was observed that the total cpm originated from light emission was lower in samples with lutein ($486x10^3$; $376x10^3$ and $281x10^3$ in 5, 15 and 25 μg of lutein, respectively) compared to the control group ($772x10^3$). These results show that lutein may act as an antioxidant protecting erythrocytes membranes from oxidative damage.

A65
IDENTIFICATION OF lncRNAs INVOLVED IN THE RESPONSE TO RHIZOBIAL SIGNALING MOLECULES

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Common bean (Phaseolus vulgaris) establishes a symbiotic relationship with soil bacteria, resulting in the formation of root nodules in which bacteria are allocated and atmospheric nitrogen reduction takes place. In an effort to dissect the molecular responses mediated by rhizobial signaling molecules, we sought for long non-coding RNAs (lncRNAs) differentially expressed in response to rhizobial strains with different symbiotic efficiency, as well as with mutants defective in the synthesis of signaling molecules involved in the interaction. Here, we show that the percentage of lncRNAs is significantly higher in the population of transcripts differentially expressed than in the whole transcriptome of common bean. Notably, the majority of the differentially expressed lncRNAs are down-regulated in response to rhizobia. This down-regulation is mainly dependant on the Nod Factor, but also influenced by the presence of exo- and lipo-polysaccharides. In addition, we identified a group of lncRNAs differentially expressed in roots inoculated with an efficient strain of Rhizobium etli (SC15) as compared with a less efficient one (55N1). When we integrated the lncRNA data with small RNA libraries obtained from the same samples, we identified 24-mer siRNAs associated to the loci that produce these differentially expressed lncRNAs, suggesting that they might function as cis-acting modulators of transcription via chromatin remodeling. These lncRNAs will be further characterized by reverse genetics to elucidate their role in the nodulation process, and particularly, in the efficiency of the interaction in the common bean model.

A66
TRANSPORTERS OF AMINO ACIDS AND DERIVATIVES FROM *Trypanosoma cruzi* AS POTENTIAL THERAPEUTIC TARGETS

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T. cruzi has a metabolism largely based on amino acid consumption, mainly proline, which constitutes the main carbon and energy source in the insect stage of the parasite life cycle. In addition, proline participates in the progression of life cycle in the host cells, and it is also involved in oxidative stress and trypanocidal drugs resistance. Polyamines are essential molecules for all living organisms. T. cruzi is unable to synthetize de novo these molecules, so its acquisition relies exclusively in transport mechanisms. Nowadays there are only two drugs approved for treatment of Chagas disease with limited efficacy and severe side effects. Since amino acids and polyamines participate in a variety of metabolic routes leading to many crucial compounds for survival of T. cruzi, transporters and enzymes related to their metabolism become interesting targets. In this work we evaluated gentian violet (GV) and isotretinoin as trypanocidal drugs targeting transporters from the T. cruzi Amino Acid/Auxin Permeases (TcAAAP) family. GV was used for several years as a blood additive for prevention of transfusion transmitted Chagas disease and it was reported that inhibition of protein synthesis by GV could be due to inhibition of amino acid uptake. Our results showed that GV enters at least in part through the proline permease TcAAAP069. On the other hand, using a combined in silico strategy for drug repositioning, isotretinoin, a safe drug used for acne treatment, was selected for in vitro studies. Our results indicate that isotretinoin acts as a trypanocidal drug through the TcAAAP transporter family. Taken together, the results suggest that this permeases family constitutes an interesting target for drug development.

A67
IN VITRO ENDOTHELIAL CELL RESPONSE TO Li-CONTAINING 45S5 BIOACTIVE GLASS SCAFFOLDS

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Bioactive glasses (BGs) can be used for the manufacture of three-dimensional porous matrices, known generically as scaffolds. The functions of these scaffolds include providing a temporary biocompatible mechanical support and promoting the biological processes of repair and/or regeneration of tissues, serving as scaffolding for cells and growth factors involved in the repair process. Since lithium (Li^+) plays roles in angiogenesis, the controlled and localized release of Li^+ ions from BG based-scaffolds represents a promising alternative therapy for regenerative medicine of tissues that require high degree of vascularization. Here, scaffolds from a melt-derived 45S5 BG (composition in wt%: 45% SiO_2, 24.5% Na_2O, 24.5% CaO, and 6% P_2O_5) in which Na_2O was partially substituted by 5 wt% Li_2O (45S5.5Li) were investigated. The results demonstrate that human umbilical vein endothelial cells have greater migratory and proliferative response and ability to form tubules in vitro when stimulated with the ionic dissolution products released from 45S5.5Li BG scaffolds. These findings are relevant in tissue engineering because the 45S5.5Li BG-derived scaffolds would act as useful inorganic agents to improve tissue repair and regeneration, ultimately stimulating angiogenesis.

ECOTOXICOLOGY

A68
CONTRIBUTION OF PROTEASES IN THE HEMOSTATIC ALTERATIONS INDUCED BY *Bothrops alternatus* VENOM

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Bothrops alternatus is a medical important snake in central and northern Argentina. Bothropic envenoming causes proteolysis of tissues, bleeding and coagulation disorders. Coagulating enzymes belong to two proteases families: snake venom serine proteinases (SVSPs) with thrombin-like activity and metalloproteinases (SVMPs) mainly as prothrombin activators; these latest representing more of 50% of B. alternates venom components. In this work, the contribution of metalloproteinases in the hemostatic alterations was studied. The whole venom (10mg/mL) was incubated with an inhibitor of SVMPs, EDTA-Na_2 (10mM), the excess of inhibitor was removed by passing the mixture on Sephadex G-25 column (venom without inhibitor was subjected to the same process). Clotting time (CT) was recorded using citrated plasma or fibrinogen (3.5mg/mL) incubated with venom or venom-EDTA-Na_2 (venom concentration 270 µg/mL).Results showed that venom-EDTA-Na_2 was able to cause a delay of 3.5 times of the CT on citrated plasma compared with venom alone. However, venom-EDTA-Na_2 did not alter fibrinogen CT. Besides, venom pre-incubated with specific antibodies anti-baltergin (baltergin is a SVMPs isolated from this venom) was assayed on citrated plasma. In this case, CT showed a delay of 3.1 times compared with crude venom. Results suggest the presence of both proteases families in B. alternatus venom: metalloproteinases acting mainly as pro-coagulant factors. Because of SVMPs are the most important components of this venom, they become relevant targets for the development of new therapeutic agents.

A69
EVADING THE COLD: CHANGES IN THE ACTIVITY RATE OF *Leptuca uruguayensis*THROUGHOUT THE YEAR IN A TEMPERATE ESTUARY

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Fiddler crabs are usually found on tropical and subtropical estuaries, although some fiddler crabs have extended their distribution and are able to inhabit temperate estuaries. The aim of this study was to evaluate the variation on activity rate of the fiddler crab *Leptuca uruguayensis*, measuring the proportion of active crabs on the sediment surface throughout the year. Sampling was carried out monthly in Samborombón bay, Argentina. Population density was estimated by digging and density of active crabs was measured with a video camera by shooting the surface. Total amount of fiddler crabs outside their burrows, performing any type of activity, was registered from the images. Two-way ANOVA was used to compare the population density with density of active crabs, throughout the year. Density of crabs, estimated by digging, was larger than density of active crabs in most of the months except for August, September and October, in which densities were similar, suggesting a high activity rate. During the months of May, June and July, crabs were not observed on the surface indicating that they were inactive over this period. When the sediment temperature reached about 20°C, crabs emerged and reached maximum activity. The temperatures of winter are probably below the optimal range for *Leptuca uruguayensis*, representing a source of stress. The reduction of activity on the surface seems to be a strategy to save energy during the stressful period of lower environmental temperatures.

A70
VIABILITY OF SEED FROM THE NATIVE PALMS FOUND ON THE DIET OF WILD CARNIVOROUS: FINE TUNNING THE TECHNIQUE

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Between 2004 and 2012 as part of the project "Conservación de los Carnívoros del Nordeste Argentino" it was collected feces of *Chrysocyon brachyurus*, *Procyon cancrivorus*, *Cerdocyon thous* y *Pseudalopex gymnocercus*. It was conducted a conventional diet study. A subsample of palms seed (N=140) was taken in order to observe the integrity and viability of them. Of the whole sample analyzed the 27% has seed (8 seeds/feces). The seeds not have any damage caused by the depredation. The presence of *Syagrus ramanzoffiana* over *Butia yatay* was recorded in the ratio 3:1. The 21% of the seeds were attacked by insects of

Bruchidae family and by fungi, leaving unviable the seeds. The rest was cut longitudinally, hydrated during 24 hours and immersed in TTC solution (0.5%) for the period of 48 hours at temperatures between 36-38 °C in total darkness. The 14.5% was nonviable, 24.5% was viable, and in the 40% the endosperm was stained but the embryo could not be observed. These preliminary contributions allow us to fine tunning the viability technique implemented and show the role played by carnivores as dispersers. The results obtained so far will be contrasted with subsequent germination tests. This project was supported by: Amnevillé Zoo, Doué la Fontaine Zoo, Zoo des Sables d`Olonne, Abilene Zoo, Cerza Conservation, John Ball Zoological Garden, Friends of Dickerson Park-SSPMW/IUCN, Brookfield Zoo, Idea Wild, WAZA, Safari de Peaugres, ZACC, ZACC 2016 Steering Committee. WAZA Project: 06031. SGCyT (UNS), PGI 24B/198.

A71
ISOLATION OF CROTAMINE FROM VENOM OF ARGENTINIAN RATTLESNAKE

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Crotamine is a small cationic peptide originally found in the venom of the South American rattlesnake *Crotalus durissus terrificus*. It is a 5-kDa mio-neurotoxin that has been shown to possess analgesic effects. At final concentration ranging from 0,1 to 10 □M, it was not cytotoxic to normal cells of different types (e.g., muscle cells, human endothelial cells), even after 72 h of exposure, according to works of brazilian authors. Considering regional variation not only between species, but also within a single species, it is our interest to isolate and characterize crotamine from specimens living in the Argentine northeast, which has not been studied yet. In this work we purified crotamine by size-exclusion chromatography and ultrafiltration. When injected i.p. (8,75 - 280 ng/Kg body mass) in adult male CF1 mice, it induced a time-dose dependent high analgesic effect by the acetic acid-induced writhing method, where 35 ng/Kg was the minor dose that showed effect. By in vitro assays, at final concentration of 0,53 □M, the crotamine isolated from argentinean rattlesnakes, exhibited toxic activity on C2C12 cells, in contrast to the behavior of that obtained from brazilian snake venoms. These preliminary findings highlight differences between crotamines purified from species from distinct geographic areas. Further study is required to elucidate the structure and the properties of the small protein here isolated.

A72
POTENTIAL ANTITUMOR ACTIVITY OF A BASIC PHOSPHOLIPASE A_2 FROM *Bothrops diporus* VENOM

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Many toxins from snake venoms have been investigated as possible prototypes for cancer treatment. In particular, different types of phospholipases have been shown to possess antitumor and antiangiogenic properties, such as acidic and basic PLA_2s, and synthetic peptides derived from PLA_2 homologues. In this work, a basic PLA_2 was isolated from Bothrops diporus (yarará chica) venom and its potential antitumor effect in vitro was studied. Purification was made by a two-step procedure utilizing ion exchange (HiTrap SP XL-AKTAprime) and gel filtration chromatography (Sephadex G-75). 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 normal (NMuMG) and a tumoral (LM3) epithelial cell lines. Briefly, cells were exposed to different amounts of PLA_2 (10-250 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. Results indicate that the isolated enzyme PLA_2 induces a dose-dependent detachment of cells in both lines, but tumoral LM3 cell line showed to be more sensitivity to the enzyme cytotoxic action (CC_{50}= 13.3 μg/mL) than normal cell line NMuMG (CC_{50}= 74.2 μg/mL). Further studies will be necessaries to demonstrate if this selectivity for tumor cells could be an advantage in the investigation of novel therapeutic agents.

A73

COMPARATIVE MYOGENESIS (EARLY PHASE) IN MICE AFTER EXPERIMENTAL INTOXICATION INDUCED BY BOTHROPIC VENOMS

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Muscle regeneration after *Bothrops* sp envenomation is a phenomena of great interest since their best knowledge would improve the conventional serum therapy. We proposed a comparative study about myogenesis in mouse gastrocnemius after two *Bothrops* sp venom intramuscularly injected. Groups of four CF-1 mice (18-20 gr) were injected at the upper two-thirds of the right gastrocnemius with 100 or 60 µg of crude venoms dissolved in 0.1 ml of PBS, pH 7.2, respectively. Control mice received PBS alone. After 7 days, mice were sacrificed. Samples of injected muscles were dissected and then examined using standard histopathologic processing with H&E. Preliminary histological observations at 7 days for both LD50 of Ba or Bd exhibited characteristics of the initial phase of muscle recovery after drastic morphological alterations like haemorrhage, necrosis and intense infiltrate inflammatory polymorphonuclear. At the beginning stage of myogenesis, only few newly regenerating centrally located fibres of small size appeared. Besides, abundant necrotic myofibres and extensive inflammatory infiltrate predominated in the vicinity for Bd. Meanwhile, after Ba envenomation, the microenvironment anticipates effective regenerative events since the removal of necrotic material was well advanced by the seventh day. Areas of muscle fibres without damage were also present. Venom composition can explain the above differences. The crucial difference between the *Bothrops* venom species is the proteases (svMPs) composition whereas Ba venom comprises mainly P-III class metalloproteinases (highly haemorrhagic). Contrary, in Bd prevailed svMPs P-I class (weakly haemorrhagic) and also myotoxins.

A74

ISOLATION OF PHOSPHODIESTERASE FROM THE VENOM OF *Crotalus durissus terrificus*

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Phosphodiesterases (PDEs) belong to a super-family of enzymes that have multiple roles in the metabolism of nucleotides. Snake venoms contain PDE (e.g. *Trimeresurus stejnegeri*, *Daboia russelli russelli*,*Bothrops jararaca*) but their function is poorly understood at the present. In this work a PDE was isolated from C.d.t venom (CDT-PDE) by ultrafiltration process in 50 KDa membranes. After that, proteins with a molecular mass more than 50KDa were concentrated and then purified by ion-exchange chromatographies (HiTrap Q-FF and SP XL). Homogeneity of the pool was verified by SDS-PAGE. Two bands around 100 KDa were analyzed by mass spectrometry (Q Exactive LC-MS/MS system) and the study of fragment peptides determined the presence of two fosfodiesterase isoforms (96 and 91 KDa). The activity of the CDT-PDEs on platelet aggregation was evaluated. When platelet rich plasma ($350x10^3$ /µl) was stimulated with 10 mM of ADP, platelet aggregation was completed by around 40%. However, in presence of 0.16µg of CDT-PDEs, the extent of aggregation reached at most 10%. This inhibitory effect is characteristic of other snake PDEs. This preliminary study will be expanded to characterize this enzyme of biomedical interest.

A75

EXPOSURE TO BPA DISRUPTS WNT SIGNALING PATHWAY DURING POSTNATAL DIFFERENTIATION OF THE OVIDUCT IN BROAD-SNOUTED CAIMAN (*Caiman latirostris*)

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Wnt molecules and β-catenin control postnatal female reproductive tract development through canonical (β-catenin -dependent) or non-canonical pathways. These molecules are sensitive to endocrine-disrupting compounds (EDCs) and the oviduct is a target organ of EDCs action. Our aims were to establish the ontogeny of temporal and spatial expression patterns of wnt-5a, wnt-7a and β-catenin in C. latirostris oviducts from neonatal to pre-pubertal juvenile caimans, and to evaluate the effect of BPA early postnatal exposure. C. latirostris females raised in control conditions were euthanized at neonatal, early and late postnatal or juvenile stages. Early postnatal caimans were injected (sc) twice, 7 days apart, with 17-β estradiol (E2) (0.014 or 1.4ppm) or BPA (1.4 or 140 ppm) and euthanized 7 days after last injection. Oviductal wnt5a, wnt7a and β-catenin protein expressions were assessed by immunohistochemistry. Epithelial wnt-7a and subepithelial wnt-5a levels increased as oviduct differentiation advances showing a mutual feedback in the juvenile stage. Epithelial membrane-associated β-catenin expression was not correlated to wnts levels. All the treatments, but BPA 1.4ppm reduced wnt-7a expression, whereas E2 1.4ppm and BPA 1.4ppm decreased wnt-5a levels. BPA 1.4ppm also modified CTNNB expression. Our results suggest that postnatal development of C. latirostris oviduct is controlled by wnt signaling pathway and that early postnatal exposure to xenoestrogens could disrupt this process leading to histofunctional changes that could impair reproductive health later in life.

NEUROSCIENCE-ENDOCRINOLOGY-METABOLISM

A76

DIFFERENTIAL DEVELOPMENT OF FEMALE EMBRYONIC GONADS IN TEMPERATURE AND HORMONAL SEX DETERMINATION: ANALYSIS OF HORMONE RECEPTORS AND AROMATASE EXPRESSION IN *Caiman latirostris*

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Sex determination is a process that guides bipotential gonads (undifferentiated) to develop toward testis or ovary. Caiman latirostris is a reptilian species that exhibits temperature-dependent sex determination (TSD). Also the female phenotype can be achieved after in ovo estrogen exposure; this is known as hormone-dependent sex determination (HSD). In stage 22, 24 and 27 of embryonic development (from the beginning to the end of embryonic gonadal differentiation) we assessed by IHC the expression of estrogen receptor alpha (ERα) and progesterone receptor (PR) in gonads of embryos obtained from eggs incubated at temperature producing females (TSD) or at temperature producing males plus a dose of 17β-estradiol -E_2- administered at stage 20 that overrides the temperature effect and produce the female phenotype (HSD). Aromatase expression was evaluated by IHC in the ovarian clusters (previously identified by VASA) using a polyclonal antibody generated in our laboratory. HSD group showed higher expression of ERα in stage 22 while PR expression was significantly increased towards the end of embryonic development compared to TSD. In addition, we detected a specific Aromatase immunoreactivity and the expression of the enzyme was higher in the gonads of HSD females throughout development. Our results demonstrate that in caimans, embryonic female gonads obtained by temperature or hormonal sex determination exhibit different expression patterns of key molecules associated with ovarian development and function.

A77

OVERCONSUMPTION OF SUCROSE DURING THE JUVENILE STAGE IMPRINTS LONG-TERM CHANGES IN THE NUCLEUS ACCUMBENS

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Sucrose consumption has increased dramatically in our society. This phenomenon is mainly associated with increased obesity and diabetes, among other metabolic disorders. Of particular interest are the youngest populations since they have the highest sugar consumption and, during this period of life, profound changes occur as part of the maturation of the CNS. However, little is known about the impact of overconsumption of sucrose on the developing CNS. Here we studied the effect of unlimited access of sugary water during the juvenile stage (childhood-adolescence) on the expression of neuronal proteins involved in the structure, function and inflammatory responses in areas belonging to the limbic system, system related to motivation and reward. In parallel, the effects of excessive sucrose consumption were tested in adulthood. The long-term expression of cFos, p-AKT, AKT-T, GFAP, NeuN and PCNA were analyzed by Western blot in the nucleus accumbens (nAcc), the medial prefrontal cortex (mPFC) and the ventral hippocampus (HIPv). We found that the nAcc is particularly sensitive to the overconsumption of sucrose at early stages of development, but not in adulthood. cFOS was increased ($p<0.05$) indicating an over-stimulation of that area in animals treated with sucrose in the juvenile stage. With regard to structural factors, the marker of mature neurons NeuN was found decreased, while the marker of proliferating cells, PCNA was increased ($p<0.05$). None of these changes was detected in animals ingesting sucrose in adulthood. None of the other parameters studied was found modified by treatment. All these results suggest that consumption of sucrose at early stages of development produces long-term effects on the limbic system, particularly on the nAcc (PIP 0243, Fundación Rene Barón).

A78

THE PINEAL GLAND OF MALE VISCACHA (*Lagostomus maximus maximus*). A SEASONAL AND AGE-RELATED STUDY OF THE INTERSTITIAL CELLS

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The pineal gland of viscacha exhibits histophysiological variations throughout the year, with periods of minimal activity in summer and maximal activity in winter. The purpose of this work was to analyze the interstitial cells (ICs) in the pineal gland of male viscachas in relation to season and age. The glio-fibrillary acidic protein (GFAP), the S-100 protein, and vimentin were used as markers. Immunohistochemistry (IHC) and double-IHC were performed. GFAP was present only in the cytoplasm, while

the S-100 protein was localized within both, IC nucleus and cytoplasm. Vimentin was expressed in some ICs, besides endothelial cells and perivascular spaces. In the adult males, the S-100 protein and GFAP exhibited seasonal variations with lower values of immunopositive area percentage in summer and higher values in winter, whereas the immature ones showed the lowest values for all the adult groups studied. Colocalization of S-100 protein and GFAP was also observed. The ICs exhibited differential expression for the analyze proteins, supporting the hypothesis of the neuroectodermal origin. The ICs generate an intraglandular communication network, suggesting its participation in the glandular activity regulation processes. Double-IHC indicates ICs in different functional stages, probably related to the needs of the cellular microenvironment. The morphometric variations in the proteins analyzed probed to be more salient in the adult males compared with the immature viscachas. These differences can be explained in relation to seasonal needs of the adult pineal gland that depend on the environmental photoperiod.

A79
EFFECTS OF GABAB ANALOGS ON BRAIN KISS1 EXPRESSION IN ADULT MALE MICE

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Our previous results have shown that in adult $GABA_{B1}$KO male mice, which lack of $GABA_B$ receptors, Kiss1 mRNA expression was similar to wild type males in hypothalamic nuclei [arcuate (ARC) and anteroventral periventricular nucleus]. However, Kiss1 mRNA expression was markedly increased in extra-hypothalamic areas that are related to reproduction, such as medial amygdala (MeA), bed nucleus of the stria terminalis and lateral septum. To determine whether these characteristics were imprinted during development or could be mimicked in adulthood, here we studied the effects of the administration of a GABAB antagonist or a GABAB agonist on brain Kiss1 expression in adult mice. Adult Balb/c male mice were sc injected with CGP55845 (CGP, 1 mg/kg, a GABAB antagonist), Baclofen (Baclo, 5 mg/kg, a GABAB agonist) or saline as control (Sal) for 5 days, three times/day (8AM, 1PM, 6PM). Males were sacrificed at 3PM (after two injections on day 5). Serum samples and testes were obtained to determine hormone levels by RIA. Brains were frozen and 500µm slices were obtained on a cryostat. ARC and MeA were micropunched and Kiss1 mRNA expression was evaluated by qPCR (cyclophilin B, control gene). Baclo increased serum LH ($p<0.02$) and decreased serum prolactin compared to Sal ($p<0.01$) whereas CGP had no effect. However, neither CGP nor Baclo were able to modulate serum and testicular testosterone or Kiss1 expression in ARC (AU: Sal 1.18±0.10; Baclo 0.86±0.08; CGP 1.27 ±0.22, NS) or MeA (AU: Sal 1.23±0.23; Baclo 1.16±0.16; CGP 1.06±0.09, NS) in these mice. We conclude that these GABAB analogs cannot mimic during adulthood the effect on Kiss1 expression we observed in extra-hypothalamic areas of adult GABABKO mice (CONICET, ANPCYT, UBA, Fundación René Barón).

A80
EFFECTS OF A GABAB ANTAGONIST ON HYPOTHALAMIC KISS1 EXPRESSION IN NEONATAL MICE

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We have previously shown that in neonatal female $GABA_{B1}$KO mice, lacking functional $GABA_B$ receptors, Kiss1 mRNA expression in the arcuate nucleus (ARC) is significantly lower that in WT female littermates, abolishing the normal sex differences observed at this age (females > males). Here we aimed to establish whether pharmacological intervention immediately postnatally could mimic the differences in ARC Kiss1 expression. Neonatal Balb/c mice were subcutaneously injected with CGP55845 (CGP, 1 mg/kg, a GABAB antagonist) or saline from postnatal day 2 (PND2) to PND6±1, three times/day (8AM, 1PM, 6PM). Mice were sacrificed at 3PM (after two injections on that day). Brains were frozen and 400 µm slices were obtained on a cryostat. ARC and anteroventral periventricular nucleus (AVPV) were micropunched and Kiss1 mRNA expression was assesed by qPCR (control gene: Cyclophilin B). Serum and gonads were collected for hormonal measurements. CGP significantly decreased ARC Kiss1 expression in both sexes (AU: ♀-Sal: 4.7±1.7, ♀-CGP: 3.0±1.1, ♂-Sal: 1.1±0.2, ♂-CGP:0.5±0.1; CGP≠SAL: $p<0.04$, sex: $p<0.001$). No differences were observed in ARC estrogen receptor α mRNA expression. PRL serum titers were higher in males than in females but not affected by treatment. No differences were observed in testicular testosterone contents between treatments. Ovarian estradiol content was significantly increased in CGP-treated females (pg/mg: ♀-Sal: 33.5±12.2 vs ♀-CGP:186.3±72.2, $p<0.01$). We conclude that at this early age a GABAB antagonist can modulate ARC Kiss1 expression in mice. (CONICET, ANPCYT, UBA, Fundación René Barón).

A81

EXPRESSION OF NEURAL CREST LINAGE MARKER IN OSTEOGENIC TISSUE OF THE ARMADILLO *Dasypus hybridus* (XENARTHRA, CINGULATA, DASYPODIDAE) FETUS DERMIS

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Neural crest cells give rise to various cell populations in the adult vertebrate including facial bones. In some reptiles, such as turtles and crocodiles, neural crest is involved in the origin of the osteoderms (dermis ossifications). In modern mammals, osteoderms are only present in the Dasypodidae (armadillos, tatus, etc).skin The osteoderm formation is a complex process including both metaplastic and intra-membranosous ossification mechanisms. At present there are not studies about a possible neural crest origin of the of the Dasypodidae osteordems. In this work we focus on Dasypus hybridus,a Dasypodidae species, to assess the presence of a neural crest marker (the HNK-1 epitope), which has been previously found in the osteoderm precursor tissue of reptiles. Samples of dorsal and ventral skin of fetus and neonates of D. hybridus were processed for immunohistochemistry using a HNK-1 antiobody. The ventral skin mesenchymal or connective tissues were negative, but in the dorsal region of a fetus (total length 90.8 mm) positive cells were found in the subepidermal region of the undifferentiated dermis. Positive cells were absent in the region between the epidermal scales, where the osteoderms never develops. These results are the first evidence of a potential neural crest origin of poscranial ossifications in mammals. Further works are in progress, using others neural crest markers and alternative designs to demonstrate negative melanocyte lineages in these cells.

A82

EFFECTS OF NATIVE PLANTS DECOCTION FROM CUYO ON SERUM PARAMETERS OF RATS WITH EXPERIMENTAL DIABETES

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Diabetes mellitus is a chronic disease with great global distribution, thus the search of new therapeutic alternatives is constant. Several studies indicate that 85% of world population still uses “medicinal plants” to be healthy. In particular, in the region of Cuyo (Argentine), Oxalis erythrorhiza (Oe) and Tessaria absinthiodes (Ta) species are consumed to regulate the glycaemia (Glu) and the cholesterol level (Chol), although those effects lack of scientific support. Male rats (SD) of 42 days, controls (C, i.p. vehicle) or with experimental diabetes (D, i.p. streptozotocin 30mg/Kg) received 10% decoctions of Oe (DOe and COe), Ta (DTa and CTa), or water (DW and CW) like only drink for 4 weeks. Glu, Chol and triglycerides (TG) were determined on samples obtained weekly with colorimetric kits. At the end of the treatment, Glu and TG were higher in the D than the C (200% and 170% respectively; $p<0.05$), while Chol was lower (30%; $p<0.05$). Glu values on DOe and DTa were lower than DW (34% and 30% respectively; $p<0.05$), and in these groups a increasing TG and a decreasing Chol trend was observed. The decoctions intake did not alter plasmatic parameters on C groups. These preliminary results show that Oe and Ta could have regulatory effects on Glu. To asses some other properties in these native plants decoctions, a longer treatment and two other dilutions are being performed with the same experimental model. (CAM-2 SECITI-UCCUYO 2015; PIP 0243;PIO-SECITI150 2015-0100022).

A83

ROLE OF NOTCH PATHWAY IN ANGIOGENESIS REGULATION IN BOVINE MAMMARY GLAND

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Notch pathway is associated with stem cells maintenance, proliferation, angiogenesis and cell differentiation. These processes occur in bovine mammary gland development. Gastrointestinal parasites affect Holstein heifer development. Hereby we studied Notch receptor expression (Notch 1-4) and its role in angiogenesis along development in control (C) and anthelminthic drug treated (T) heifer mammary glands. We determined Notch 1-4 and the endothelial cells marker CD34 expression by immunohistochemically studies. We observed that these proteins are expressed in glandular parenchyma and in stromal cells at different stages of heifer development. We studied Notch receptor expression levels in its active (80 KDa) and membrane (110 KDa) forms by Western blot and we found increased the Notch 3 active domain and a reduction of Notch 2 active and membrane domains at 40 weeks of age. Notch 4 receptor expression decreased in heifers at 30 and 40 weeks of age. Moreover we determined Hes-1 mRNA expression, a target gen of the Notch pathway, and we found an increment of this gen expression at 40 weeks of age. Real Time studies showed that the expression of the endothelial cell markers VEGF and Endocan increased at 40

weeks of age. The expression of Notch 1-4 receptors and Hes-1 target gen suggests an activated Notch pathway in bovine mammary gland. The increase in VEGF (p= 0,014) and Endocan mRNA levels, high Notch 3 expression (p= 7,86E-07) and low Notch 2 (p=3,23E-05) and 4 (p=0,032) expression at 40 weeks of age suggests that Notch 2, 3 and 4 might be involved in angiogenesis regulation at that time of development.

REPRODUCTION III

A84
COMPARISON OF TWO PROCESSING TECHNIQUES FOR CRYOPRESERVED PORCINE SPERM. EFFECT ON ROUTINE AND FUNCTIONAL PARAMETERS

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Cryopreservation increases reactive oxygen species (ROS) that is associated with oxidative stress and involves changes that affects sperm membrane characteristics and causes damage and inhibition of cellular mechanisms. Different techniques were developed to improve post-thaw sperm quality. Alpha-tocopherol protect sperm membranes from ROS damage and improves boar sperm functionality. Sephadex filtration removes dead and abnormal spermatozoa, increasing post-thaw semen quality. The aim of this study was to compare two different processing techniques: cryopreservation with alpha-tocopherol added to freezing extender and post-thaw simple washing (VE-SW) or cryopreservation without alpha-tocopherol and post-thaw Sephadex filtration (C-SF), with cryopreservation without alpha-tocopherol and post-thaw simple washing (C-SW), evaluated by routine and functional sperm parameters. Motility (MOT), sperm membrane integrity (MI) and acrosome integrity (AI) were used as routine sperm parameters and in vitro capacitation and acrosome reaction induction were evaluated by incubating sperm in TBM medium with bicarbonate and follicular fluid as capacitation and acrosome reaction inducers, respectively. Routine quality parameters were significantly improved by Sephadex selection (MOT: 54 vs. 39 and 37%, for C-SF, VE-SW and C-SW, respectively; MI: 55 vs. 39 and 30%, for C-SF, VE-SW and C-SW, respectively; AI: 47 vs. 40 and 30%, for C-SF, VE-SW and C-SW, respectively). In vitro capacitation response was significantly higher in C-SF samples. Our results demonstrated that the quality of cryopreserved porcine sperm can be improved by the utilization of these processing techniques.

A85
ELEVATED PARENTAL REPRODUCTIVE EFFORT DECREASES IMMUNE FUNCTION IN BROWN SKUA (*Stercorarius antarcticus*) AND AFFECTS CHICKS DEVELOPMENT

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In birds, feeding and caring for chicks have been assumed to be the costliest of the reproductive phases. Life-history theory predicts that increased reproductive effort should lead to a fitness cost and that this cost may be caused by a temporal decrease of body condition and suppression of immune function in hard-working individuals. Brown skuas show a different behaviour during breeding, being males more active in the maintenance of the nest. We used hematological values to assess the nutritional and health status in adult Brown skuas during breeding and its effect on offsprings, in order to understand the physiological background responsible for the trade off between reproductive effort, nutritional and health status. Blood samples were obtained in three different moments from adults: In (incubation), Pi (after egg hatching) and Pii (during chick rearing), and only during Pi and Pii from chicks. Total proteins, serum globulins, A:G ratio, IgY and electrolytes (Na, K, Cl and phophorus) levels were determined. Differences in total proteins, uric acid, IgY and γ-globulin fraction levels were observed in adult males and females through the reproductive stages. In addition, nutritional and immunological consequences (decrease of γ-globulin fraction) were observed in growing chicks. These results indicate that there is a close relationship between the reproductive effort performed by adults –inparticular by males-, the decrease in body condition during these stages and the health status of the offsprings during development.

A86

ESTRADIOL AND PROGESTERONE REGULATES THE VASCULARIZATION PROCESS OF THE FIRST TRIMESTER TROPHOBLAST THROUGH THE LPA/LPA3 SYSTEM

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Remodelling of the spiral arteries by the trophoblast is crucial during human implantation. Previously, we observed that lysophosphatidic acid (LPA), a small and bioactive phospholipid, enhances vascularization of first trimester trophoblast cells by increasing tubulogenesis through LPA3 (an LPA receptor). Estradiol (E2) and progesterone (P4) are the master hormones that orchestrate the events that take place during implantation. Our objective was to study if E2 and P4 regulate the action of LPA/LPA3 in vascularization of trophoblasts. First trimester trophoblast cells (HTR-8/SVneo line) were seeded on a reduced growth factor basement membrane matrix (Geltrex®), incubated with E2 (10^{-8}, 10^{-9} and 10^{-10} M) or P4 (10^{-6}, 10^{-7} and 10^{-8} M), in the presence of a selective LPA3 antagonist (DGPP 10^{-4} M) or a non-selective LPA3 antagonist (8 Br-LPA $5x10^{-6}$ M), and assayed for tube formation during 6h. 8 Br-LPA antagonizes LPA1, 2, 3 and 4 receptors. We observed that the incubation with E2 (10^{-8} M) or with P4 (10^{-7} M) increased tube formation ($p<0.05$) of HTR-8/SVneo cells. While the co-incubation of E2 with DGPP partially reversed tubulogenesis, the treatment with E2 + 8 Br-LPA completely blocked the effect. Both DGPP and 8 Br-LPA decreased P4 stimulation to control level. Our results suggest that E2 and P4 regulate the vascularization of first trimester trophoblasts through LPA and LPA3 receptor.

A87

EVALUATION OF A5B1 INTEGRIN AS POSSIBLE MOLECULAR MARKER OF FERTILIZING ABILITY OF BOVINE CRYOPRESERVED SPERM

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Sperm contribution to fertilization is estimated through spermogram evaluation. However, results from those analysis often do not correlate with fertility. Therefore, the identification of potential markers of sperm functionality would strengthen the proposal of new therapies for infertility. Previous results indicated that α5β1 integrin play a key role in fertilization events in bovines. The aim of this study was to investigate whether there is any relationship between α5β1 expression and the fertilizing ability in vitro, in bovines. We studied the localization of α5β1 in bull sperm by immunocytochemistry. Integrin was localized mainly at acrosomal and postacrosomal regions. When sperm were incubated for 45 min in the presence of fibronectin (integrin ligand) an increase of fluorescence intensity was detected by flow cytometry, suggesting an integrin activation. Then, we analysed the fertilizing ability of sperm samples by in vitro fertilization (IVF) according to the rate of cleavage. Sperm samples that showed optimal IVF rates presented higher percentage of sperm expressing α5β1 than those that presented lower IVF rates. These results suggest a possible correlation between the integrin expression and the fertilizing ability of a sperm sample in bovines.

A88

FATTY ACID B-OXIDATION, ITS RELATIOSHIP WITH BOVINE OOCYTE ATP CONTENT, MATURATION AND DEVELOPMENTAL COMPETENCE

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The aim of this work was to evaluate β-oxidation of fatty acids in bovine oocyte in vitro maturation (IVM) and it effect on oocyte ATP content, maturation process and developmental competence. Cumulus-oocyte complexes (COCs) were obtained by aspiration from slaughtered cows ovaries. IVM was performed in 199 + 5% FBS + FSH + LH (Control) supplemented with a β-oxidation inhibitor or stimulator, etomoxir (E) or L-carnitine (L-C), respectively. In vitro fertilization (IVF) and embryo development was performed in mSOF. ATP content was measured in matured-denuded oocytes using a kit based in the luciferin-luciferase reaction. Meiotic maturation was evaluated by the presence of the chromosome plate in metaphase II by Hoechst stain. IVF was evaluated by the cleavage rate and embryo development by the blastocyst rate at day 7 following insemination. COCs matured with E showed a significant decrease in oocyte ATP content and in nuclear maturation rates ($p<0,05$) while supplementation with L-C did not modify the ATP content, maturation, cleavage and blastocyst rates. These results show that the stimulation of β-oxidation did not improve the development competence of the bovine oocyte. However, the data suggest that the oocyte nuclear maturation depends on fatty acid β-oxidation and this process might be related with the ATP content in the bovine oocyte.

A89
LYSINE ACETYLATION AS A MODULATOR OF SPERM CAPACITATION

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Mammalian sperm are unable to fertilize the egg before undergoing a series of biochemical and physiological changes in the female reproductive tract, collectively known as capacitation. Functionally, capacitation is associated with changes in the sperm motility (hyperactivation) and with their ability to undergo the acrosome reaction. At the molecular level, capacitation correlates with activation of the cAMP-PKA pathway, increase in intracellular pH and Ca^{2+} concentration, hyperpolarization of the plasma membrane potential, loss of cholesterol and other lipid modifications and increase in protein tyrosine phosphorylation. How these signaling pathways interact to induce hiperactivation and the acrosome reaction is not well understood. Since mature sperm are transcriptionally and translationally silent, they rely on postranslational modifications (PTM) of proteins more than any other cell type. Therefore, it is an exceptional model for the study of signaling pathways based on PTM. The importance of phosphorylation, an essential PTM in sperm physiology has been well established. Acetylation as a broad and abundant PTM comparable with phosphorylation, however, has not been well analyzed. Recently, two groups identified 576 and 456 acetylated proteins in capacitated and non capacitated human sperm respectively, 250 were present in both conditions. In the present work, we studied the role of acetylation in capacitation of mouse and human sperm. WB analysis with anti-acetyl lysine antibodies showed lysine acetylation of lots of proteins spanning a wide mass range. Acetylation of proteins was associated with PKA activity. In addition, this PMT is necessary for hyperpolarization of the sperm plasma membrane, which is a prerequisite for acrosome reaction. These results point towards a key role of lysine acetylation in sperm capacitation.

A90
STUDY OF THE MECHANISMS INVOLVED IN CRISP2$^{-/-}$ SPERM FERTILIZING DEFECTS

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Testicular Cysteine-RIch Secretory Protein 2 (CRISP2) is present in sperm and participates in gamete fusion and penetration of both zona pellucida (ZP) and cumulus oophorus during fertilization. In addition, Crisp2$^{-/-}$ sperm exhibit lower percentages of hyperactivation, a vigorous motility required for the penetration of the egg coats, and higher intracellular Ca^{2+}(iCa^{2+}) levels after capacitation, compared to controls. In the present work, we further investigated the mechanisms underlying these Crisp2$^{-/-}$ sperm defects. ZP-intact eggs treated with reduced glutathione to destabilize the ZP prior to their insemination, produced a significant increase in the fertilization levels corresponding to Crisp2$^{-/-}$ sperm, supporting that the lower fertilizing ability of these cells is linked to their lower levels of hyperactivation. We then evaluated iCa^{2+}levels by flow cytometry in mutant sperm incubated in capacitating media containing either an inhibitor of CatSper, the main sperm membrane Ca^{2+} channel, or lower concentration of $CaCl_2$. Exposure of sperm to any of these conditions produced a reversion of the deregulated iCa^{2+} of Crisp2$^{-/-}$ cells, indicating that the higher iCa^{2+} levels of the mutant sperm are dependent on extracellular Ca^{2+}. However, when these treated sperm exhibiting normal iCa^{2+}were subjected to in vitro fertilization, no improvement in the fertilization rates was observed. Together, these observations support the relevance of CRISP2 for the regulation of the complex and fine-tuned Ca^{2+} regulating system operating in the fertilizing sperm.

A91
NEURAL CADHERIN IN MURINE SPERM AND COCs AND ITS PARTICIPATION IN FERTILIZATION

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Mammalian fertilization involves an organized sequence of molecular events throughout the spermatozoan journey to the fertilization site. Since gamete interaction implicates adhesion events and presence of Ca2+ions, the involvement of the Ca+2-dependent adhesion protein Neural Cadherin (N-Cadherin) was studied. We previously reported the expression of N-cadherin in both human sperm and oocytes and showed evidence of its participation in fertilization, describing the ability of specific antibodies to impair gamete interaction. The present study was designed to evaluate the expression of N-cadherin in murine gametes and reproductive tissues and its involvement in fertilization-related events. N-cadherin mRNA expression was determined by qRT-PCR in adult testis and epididymis, as well as in ovary, GV- and MII-oocytes, finding the highest levels in testis and MII oocytes. Western immunoblot analysis revealed the presence of the 135 KDa mature protein in gonads and gametes. By fluorescence immunocytochemistry, N-cadherin was detected in the acrosomal cap of testicular sperm and in the acrosomal region and equatorial segment of acrosome-intact non-capacitated and capacitated (FITCPSA/ anti-Ncadherin) epididymal sperm. Contrastingly, Progesterone-induced acrosome-reacted sperm showed N-cadherin signal mainly localized in the equatorial segment. Immunodetection of N-cadherin was confirmed in mature cumulus cells and MII-oocytes. In spermoocyte interaction assays, gamete preincubation with specific N-cadherin antibodies (H-63, StaCruz & GC-4, SIGMA) resulted in

decreased COCs fertilization (48.2% of control, $p<0.05$) and sperm-oocyte fusion (60.2%, $p<0.05$). The present report thoroughly describes N-cadherin expression in murine gametes and reproductive tissues and shows evidence of its participation in fertilization.

A92
FOLLICULAR DEVELOPMENT AND OVARIAN MATURATION OF *Cnesterodon decemmaculatus* (CYPRINODONTIFORMES: POECILIIDAE)

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Viviparity, or internal fertilization refers to development of the embryo inside the female reproductive system, is the mode of reproduction for approximately3% of all living teleost. The madrecita, *Cnesterodon decemmaculatus*, is a Neotropical species of poeciliid native to South America. In the last years, this species has been used as a test organism for the biomonitoring of polluted freshwater environments. This work provides information on the reproductive biology of *C. decemmaculatus*, identifying and characterizing the follicular development stages and its organization within the ovary. Females from 0.8 to 3.3 cm in total lenght (TL) were used. Samples (small specimens or ovaries) were fixed by immersion in 10 % buffered formalin and routinely processed and embedded in paraffin wax. Histological sections of3 - 4 µm were prepared according to the standard protocol, and then stained by thehematoxylin-eosin and Masson's trichrome methods. The histological analysis showed six stages of follicular development. Only stages I, II and III predominatedin females to size ≤ 2 cm TL; whereas stages IV, V and VI were observed in females to size ≥ 2 cm LT. Stages I and II are considered previtellogenic follicles, in which oocytes have nucleoli toward the periphery of the nucleus and basophilic cytoplasm. Stage III is an early vitellogenic follicle, in which small lipid yolk vesicles in the cytoplasm were observed. Stages IV, V and VI are characterized by progressive accumulation of proteic yolk globules. The results of this analysis suggest that *C. decemmaculatus* presents a relationship between total length and gonadal maturity; besides, presents a gonadal development of synchronous type which is evidenced by the presence of ovarian follicles in a same stage of development.

A93
POTENTIAL USE OF A SPECIFIC CATSPER INHIBITOR FOR NON-HORMONAL CONTRACEPTIVE DEVELOPMENT

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Hormonal contraceptive methods are known to produce multiple side effects. Thus, there is a need to develop new and safer non-hormonal options. Contraceptive methods specifically targeting sperm function constitute an attractive approach for both male and female contraception. CatSper, the principal mammalian sperm calcium channel, is involved in the development of hyperactivation, a vigorous motility essential for fertilization and male fertility. Considering that CatSper is only expressed in sperm, it represents an excellent target for non-hormonal contraception. Based on this, in the present work, we evaluated the effect of HC, a specific Catsper inhibitor, on different sperm functional parameters. Our results show that exposure of cauda epididymal mouse sperm to different concentrations of HC (1, 5, 10 and 20 uM) during capacitation did not affect sperm viability but produced a significant decrease in their motility at concentrations ≥ 5 uM. A time course study using HC 10 uM showed that this effect becomes evident after 30 minutes of incubation, reaching levels lower than 5% at the end of capacitation (90 min). Whereas protein tyrosine phosphorylation, a capacitation-associated event, markedly decreased by exposure to > 5 uM, neither the spontaneous nor the ionophore-induced acrosome reaction were affected at any of the HC concentrations employed. Experiments in which cumulus oocytes complexes or zona-free eggs were co-incubated with sperm capacitated in the presence of HC (1 to 20 uM) showed a significant decrease in the percentage of penetrated eggs at 5 uM with a complete inhibition at ≥ 10 uM. The fact that the presence of HC only during gamete co-incubation neither affected fertilization nor egg penetrability confirmed that HC mainly affects sperm capacitation. Together, these results indicate that HC is specifically interfering with the sperm fertilizing ability, supporting its potential use for non-hormonal contraceptive development.

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