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"CONVENTIONAL AND UNCONVENTIONAL MODELS FOR THE STUDY IN BIOLOGY"

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LECTURES

A1
NON-CONVENTIONAL ANIMAL MODELS AND ONE HEALTH APPROACH. HOW MUCH WE HAVE LEARNED REGARDING ENDOCRINE DISRUPTION FROM *CAIMAN LATIROSTRIS*?

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One Health defines the profound interactions that exist across humans, animals, plants, and ecological health. In this context, a greater understanding of the biodiversity-health link should reinforce biodiversity conservation as a strategy for health promotion for people and other living beings. Areas such as animals as sentinels for environmental agent and contaminants detection and organism responses support the One Health concept. Endocrine-disrupting chemicals (EDC), which include a large variety of natural and man-made chemicals frequently used worldwide, behave as endogenous hormones or as inhibitors of their actions. Many years before the One Health approach gained popularity, we have been working on the effects of EDC exposure on Caiman. This lecture aims to highlight the value of the native South American crocodilian species *Caiman latirostris* as a sentinel species of EDC pollution and to summarize how much we have learned from this caiman in the last two decades. Caiman occurrence in tropical and sub-tropical wetland ecosystems and their ecological and physiological features, render them vulnerable to exposure to EDC, such as agrochemical pesticides and microplastic components, at all life stages. The results reviewed will provide important insights into the effects of EDC at cellular, tissue, and organ levels in the reproductive system of caimans. Alterations related to male and female reproductive systems will be highlighted because they could have negative consequences for the species' population dynamics. We expect that the presented evidence can contribute not only to the knowledge of the effects of EDCs on wild species but also to warn government control agencies and EDC users and producers to use EDC responsibly as a tool for the preservation of natural ecosystems.

BIOLOGY SOCIETIES' SYMPOSIA

A2
INNOVATION IN THE DIAGNOSIS OF PARKINSON'S DISEASE: FROM CONVENTIONAL METHODS TO NANOBODIES

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Synucleinopathies, such as Parkinson's Disease (PD), are severe neurodegenerative disorders characterized by the accumulation of misfolded α-synuclein ($PFF_{\alpha Syn}$) in the brain. PD is a progressive disorder with an increasing prevalence and no known cure; current treatments focus on alleviating symptoms. Clinical diagnosis occurs years after the onset of $PFF_{\alpha Syn}$, coinciding with an irreversible loss of dopaminergic neurons (60-80%), which limits the effectiveness of any intervention. The lack of methods for early and accurate diagnosis has been a significant obstacle in the development of effective treatments. Recently, seed amplification assays (SAAs) have emerged as a revolutionary technique, allowing for the precise detection of small amounts of abnormal α-synuclein in human biological fluids. This innovative technology, which is already being applied in diagnostics, provides highly sensitive and specific detection of the PD biomarker in cerebrospinal fluid. In our laboratory, we have developed early diagnostic strategies for PD, such as the use of modified tetracyclines for the specific detection of $PFF_{\alpha Syn}$, validated through conventional techniques (ELISA and electrochemical impedance spectroscopy), which resulted in an international patent (PCT/IB2023/054715). Another promising strategy involves immunodiagnosis with antibodies designed to recognize $PFF_{\alpha Syn}$, although success has been limited so far. In this context, we are investigating the use of nanobodies (single-domain antibodies derived from *Camelidae*), which, due to their small size and ability to penetrate tissues, including the brain, are emerging as suitable candidates for both the diagnosis and treatment of PD. In conclusion, this work addresses the challenges of PD and focuses on the technical foundations of SAA and the diagnostic developments that we are currently undertaking.

A3
APPROACHES TO DIFFERENT CONVENTIONAL AND UNCONVENTIONAL MODELS IN AGRONOMY AND MANAGEMENT OF SPONTANEITIES

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Since Agronomy involves knowledge from various formal and experimental sciences (and within these, natural and social), there is a variety of experimental models that are applied in its studies. In this presentation, an experimental model will be understood as any system capable of representing, either partially or fully, the process intended to be studied, including both mathematical and biological models at any level of life organization. As a conventional model, the tomato crop (*Solanum lycopersicum*) will be considered at the molecule, the organ, the organism, and the population levels of organization. Results from integrative studies will be discussed. Starting from transcriptomic analyses of its fruits, a cluster of genes from the sHSP (small heat shock proteins) family located on chromosome 6 with differential expression between maturity stages and genotypes was identified. The genomic comparison between two parents of various breeding populations allowed the development of sequence-specific structural DNA markers based on their function. The molecular characterization of two populations derived from both parents demonstrated a Mendelian inheritance of the developed markers. The phenotypic evaluation of fruit quality revealed a wide genetic variability, with significant values of narrow-sense heritability for agronomically important traits such as weight, shelf life, acidity, and soluble solids content. The integration of these molecular and phenotypic datasets was achieved through an original development, the Mixed Dual Multiple Factorial Analysis, identifying associations between the developed markers and the quantitative traits. Finally, the associations detected by this three-way analysis were verified by the conventional statistical-mathematical ANOVA model, validating 6 QTLs (quantitative trait loci) that will allow the assisted selection in future generations of the breeding plan carried out for over 30 years by the GMT (Breeding Tomato Group, IICAR). As a non-conventional model and management of spontaneities, *Musa acuminata* Cavendish AAA group will be used. Results on the micropropagation of selected genotypes and the establishment of a germplasm bank will be discussed, considering that in a strictly asexual autopolyploid, it is necessary to have protocols that guarantee the sanitary and genetic quality of the cultivated materials, as well as to conserve the genetic variability originated by *de novo* mutation in the fields of family farmers in Formosa, from which the first Argentine variety of fresh-market banana.

A4
ANIMAL MODELS FOR THE STUDY OF MOOD DISORDERS

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An animal model is a non-human species used in biomedical research because it can replicate certain aspects of a biological process or disease found in humans. Animal models (e.g., mouse, rat, zebrafish, etc.) have an anatomy, physiology, or response to a pathogen that is similar enough to that of humans to extrapolate the results of animal model studies to better understand human physiology and pathology. Through animal models, researchers can conduct experiments that would otherwise be impractical or ethically prohibited in humans. Animal models are currently used in virtually every field of biomedical research, including but not limited to basic biology, immunology and infectious diseases, oncology, and behavior. The use of animal models to address questions in neuroscience (psychopathologies or neurodegenerative diseases) is widely reported in the scientific literature. Anxiety and depression disorders are the most prevalent psychiatric disorders in the human population and often co-occur (comorbidity). It is estimated that more than 300 million people worldwide suffer from one or both mood disorders. Stressful situations or events have been implicated in triggering anxiety and depression episodes in humans, so it is not surprising that depression- and anxiety-like symptoms can be observed in animal models subjected to stress. Throughout the talk, we will discuss some animal models, especially in rodents, used to study the neurobiology of mood disorders. Additionally, we will briefly analyze some behavioral tests used to assess depression- and anxiety-like states.

A5
HOUSE SPARROWS (*PASSER DOMESTICUS*) AND DOVES (*ZENAIDA AURICULATA*) AS LABORATORY MODELS TO STUDY ENVIRONMENTAL STRESSORS

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In recent decades, nontraditional animal models, particularly birds, have gained importance in various scientific fields. Wild birds are increasingly utilized to study areas such as neurobiology, aging, environmental endocrinology, and physiology. Their diverse physiological adaptations and ecological roles make birds excellent laboratory models for examining animal physiology and stress. Birds occupy a wide range of trophic levels, are widely distributed, and are sensitive to environmental changes, which make them as effective bioindicators of environmental health. In our research, we used house sparrows and doves to evaluate the plasticity and adequacy of health status and digestive systems in response to environmental stressors, including fasting, heat stress, and contaminants. In house sparrows, we observed elevated levels of corticosterone and an increased heterophil/lymphocyte (H/L) ratio during fasting. The biochemical parameters reflected a classical model of fasting observed in other vertebrate animals. We noted an increase in digestive enzyme activity during fasting to improve the refeeding process. While heat stress resulted in elevated H/L ratios and plasma uric acid levels. Notably, capsaicin, a stress mitigation additive, led to a decrease in the H/L ratio in heat-stressed animals, although we observed no significant change in digestive enzyme activity under heat stress. In others studies we evaluated the role of corticosterone as a

component of the stress response and found effects on body condition and a reduction in plasma uric acid concentration, but no impact on digestive enzyme activities. Regarding the effects of contaminants such as lead, we noted a decrease in ALAD enzyme levels in the blood. Chronic exposure resulted in physiological stress, evidenced by H/L ratio inversion, along with minor changes in antioxidant enzyme activity. Additionally, we found a decrease in digestive enzyme activity in the presence of lead. In doves, exposure to contaminants (lead and the pesticide atrazine) affected biochemical parameters, the H/L index, and induced histological and morphometric alterations in the liver and small intestine; however, we did not observe any effects on enzyme activities. Overall, the versatility and ecological significance of birds make them necessary for advancing our understanding of animal physiology and stress responses, thereby contributing to ecological (ecophysiology and ecotoxicology) and conservation research in future scenarios of climate change and global contamination. Enhancing knowledge in this field has important implications for animal and human health.

YOUNG SCIENTISTS SYMPOSIUM

A6
GENE EDITED PIGS FOR XENOTRANSPLANTATION: MADE IN ARGENTINA

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The shortage of human organs for treating patients with terminal diseases is widely known. Xenotransplantation emerges as a strategy that could provide an unlimited supply of organs. However, multiple genetic modifications in pigs are necessary to overcome immune rejection and physiological incompatibilities between species. Our objective was to obtain pigs with the three genes responsible for hyperacute immune rejection (GGTA1, CMAH, β4GalNT2) and the growth hormone receptor (GHR) knocked out (KO). To achieve this, in vitro (IVF) or in vivo produced porcine embryos were genetically modified through microinjection of the CRISPR-Cas9 system. The following day, they were surgically transferred to the oviduct of previously synchronized nulliparous recipients (100-150 IVF embryos and 50-70 embryos produced in vivo). Pregnancy was confirmed in 5 out of 6 females transferred with IVF-produced embryos by day 28, though none of these pregnancies went to term. In contrast, of the 5 females transferred with in vivo-produced embryos, only one became pregnant and gave birth to 5 live piglets (2 males and 3 females). All animals presented biallelic mutations for the GGTA1 gene, and one male was mosaic for both GGTA1 and GHR. Additionally, two females showed mutations in the GHR gene, one with a biallelic mutation and the other with a monoallelic mutation for this gene. Unfortunately, no edits were observed in the CMAH and β4GalNT2 genes. Phenotypically, the absence of α-1,3-galactose residues, a product of the enzyme encoded by GGTA1, was confirmed in skin samples from these animals. Additionally, the female with a biallelic mutation for GHR was significantly smaller. Once the animals reached sexual maturity, they were bred, and the resulting F1 litters were analyzed. The male mosaic for GGTA1 (with three mutated alleles) and for GHR (with two mutated alleles and one wild type) was used. Mating 1 was conducted with a female with a biallelic homozygous mutation for GGTA1 (two alleles with the same mutation) and heterozygous for GHR (one mutated allele and one wild type). Mating 2 was carried out with the female who exhibited biallelic mutations for both GGTA1 and GHR. Pregnancy was confirmed at 28 days in both females, and live piglets obtained inherited some of the mutated alleles for both genes from their parents. In the offspring from mating 1, the maternal mutated allele for the GGTA1 gene could not be identified, highlighting the presence of another mutated allele that could not be amplified by PCR in the F0 female. In conclusion, it was demonstrated that the microinjection of in vivo-produced zygotes allowed the production of genetically edited animals for GGTA1 and GHR. Additionally, the fertility of the F0 animals was confirmed with the birth of two litters of F1 animals. Most of the offspring inherited the known mutations from their parents, resulting in homogeneous double KO animals for both genes. Finally, the female from mating 1 may carry an unidentified mutated allele that was inherited by her offspring and requires further characterization.

A7
EXPLORING THE MOLECULAR BASIS OF SENSORY PHYSIOLOGY IN TRIATOMINES AND BEES

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Our research focuses on identifying genes with pivotal roles in insect neurophysiology and behavior, aiming to develop targeted behavioral manipulation systems for managing human disease vectors and supporting beneficial insects. We primarily use triatomines, especially *Rhodnius prolixus*, and have recently incorporated *Apis mellifera* into our studies. Triatomines transmit Chagas disease, a neglected illness affecting 6–8 million people in Latin America. Due to the rise of insecticide-resistant populations and insecticide toxicity, alternative control methods are urgently needed. In this context, behavioral manipulation strategies present a more specific, environmentally friendly alternative that does not foster resistance. Through *R. prolixus* genome sequences, we identified and analyzed various families of sensory receptors. Using qPCR and RNA sequencing, we demonstrated how physiological conditions, nutritional status, and age influence the expression of specific antennal receptors, a key sensory organ in insects. RNAi assays have further characterized the function of several sensory receptors, revealing their roles in salt perception or in the sexual behavior of *R. prolixus*

males. Honey bees (*A. mellifera*) play a critical role in pollinating crops and wild plants, essential for biodiversity and food security. Deeper insight into their olfactory system could support the development of novel methods to attract bees to targeted crops. For the first time, the expression patterns of various chemosensory receptor genes have been characterized during the larval stages of *A. mellifera,* suggesting that larvae possess the necessary molecular components to operate a functional sensory system. The availability of both genomes, combined with advances in sequencing and gene-editing technologies, makes *R. prolixus* and *A. mellifera* powerful models for exploring insect neurobiology and ecology.

SAB MEMBERS SYMPOSIUM

A8
ALTERNATIVE METHODS TO ANIMAL USE APPLIED TO PRODUCT SAFETY EVALUATION FOR REGISTRATION PURPOSES

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Toxicity tests are an essential part of the safety evaluation process during the development, registration, and approval stages for cosmetic, household hygiene, agrochemical, pharmaceutical products, and medical devices. Historically, since the early 20th century, the gold standard for conducting these tests was laboratory animals. Due to ethical concerns, however, the development of alternative methods to in vivo tests has been promoted for decades, and numerous procedures now exist to replace the use of experimental animals. Alternative methods have gained significant ground with regulatory agencies, to the point that it is now recommended that safety testing of any substance begin, wherever possible, with *in silico, in chemico, in vitro, and ex vivo* methods, leaving animal testing as a last resort. Furthermore, significant advances in cellular and molecular biology, as well as tissue engineering, have led to the creation of human cell-based in vitro models that can more efficiently represent the processes leading to an adverse event.
The Laboratory of Alternative Methods (LMA-EBAL) is fully dedicated to implementing, developing, refining, and transferring alternative methods to animal testing. Our goal is to incorporate internationally approved methodologies, improve and develop new methods for safety assessment, promote the use of alternative methods in the country, transfer knowledge, and provide services to companies that require them.
The purpose of this presentation is to provide an update on the implementation of alternative methods to animal use globally, regionally, and locally, and to share LMA-EBAL's experience in applying alternative strategies to animal testing for evaluating ocular and dermal irritation and corrosion, as well as dermal sensitization from exposure to cosmetics, agrochemicals, and household hygiene products.

A9
NANOPARTICLES AS EMERGING ENVIRONMENTAL POLLUTANTS: MECHANISMS OF CELLULAR RESPONSE TO EXPOSURE.

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Emerging environmental contaminants are compounds of different origin and chemical nature that are being widely identified in water and soil with the advance of detection techniques. These compounds have the potential to exert adverse effects on both the environment and human health, and often lack specific regulations limiting their presence in water. Emerging contaminants include various nanoparticles (NPs) synthesized for several purposes, as well as emerging micropollutants such as micro- and nanoplastics. In our laboratory we developed and synthesized magnetic NPs functionalized with oleic acid (NPMAO) for the remediation of water contaminated with organic pollutants, including polycyclic aromatic hydrocarbons (PAHs). Given the increasing production and use of magnetic NPs in multiple applications, their release into water bodies unintentionally or through specific applications is becoming more and more likely. Therefore, it is essential to determine the potential impacts of NPs at different levels, both on biota and humans. To this end, it is essential to carry out physicochemical characterization and assessment of the mechanisms of action to establish the risks associated with exposure. In this context, our aim was to evaluate the effects of anthracene (ANT), selected as a representative of PAHs, and NPMAOs on both tumorigenic and non-tumorigenic human mammary cells. We analyzed the impact of both pollutants on cell viability, apoptosis and cell cycle. In addition, we evaluated NPMAO uptake and cellular localization. Our results revealed that ANT decreased viability and proliferation only in non-tumorigenic cells, arresting the tumorigenic cell cycle in S phase, whereas in non-tumorigenic cells, the disruption occurred in G2/M phase. Furthermore, NPMAOs were observed to interact with the cell membrane of human mammary cells, decreasing viability and proliferation and significantly altering the cell cycle phase distribution of tumorigenic and non-tumorigenic mammary cells. The cytotoxicity of NPMAOs was evident in both tumorigenic and non-tumorigenic cells, with a higher sensitivity in non-tumorigenic lines. The effects derived from NPMAOs alone raise a red flag for safe uses that avoid the release of nanomaterials into the environment during remediation processes.

A10
EXPERIMENTAL APPROACHES FOR STUDYING THE EFFECTS OF ENDOCRINE DISRUPTORS IN FISH

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The increase in industrial and agricultural activities has led to the widespread presence of contaminants in aquatic environments. Many of these contaminants can interfere with the endocrine system of vertebrates, which is why they are known as endocrine disruptors. Exposure of fish to these compounds can result in alterations in development, growth, and reproduction, among other adverse effects. The approaches used to investigate the action and impact of endocrine disruptors in fish include both acute and chronic exposures in adult and larval animals. The use of in vivo and ex vivo assays allows for studying effects at the whole-organism level or at the organ and cellular levels, respectively. These different approaches will be discussed through studies conducted by our research group in the Laboratorio de Ecotoxicología Acuática on the reproductive and thyroid axes of native fish, highlighting the importance of considering the sublethal effects of contaminants on the health of aquatic organisms.

EXPERT SCIENTISTS SYMPOSIUM

A11
TELEOST FISH AS A STUNNING MODEL TO STUDY STEM CELLS, CELL LINEAGES AND TETRAGAMETIC CHIMERAS.

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In mammals, post-embryonic growth is mostly limited to the juvenile stages and generally stops once sexual maturity is reached. Even though adult mammals maintain their overall size, most organs contain active stem cells that continuously replace the many cells lost on daily basis. These adult Stem Cells (aSCs) are primarily responsible for maintaining organ and tissue homeostasis. In contrast, teleost fish have stem cells that not only contribute to cell replacement but also drive continuous growth throughout life. Fish aSCs achieve this by generating new cells that integrate into functional organs, or even by creating new organs that become part of an organism's systems. In our lab, we focus on post-embryonic stem cells in teleost fish, using genetic tools and classical experimental embryology techniques. I'll be introducing the medaka (*Oryzias latipes*) model as a way to study stem cells in living organisms and highlight some of the general advantages of working with fish in the lab. I'll also present lineage studies in various organs and discuss the differences and similarities between stem-cell-driven growth in fish and the homeostatic maintenance seen in mammals. Finally, I'll share how we're investigating the hierarchical organization of stem cell lineages by creating tetraploid chimeras and tapping into the broad phenotypic diversity among species in the *Oryzias* genus.

A12
FROM THE OUTSIDE TO THE INSIDE: A STORY ABOUT PLASTICITY

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Plasticity - the ability to make adaptive changes - is a fascinating property of the nervous system. Plasticity occurs at different time scales and in different structures, ranging from spines and boutons to axonal and dendritic arbors. While experience-dependent synaptic plasticity has been extensively studied, the mechanisms underlying structural plasticity, in particular the large-scale growth and remodelling of axons and dendritic branches in the postnatal brain, have received less attention. Our laboratory has focused on a group of circadian neurons, the small lateral ventral neurons (s-LNvs), which undergo extensive daily remodelling. We use as a model the fruit fly, *Drosophila* melanogaster, which has pioneered the field of chronobiology - as so many others - in identifying the molecular and cellular mechanisms of fundamental, highly conserved processes.
Over the years, our laboratory has shown that circadian structural remodelling is associated with rhythmic changes in the number of synaptic contacts and thus the ability to synapse with specific targets at specific times of the day. To investigate the extent to which dynamic changes in the membrane describe the underlying subcellular organisation, we used serial block scanning electron microscopy

(SBEM). Using volumetric electron micrographs of dorsal endings taken at times of day when the terminals have different levels of complexity, our work revealed some of the basic principles by which s-LNvs differentially contribute to the circadian network. In parallel, we investigated some of the cellular mechanisms underlying this unusual form of plasticity, showing that it relies on both activity-dependent and activity-independent mechanisms, and that it is not restricted to a subset of adult brain clock neurons, nor is it restricted to *Drosophila*, leading us to propose that structural plasticity is another mechanism that characterises central clock neurons.

A13
GABAERGIC ADULT NEUROGENESIS IN THE PALLIUM OF ZEBRAFISH

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The pallium is a brain region involved in the processing of cognitive functions, such as memory, learning, and emotional regulation. In fish, the pallium is divided into two primary regions: the dorsomedial pallium (Dm) and the dorsolateral pallium (Dl). Both regions exhibit significant neuronal plasticity and active neurogenesis in adulthood, a process involving the generation and integration of new neurons into brain circuits. While many studies have focused on neural stem cell (NSC) populations in these regions, little is known about the phenotypic fate of newly generated neurons. Both Dm and Dl regions mainly contain glutamatergic and GABAergic neurons, being the excitatory neurons more abundant (~90%) than the inhibitory ones (~10%). During early development, glutamatergic neurons in the pallium arise from NeuroD1+ NSCs located in the periventricular pallial zone, while GABAergic neurons originate from Zash1+ NSCs in the subpallium and in turn, they migrate tangentially towards their final position in the pallial circuits. In this study, we investigate whether GABAergic neurogenesis occurs in the adult zebrafish pallium and, if so, aim to determine the origin of the NSCs that produce these neurons.
To this end, we labeled a cohort of mitotic NSCs by administering EdU (intraperitoneally, 40 µl, 10 mM) to a transgenic *tg(gad1b:gfp)* fish line, in which GABAergic neurons express the GFP reporter. We then sacrificed fish at 1.5, 3, 8 or 16 days post-EdU administration to assess neuronal survival and lineage fate in a spatio-temporal characterization. We observed a ~50% decrease in the survival of EdU-labeled cells over time. The labeled cells displayed a layered positioning, migrating from the periventricular zone toward the parenchyma as they matured. After the chase periods, approximately 70% of EdU+ cells expressed NeuroD1, indicating a glutamatergic phenotype. In contrast, only a small percentage (~3%) of adult-born cells expressed the GABAergic marker GAD1b. A spatiotemporal analysis revealed that adult-born GABAergic neurons originated from NSCs located within the pallial periventricular zone.
As an alternative functional approach, we conducted an electrophysiological study. We performed patch-clamp recordings on GFP+ neurons at various distances from the periventricular region. This analysis showed that intrinsic and synaptic properties of GFP+ neurons exhibit spatial variation: immature neurons are located near the pallial periventricular region, while mature neuronal characteristics were exhibited by cells deeper within the parenchyma. This finding support a radial migration of GABAergic adult-born neurons from the pallial periventricular zone. Overall, our results indicate that GABAergic neurons are indeed generated in the adult zebrafish pallium, migrating radially from the periventricular zone toward the parenchyma.

A14
ANURANS AS NON-TRADITIONAL EXPERIMENTAL ANIMALS: THE CASE OF A SOUTH AMERICAN TREE FROG

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For several decades, anurans have been considered advantageous animals for studying the neurobiological bases of reproductive behavior. During courtship, males produce vocalizations and gather in choruses to attract females. The neural basis of these vocalizations lies in circuits located across different brain regions. Additionally, the modulatory role of hormones, such as hypothalamic nonapeptides (e.g., vasotocin, AVT), on these vocalizations is well established. *Boana pulchella* is a long breeding hylid anuran found in southern South America. In Uruguay, it is abundant and widely distributed throughout the country. During courtship nights, males grouped in choruses emit three types of vocal notes. Our research is conducted in nature at two scales: (1) population level, through passive acoustic monitoring of annual chorusing activity and its relationship with various physical factors, and (2) individual level, through acoustic recordings of males. Our results show that chorusing activity occurs in temperate and warm months and correlates with temperature and photoperiod. Additionally, individual acoustic recordings reveal a flexible temporal pattern of vocalization according to the social context. To investigate the potential effect of AVT on male vocal behavior, we conducted pharmacological field trials, finding that AVT antagonists reduce the likelihood of calling in treated males compared to untreated ones. These approaches, applied to a non-traditional experimental animal, have deepened our understanding of the neuroendocrine foundations of reproductive behavior in vertebrates.

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SHORT COMMUNICATIONS

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 1

A15

PRENATAL MILD CHRONIC STRESS IMPACTS THE GROWTH AND NEURODEVELOPMENT OF THE OFFSPRING

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Lifestyle modulates maternal, placental and fetal physiology. We have previously observed that exposure of female mice before and during gestation to a chronic mild stress protocol affects the vascular and immunological processes at the maternal-fetal interface. Additionally, we found that these alterations were paralleled by a reduction in fetal weight on day 15 of gestation. Based on these findings, our objective was to assess the consequences of the exposure to chronic mild prenatal stress on the development and growth of the offspring after birth.

For this, BALB/c female mice were housed in cages with half the bedding material (stressed group) compared to the control group. Furthermore, every other day, the stressed females were placed on a lid that was shaken in an 8-shaped pattern for one minute. This protocol was carried out until the day before delivery.

First, the pups were weighed on postnatal day 1 and then weekly until 6 weeks of age. Pups from stressed mothers showed lower weight on postnatal day 1, and this difference persisted up to 6 weeks of age. Additionally, the early neonatal mortality rate was higher in litters born to stressed females.

Afterward, we evaluated developmental parameters such as ear pinna separation, teeth eruption and eye-opening. We found that 10-20% of the pups from stressed mothers showed a delay in the appearance of these developmental milestones compared to pups from control mothers. Furthermore, we observed dysregulations in metabolism-associated parameters and behavioral tests.

In conclusion, our results suggest that while mild chronic prenatal stress is not associated with embryonic resorptions or preterm birth, intrauterine exposure to this protocol causes alterations in the fetuses that impact the health of the offspring after birth.

A16

INCREASE IN THE EFFICIENCY OF SPERM PREPARATION THROUGH THE USE OF NEW REGULATORS OF CYCLIC ADENOSINE MONOPHOSPHATE

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Activation of the cyclic adenosine monophosphate (cAMP) pathway is a fundamental event in the acquisition of sperm fertilizing capacity. Both cAMP analogs and phosphodiesterase (PDE) enzyme inhibitors have been used *in vitro* to promote sperm motility. However, results are variable among different patients and unwanted effects, such as premature acrosomal exocytosis (AE) have been observed. In previous studies from our laboratory we reported that cBiMPs (cAMP analogue) and TAK-063 (PDE10A inhibitor) increase the motility and hyperactivation of human sperm, without inducing the AE or affecting DNA fragmentation. This effect was more potent than that of other known cAMP analogues and PDE inhibitors. The objective of our work was to evaluate whether cBiMPs or TAK-063 can be used to improve the efficiency of human semen sample preparation. Fresh and cryopreserved semen samples were used, obtained under donors´ written consent. They were subjected to sperm preparation techniques in the presence of cBiMPs (30 µM), TAK-063 (1 µM) or DMSO (0.2 %, control). We calculated the percentage of sperm recovery after *swim-up* separation ([volume x concentration x total motility of the recovered aliquot] / [volume x concentration x total motility of the used aliquot] x 100) and the percentage of progressively motile sperm, in samples in which the seminal plasma was removed by washing. The addition of cBiMPs and TAK-063 significantly increased ($P<0.01$) sperm recovery compared to the control, in fresh (n=10 for cBiMPs, n=7 for TAK-063) and frozen/thawed samples (n=8). Moreover, the use of these compounds for 1 h led higher ($P<0.01$) percentages of progressively motile sperm after washing in both types of samples, showing a 2.8± 0.5 and 2.6±0.4-fold increase for cBiMPs and TAK-063 compared to the

control in fresh samples (n=25 and n=21, respectively) and a 1.5±0.1 and 1.8±0.1-fold increase for cBiMPs and TAK-063 in cryopreserved samples (n=12). The enhancing effect of cBiMPs and TAK-063 on sperm motility and on the efficiency of sperm preparation would lead to their use during the andrological practice, favoring the implementation of low-complexity assisted reproduction techniques. These compounds could also be used to select viable sperm during ICSI protocols, contributing to the treatment of male infertility. This work had the financial support from the CONICET, the ANPCyT, and the René Barón, Williams and Honorio Bigand Foundations.

A17
ROLE OF TROPHOBLAST EXTRACELLULAR VESICLES IN THE REGULATION OF THE PLACENTAL MICROVASCULAR CELL MIGRATION: ALTERATIONS UNDER HYPOXIA-REOXYGENATION

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During pregnancy, gas and nutrient exchange between the mother and fetus occurs within the microvasculature that establishes the placental vascular network of the fetal capillaries of chorionic villi. Therefore, proper microvascular development is essential for successful fetal growth. This process occurs through placental angiogenesis and it is regulated by trophoblast paracrine signaling, which constitutes the trophoblast secretome (composed of soluble molecules and extracellular vesicles - EVs). Preeclampsia (PE) is a severe pregnancy complication characterized by hypertension, proteinuria and damage to multiple organs. Incomplete remodeling of the uterine spiral arteries leads to placental ischemia-reperfusion injury (generating hypoxia-reoxygenation -HR- episodes) and, contributing to inflammation, oxidative stress and endothelial dysfunction. In PE, the placental microvasculature exhibits significant alterations in both architecture and function, and there is an increase in the release of placental EVs which may induce endothelial damage. In this study, we used HR as a model to investigate the damage observed in PE. Placentas from healthy full-term pregnancies (n=4) were obtained from the Naval Hospital. Placental explants were cultured under normoxia (N) and HR conditions, and tissue viability was assessed by MTT assay. Placental EVs were isolated by differential centrifugation, filtration and ultracentrifugation, and characterized using DLS, NTA, electron microscopy and western blot. Primary cultures of human placental microvascular endothelial cells (hPMEC) obtained from healthy placental villi were cultured with or without placental EVs or EV-depleted culture supernatant (SN). Cell migration was evaluated using the wound healing assay. Placental explant viability was not affected by HR. The secretome derived from trophoblasts cultured under N modulated hPMEC migration. Both EVs and EV-depleted SN derived from trophoblasts cultured under HR significantly reduced hPMEC migration compared to hPMEC cultured with EVs and/or SN from explants cultured under N ($p<0.05$). These results suggest that both EVs and soluble factors derived from trophoblasts are involved in trophoblast-placental microvasculature communication and modulate hPMEC migration. This modulation appears to be altered under HR conditions, as observed in PE.

A18
SOUTH AMERICAN CAMELIDS AS MODELS FOR STUDING EARLY MATERNAL-EMBRYO DIALOGUE

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A unique reproductive feature of South American camelids is that 98% of pregnancies are established in the left uterine horn (LUH), despite both ovaries contributing almost equally to ovulation. It has been suggested that the right uterine horn (RUH) may not be capable of maintaining pregnancy, forcing embryos originating from the right ovary to migrate to the LUH for implantation and survival. However, no differences have been identified at the ultrasonographic, histological, or subcellular levels to explain this pattern of migration and implantation. Our hypothesis is that the proteomes of the LUH and RUH during the peri-implantation phase are different, generating an optimal environment in the LUH and/or a suboptimal environment in the RUH for the embryo. Our objective was to characterize the proteome of the uterine horns of alpacas on day 15 post-mating (dpm), after the maternal recognition of pregnancy has already occurs (8-10 days post-ovulation, dpo) but before implantation (20 dpo). The study was conducted with alpacas from the Veterinary Research Center of the Universidad Nacional Mayor de San Marcos, Peru. Three 2-year-old females were mated with fertile males and slaughtered at 15 dpm. The uterine horns were preserved in RNA-later for mechanical lysis in a buffer containing 4% SDS and protease inhibitors. The proteins were analyzed by SDS-PAGE, in-gel digestion, and mass spectrometry (LC-MS/MS), and the data processed using ProteomeDiscoverer v1.4 and v2.2, Perseus v2.0.7.0, Metascape v3.5, Proteomaps v2.0, and Cytoscape v3.10.1. A total of 1,728 proteins were detected in the LUH and 1,983 in the RUH, of which 1,598 were common to both horns. Statistical analysis identified 33 proteins significantly increased in the LUH and 69 in the RUH ($p \leq 0.05$). The analyses revealed functional differences between the two uterine horns: the RUH showed enrichment in processes related to RNA splicing, steroid metabolism, phospholipid synthesis, and proteolysis. Notably, ubiquitin-mediated proteolysis is relevant in the embryo-maternal dialogue, as it is enriched in patients with recurrent implantation failure. On the other hand, the proteins of the LUH were associated with cytoskeletal organization,

cell migration, biosynthetic processes, and binding to Major Histocompatibility Complex I (MHC) proteins. During pregnancy, the maternal immune system tolerates the embryo through immunological communication, involving MHC I proteins. These differences could explain the differential implantation in South American camelids. However, further studies are needed to characterize the proteins involved in these processes and determine their role in embryo implantation and/or infertility.

A19
PARTICIPATION OF AMINO ACIDS IN REFRIGERATED PORCINE SPERM CAPACITATION

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It has been suggested that boar spermatozoa could utilize various energy substrates to maintain their metabolic functions. However, the mechanisms of production, control, and use of different oxidative substrates to generate the energy required for successful fertilization are not fully understood. The aim of this study was to investigate the role of exogenous amino acids (Aa) in the *in vitro* capacitation of refrigerated porcine spermatozoa. Samples (n=5) were fractionated and incubated at 38°C under capacitating conditions (with bicarbonate as an inducer) in different media: TBM (glucose + pyruvate), TBMns (without glucose or pyruvate), TBM + Aa, TBMns + Aa, TBMns + Aa + salicylate (1, 5, or 10 µM, an inhibitor of oxidative deamination). Motility was assessed by optical microscopy, viability by the supravital technique using trypan blue, ammonium production by spectrophotometry, and capacitation by the fluorescent technique of chlortetracycline and by the response to induction of the acrosome reaction (with 30% porcine follicular fluid) evaluated using differential interference contrast and trypan blue. The data were analyzed by ANOVA and compared using the Bonferroni test, considering $p<0.05$ as statistically significant. In TBMns, spermatozoa maintained motility but did not achieve capacitation (versus TBM, $p<0.05$). The addition of amino acids to a medium without glucose or pyruvate (TBMns + Aa) did not increase the percentage of capacitated spermatozoa. Sperm motility significantly decreased when amino acids were used as the only oxidative substrate present in the capacitation medium (versus TBMns, $p<0.05$), but this effect was not observed when amino acids were added to a medium with classical oxidative substrates (TBM + Aa). Ammonium production was higher in treatments containing amino acids and decreased in a dose-dependent manner with the addition of salicylate ($p<0.05$). Although boar spermatozoa have the ability to oxidatively deaminate exogenous amino acids, these compounds can not be used as the sole oxidative substrates to produce the energy needed to sustain sperm motility and capacitation. Future studies on the use of other exogenous or endogenous oxidative substrates will complement these results and help to elucidate the metabolic pathways used by refrigerated boar spermatozoa to obtain the energy required for sperm capacitation.

A20
IMPACT OF SPERM AND EMBRYO CRYOPRESERVATION ON OFFSPRING HEALTH IN MICE

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Sperm freezing and embryo vitrification are widely used techniques in both clinical and agro-productive settings. Although their high efficiency has been demonstrated, the exposure of spermatozoa and embryos to cryoprotectants and low temperatures could generate epigenetic damage that may impact on embryo development and the health of individuals born from these techniques. Taking this into account, the aim of our study was to evaluate, using a mouse model, the effect of sperm and embryo cryopreservation on the expression of key genes involved in the epigenetic remodeling that occurs during embryo development, as well as its impact on the offspring health. For this, we established three groups of blastocysts to be analyzed: 1) fresh, obtained by *in vitro* fertilization (IVF) with frozen sperm, 2) vitrified, obtained by IVF with fresh gametes, and 3) fresh, obtained by IVF with fresh gametes (control group). First, we assessed the expression of genes related to epigenetic remodeling by RT-qPCR: DNA methyltransferases (*Dnmt1, Dnmt3a/b, and Dnmt3l*); methylation regulators (*Zfp57 and Dppa3*); histone-modifying enzymes (*Ash1l, Kmt2a, Kat5, and Kat2a*); paternally (*H19 and Igf2r*) and maternally (*Peg3 and Snrpn*) imprinted genes, and pluripotency markers (*Nanog, Pou5f1, Sox2,* and *Cdx2*). We observed that blastocysts obtained from frozen sperm showed a decrease in the relative mRNA levels of the *H19, Ash1l*, and *Kat2a* genes ($p<0.05$), while vitrified blastocysts showed a decrease in *Igf2r, Dppa3,* and *Kat2a* ($p<0.05$). Subsequently, blastocysts from the different experimental groups were transferred to pseudopregnant females, and parameters related to the postnatal and sexual development of the born individuals were analyzed. We observed a slight delay in eye opening in pups born from frozen sperm ($p<0.05$) and in incisor eruption in those born from vitrified blastocysts ($p<0.05$). However, no differences in weight gain, ear detachment, fur development, testicular descent in males, and vaginal opening and estrous cycle in females were found. Finally, upon reaching adulthood, we analyzed the reproductive phenotype of these animals, observing that, although fertility was not affected (pregnancy duration and litter size), the offspring of males generated from frozen sperm had a lower birth weight ($p<0.05$). In conclusion, our results suggest that

cryopreservation could induce an epigenetic imbalance in embryos, although this would not significantly impact the health of the born pups until adulthood. However, in males born from frozen sperm, the effects might manifest in the second generation of animals.

A21
OVERCONSUMPTION OF *STEVIA REBAUDIANA* AND REPRODUCTIVE CAPACITY

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In recent times, sucrose in food and beverages has been replaced by non-caloric sweeteners, which can be either artificial or natural. Among the latter is stevia, derived from the plant *Stevia rebaudiana* Bertoni (Asteraceae), native to the tropical regions of South America. Although there is controversy regarding its consumption, several studies suggest that stevia affects reproductive capacity, while others highlight its beneficial properties. We previously reported that stevia administration in female rats leads to changes in the estrous cycle, uterine size, and pregnancy capacity, among other aspects. The objective of this study was to evaluate whether these alterations are correlated with hormonal imbalances (FSH and LH). Female rats (SD) were given water (CON group, n=8) or stevia-sweetened water (STE group, n=8) from postnatal day 21 (PND21) to PND90. Estrous cycles were evaluated between PND40-72 by observing vaginal smears, determining the number of proestrus, estrus, metestrus, and diestrus phases, as well as the number of normal cycles. The females were weighed and sacrificed during estrus after 6 hours of fasting. Trunk blood was collected, and the uteri were removed, dried, and weighed to calculate the uterine weight/body weight index (UI). Serum levels of FSH and LH were determined using RIA. The STE group showed fewer proestrus phases (24.14%; p=0.0256) but a greater number of estrus phases (22.58%; p=0.0102) compared to the CON group. No differences were found in the number of normal cycles. No differences in body weight were recorded, but the STE group had a lower UI compared to the CON group (24.40%; p=0.0006). No differences were found in FSH and LH levels. In the CON group, positive correlations were observed between FSH and LH (p=0.0264; R^2=0.588), between UI and FSH (p=0.0452; R^2=0.515), and between UI and LH (p=0.019; R^2=0.628), while a negative correlation was found between UI and the number of normal cycles (p=0.0091; R^2=0.705). In contrast, in the STE group, only a negative correlation was observed between UI and the number of proestrus phases (p=0.0219; R^2=0.612).These results not only support our previous findings but also suggest that the effects of this sweetener may be associated with endocrine dysregulation. Additional studies are necessary to explore the various mechanisms involved. We consider these findings relevant to medical recommendations regarding the consumption of *S. rebaudiana* (PICT2019-623).

A22
EVALUATION OF EXTRACELLULAR VESICLE ISOLATION METHODS FROM HUMAN SEMINAL PLASMA AND THEIR INCORPORATION INTO SPERMATOZOA

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Extracellular vesicles (EVs) present in human seminal plasma (SP) have garnered significant interest due to their potential role in modulating sperm function and male fertility. EVs have been shown to carry a variety of bioactive molecules, such as proteins, lipids, and RNAs, which influence sperm physiology, including motility, capacitation, acrosomal reaction, and viability. The aim of this study was to compare different methods for isolating EVs from the SP of normozoospermic patients and evaluate their incorporation into spermatozoa. For this, we analyzed three EV isolation techniques: 1) double ultracentrifugation (UC); 2) size exclusion chromatography (SEC); and 3) polyethylene glycol (PEG) precipitation. EVs were characterized using western blot (WB), transmission electron microscopy (TEM), and nanoparticle tracking analysis (NTA). The incorporation into spermatozoa (which were incubated in HTF medium = non-capacitating) was evaluated through co-incubation with CFSE-labeled EVs, followed by flow cytometry. Our results indicate that both the UC and SEC methods successfully isolated EVs, as confirmed by the presence of the specific markers HSP70 and CD9, as well as TEM images showing membrane-bound nanostructures of different sizes. NTA measurements revealed that the UC method yielded a concentration of 4.8E+12 particles/mL, with particle sizes ranging from 50 to 600 nm and a median of 118.7 nm. In contrast, the SEC method provided a concentration of 3.0E+12 particles/mL, with particle sizes ranging from 50 to 800 nm and a median of 121.9 nm. Moreover, EVs obtained by both methods were incorporated into spermatozoa, as evidenced by CFSE fluorescence detection in the viable population. Interestingly, spermatozoa incubated with EVs isolated by SEC showed a higher mean fluorescence intensity (MFI) than those incubated with EVs obtained by UC (UC = 398 ± 100; SEC = 676 ± 167; mean MFI ± SEM, $p \leq 0.05$). These results suggest that SEC is an effective technique for isolating functional EVs from human seminal plasma, which are incorporated into spermatozoa at a higher rate compared to the traditional UC method and also offers a greater diversity in the size of the EVs obtained. Future studies will focus on evaluating the effects of EVs on sperm capacitation-related events and their potential impact on male fertility.

A23
THE ROLE OF AUTOPHAGY IN AN ANIMAL MODEL (PLAINS VISCACHA) WITH ACTIVE FOLLICULOGENESIS DURING PREGNANCY

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Lagostomus maximus, the plains viscacha, is a hystricomorph rodent with an extended gestation period of 5.5 months, during which follicular recruitment remains active. At mid-gestation, a decrease in progesterone levels occurs, allowing reactivation of the hypothalamic-pituitary-gonadal axis, followed by an increase in serum levels of 17-β-estradiol, follicle-stimulating hormone, and luteinizing hormone. This hormonal profile facilitates development, follicular recruitment, and a pseudo-ovulatory event for forming new corpora lutea (CL). In addition, the vizcacha exhibits a low rate of apoptosis-mediated follicular atresia, maintaining a constant and active population of germ cells. Furthermore, autophagy plays a dual role in eliminating follicles and regressing CL and in the survival of healthy follicles and CL. The study aims to analyze autophagy in a model with reduced follicular atresia and continuous folliculogenesis throughout gestation. Ovaries were collected at three gestational stages: early (n=8), mid (n=8), and term pregnancy (n=8). The expression of the autophagic proteins BECLIN 1, LC3BI-II, LAMP 1, and SQSTM1 was analyzed by immunohistochemistry. The percentage of positive follicles at different stages of development for each protein was determined. The ultrastructure of autophagic vacuoles was examined using transmission electron microscopy (TEM). The co-localization of autophagic proteins LC3B-SQSTM1 and LC3B-LAMP1 was analyzed. The results, presented as mean ± standard deviation, were subjected to analysis of variance (ANOVA) with Bonferroni post-tests for multiple comparisons, where $p < 0.05$ indicates significant differences. The expression of autophagic markers in mature follicles increased as the pregnancy progressed. The percentage of healthy follicles positive for autophagic markers tended to increase throughout gestation. The scarce atretic follicles observed at mid-gestation showed a non-significant positive decrease in marker detection. The co-localization of LC3B-SQSTM1 and LC3B-LAMP1 was primarily detected in the oocyte of mature and atretic follicles, indicating continuous autophagy. TEM revealed a low amount of autophagic vesicles and a preserved structure in healthy follicles. In contrast, in atretic follicles, a higher presence of autophagic vesicles and an altered morphology were observed throughout gestation. These findings suggest a basal or survival role of autophagy in healthy follicles as a key contributor to tissue homeostasis and follicular maturation throughout pregnancy. Conversely, autophagy may collaborate in the process of oocyte death in the scarce atretic follicles observed after pseudo-ovulation, while granulosa cells die by apoptosis.

VETERINARY AND ECOLOGY

A24

POPULATION DEVELOPMENT OF *OPHELIMUS* GALL WASPS AND THEIR PARASITOID *CLOSTEROCERUS CHAMAELEON* IN *EUCALYPTUS* TREES OF THE PAMPEAN REGION IN BUENOS AIRES

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Several species of insects native to Australia and associated with eucalyptus trees have become major pests after invading different regions of the world. One example is the gall-inducing species of the genus *Ophelimus*. In 2013, *Ophelimus maskelli* was detected in Argentina along with its parasitoid, *Closterocerus chamaeleon*. In 2017, a second unidentified species, *Ophelimus sp.,* was also recordedd. This study aimed to assess the abundance, attack levels of *Ophelimus* species, and parasitism rates by *C. chamaeleon* in eucalyptus plantations across the Pampa region of Buenos Aires province. Monthly sampling was conducted from August 2022 to January 2024 in three locations: Castilla (plantations of *E. grandis x E. camaldulensis* (GC)), Castelar *(E. viminalis, E. benthamii,* and *E. dunnii*), and 25 de Mayo (E. cinerea, *E. viminalis* and *E. tereticornis*). In each plantation, 10 trees were randomly selected from which two branches (30-40 cm) from the lower third of the canopy were collected. The number of galls per leaf (abundance) and the number of leaves with galls (attack level) were recorded. Parasitism rates were calculated based on the emergence of adults (pests and parasitoids). Data were analyzed using generalized linear mixed models (GLMMs), with Tweedie distribution for gall abundance and Bernoulli distribution for the proportion of affected leaves. The results showed that *O. maskelli* was found on GC hybrids and *E. tereticornis*, while *Ophelimus sp*. affected *E. viminalis*, *E. cinerea, E. benthamii*, and *E. dunnii* In Castilla, the GC24 clone had the highest abundance, peaking in February and March 2023, while GC9 had a peak between April and June 2023. In general, GC24 had a higher proportion of attacked leaves, except in April 2023 when no significant differences were observed. In Castelar, *E. benthamii* and *E. viminalis* showed higher gall abundance than *E. dunnii*. Significant differences were found between *E. benthamii* and *E. dunnii* in January, February, and December 2023. The overall attack level was similar among species, except in February and March 2023, when *E. dunnii* had a lower proportion of attacked leaves. In 25 de Mayo, gall abundance and attack levels were significantly higher in E. cinerea and *E. tereticornis* compared to *E. viminalis* during most of the sampling period. The parasitoid *C. chamaeleon* had parasitism rates above 96% on *O. maskelli*, but below 9% on *Ophelimus* sp. These results suggest different levels of susceptibility to *Ophelimus* pests in different eucalyptus species. The study also confirms that *C. chamaeleon* is primarily associated with *O. maskelli*, but further research is needed to understand its relationship with *Ophelimus* sp.

A25

DESCRIPTION OF THE PENILE HISTOLOGY OF *EUMOPS PATAGONICUS*: A BAT FROM NORTHEASTERN ARGENTINA

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The order Chiroptera is one of the most numerous in terms of species among mammals. This order exhibits great diversity in the morphology of their reproductive systems and gonads. In females, a wide variety of uterine morphologies is observed, as well as variations in ovarian morphology and functionality. On the other hand, in males, the testicles exhibit typical mammalian morphology, but the penile structure shows particularities unique to each species. In mammals, the penis is an organ that facilitates fertilization. It has a cylindrical shape with a body through which the urethra runs, and an erectile tissue called the corpus cavernosum, along with a distal portion, the glans, where the urethra continues and ends externally, surrounded by a second erectile tissue, the corpus spongiosum. In some species of bats, the presence of accessory erectile tissues and a penile bone or *baculum*, also found in other mammals, has been observed. The histological description of this organ is important at the phylogenetic level within this order due to the variation between species. Therefore, the objective of this study is to describe the penile morphology of *Eumops patagonicus* at the histological level, belonging to the family Molossidae, a species abundant in urban areas of northeastern Argentina. Samples of *E. patagonicus* penises were collected (n=8), processed for conventional histology, and stained with hematoxylin-eosin and Gomori's trichrome. The samples were photographed, differentiating three sections: base, middle, and tip sections. The base section corresponds to the body, while the middle and tip sections correspond to the glans. It was observed that the base of the penis is formed by the urethra, which is surrounded by the corpus cavernosum enclosed by its tunica albuginea of dense connective tissue, which emits an incomplete septum dividing it into two regions. Two peripheral nerves are observed on the sides. In the glans region, the urethra is surrounded by the corpus spongiosum, which is delimited by its tunica albuginea, and in the central region, the presence of a baculum is noted. Externally, the penis is covered by thin skin with a large number of hair follicles, which are absent in the glans that is covered whit skin. Compared to other previously described *Eumops* species, *E. patagonicus* has corpus spongiosum in the glans region accompanying the urethra. Moreover, this study reveals the presence of an incomplete septum in the corpus cavernosum, which is not observed in other Chiroptera species. This description of the penis of a member of the *Eumops* genus lays the foundation for future comparative studies between species of the same genus and family, as it has been of great importance in other taxa such as Rodentia, carnivorous Mustelids, and primates, especially the description of the presence of a baculum.

A26

COMPARATIVE *IN VIVO* AND *IN VITRO* STUDIES OF PLANTS CONTAINING SWAINSONINE

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Astragalus spp. and *Ipomea spp.* are toxic plants that affect animals and contain swainsonine (SW), an indolizidine alkaloid produced by symbiotic fungi. Additionally, *I. carnea* contains calystegines, nortropane alkaloids. Both compounds inhibit lysosomal enzymes, disrupting the normal metabolism of cells. The objective was to compare the *in vivo* intoxication and *in vitro* cytotoxicity induced by *Astragalus illini* and *Ipomea carnea*. The plants were collected in Maquinchao Manuel Choique, Río Negro, and Corrientes Capital, respectively. For the *in vivo* assay, 12 guinea pigs weighing 200 ±50 g were used, divided into three groups (G) of 4 animals each: GI (control), GII (treated with *I. carnea*), and GIII (treated with *A. illini*). Each treated group received pellets made from a mixture of dried, ground plant material and commercial balanced feed in a 50:50 ratio, while the control group received only commercial balanced feed. SW determination and quantification by HPLC-MS/MS was 0.02% in *A. illini* and 0.13% in *I. carnea*. The experiment lasted for 53 days, and prior to euthanasia, blood samples were taken for biochemical studies, and liver and brain samples were collected for histopathology. In the brainstem, the percentage of vacuolated neurons (40X) was determined in 10 representative random fields. For the *in vitro* assay, the murine malignant glioma cell line C6 (ATCC: CCL-107™) was used. The cells were maintained in DMEM medium with 10% FBS and antibiotics. Cells were exposed to aqueous extracts (AE) made from both plants, and SW quantification in each AE was 1.49 µg/mg for *A. illini* and 1.6 µg/mg for *I. carnea*. The cytotoxic concentration 50 (CC50) was determined. The results showed an increase in aspartate aminotransferase enzyme (AST) in both treated groups compared to the control (43.00±8.49 IU/L). However, GII showed a significantly higher increase in AST levels (676.5±103.35 IU/L) compared to GIII (322.00±16.40 IU/L) ($p < 0.05$). Histopathological evaluation in both treated groups revealed the presence of intracellular vacuoles in hepatocytes and Kupffer cells. A 30.6±1.92% of vacuolated neurons was observed in GII, in contrast to 6.02±4.65% recorded in GIII, while no vacuoles were found in the controls (GI). The CC50 values were 312 µM for cells exposed to the AE of *I. carnea* and 425 µM for cells exposed to the AE of *A. illini.* In conclusion, *A. illini* and *I. carnea* proved to be toxic both *in vivo* and *in vitro*, which could be attributed to the presence of swainsonine. However, the toxicity of *I. carnea* was more pronounced, which may be attributed not only to its higher concentration of swainsonine but also to the presence of calystegines, suggesting a potential synergistic interaction between the two compounds.

A27

BASAL FREQUENCY OF THE DORSAL VESSEL AND ANTERIOR MIDGUT IN *RHODNIUS PROLIXUS*: ANALYZING DIFFERENCES THROUGH YEAR SEASONS

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Since pioneering Sir V. B. Wigglesworth's research, Chagas disease vector *Rhodnius prolixus* (Hemiptera: Reduviidae), demonstrated to be a valuable experimental model. The homeostasis regulation on these organisms depends, between others, on the mechanisms that regulate hemolymph circulation, mainly during the physiologically critical process of post-prandial diuresis. The mechanisms that regulate the circulation speed varies with the physiological state, showing differences between resting conditions (starved) versus the post-ingesta diuresis process. Haemolymph circulation depends on the dorsal vessel (made up by the heart and aorta), and the anterior midgut (crop) contractions. We have previously shown that the frequency of contractions during resting condition (i.e. starved condition) presents a diurnal variation, being higher in the afternoon. Taking this into account, we decide now to study the probable existence of seasonal variations in the frequency of contractions of both organs throughout the year. We analyze the rate of contractions of both, crop and aorta in adult (male) insects obtained from a colony maintained in our laboratory and starved during 20-25 days after molting. Briefly, the insects were placed under stereoscopic microscope, the wings were removed to visualize the aorta and crop through the translucent cuticula. To counteract the stress caused by handling, after 30 minutes *Rhodnius* saline solution was applied through an abdomen incision. Then, the frequency of both organs was recorded in a 3 min-period at 5, 15 and 30 minutes. The results are expressed as number of contractions per minute and analyzed by multifactorial ANOVA. Data analyzed by semesters (i.e. spring-summer; autumn-winter) showed a higher frequency during warm months, showing a difference greater than 50% in the aorta as well in the crop. To go further in the analysis, the frequency was then evaluated for each individual season. The frequency of the aorta was 30% higher during summer (dec-jan-feb). Regarding the crop, the frequency of peristaltic waves was 25% higher in summer and autumn, in comparison with winter and spring, which did not show significant differences. Our results suggest the existence of a seasonal pattern in the basal frequency during resting conditions.

A28
AORTA FREQUENCY OF CONTRACTION UNDER HYPOOSMOTIC CONDITIONS IN *RHODNIUS PROLIXUS*: THE ENDOCRINE ROLE OF MALPIGHIAN TUBULES

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Blood intake in triatomine insects causes an osmotic shock that triggers a process of diuresis to restore homeostasis. This requires an increase of hemolymph circulation. Allatotropin (AT) stimulates the frequency of the dorsal vessel (DV) and anterior midgut (crop) contractions, increasing the speed of hemolymph circulation. Malpighian Tubules (MTs) are the main excretory system in insects. We demonstrated that under hypoosmotic conditions, MTs secrete AT to the hemolymph inducing peristaltic contractions of the rectum to mix and void urine and feces. It was also demonstrated in *Rhodnius prolixus* that AT stimulates the frequency of contractions of the aorta both during post-prandial diuresis, as well as during stress under resting conditions. The way by which the peptide acts on the DV was not still elucidated. As a first approach to understand the mechanisms ruling this process, we decided to analyze the response of the aorta under an hypoosmotic shock in two groups of insects. In one group the MTs were removed and temporary maintained in *R. prolixus* saline. A second group was sham operated (i.e. performing all the process but without removing MTs). The experiment was performed *in vivo* in adult males starved during 15-25 days after molting. After surgery, both experimental groups were maintained with the haemocel bathed in saline, and the frequency of contractions of both groups was registered. Then, MTs were relocated into the abdomen of operated insects, and the DV activity was registered. Finally, haemolymph was completely replaced with 10 µl of 80% diluted saline. In each treatment, the aorta contractions were recorded at 5, 15, 30 and 45 minutes, under stereoscopic microscope. Data were analyzed by multifactorial ANOVA. The results showed that the osmotic change causes an increase of the aorta frequency in both groups of insects suggesting that AT secreted by MTs might be modulating the frequency of aorta contractions in an endocrine way. We have recently shown that the mechano-sensitive (MS) Piezo proteins, are involved in the modulation of DV activity under resting conditions. Due that these proteins are associated with MS processes involved in the response of cells challenged to an osmotic shock, our results might be indicating that this family of proteins could be responsible of the AT secretion by MTs during post-prandial diuresis when haemolymph is highly diluted, and would be modulating the velocity of the haemolymph circulation in an endocrine way.

A29
INTERACTION BETWEEN CHEMICAL MESSENGERS ON THE CNIDOCYST DISCHARGE IN *HYDRA* SP.

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The serotonin system (5-HT) is an ancient communication system widely distributed in nature. Its role has been studied in different invertebrate groups, proving to be associated to feeding behavior, locomotion, circadian rhythms and learning. In cnidarians, the sister group of Bilateria, the physiological roles of 5-HT are not fully stablished. Preliminary experiments performed in our laboratory suggest that 5-HT has myoregulatory functions on the hypostome (the structure that contains the mouth and is extruded during feeding). The aim of the present work was to study the role of 5-HT in the mechanism of desmoneme discharge (cnidocyst associated to prey capture) in *Hydra* sp. (Cnidaria: Hydrozoa), and the signaling pathways involved. We also studied the probable interaction between 5-HT and Allatostatin-C (AST-C), a neuropeptide that proved to be inhibitory on the feeding behavior of *Hydra*. To study the effect of 5-HT we performed a dose-response assay, and then we analyze the role of Ca^{2+} (by mean the use of the chelators BAPTA/AM and EDTA) and the signaling pathways activated by 5-HT using different inhibitors and blocking compounds of the proteins acting in canonic pathways of GPCRs (SCH202676- hydrobromide, Melitina, Xetospongina C y U73122). Regarding the interaction between 5-HT and AST-C, one group of polyps previously incubated with AST-C, was treated with both compounds simultaneously. To better understand the effect of AST-C, a second group of hydroids preincubated with AST-C was treated with a natural stimulator of the discharge, the reduced glutathione (GSH) and AST-C. In all the experiments, the number of desmonemes discharged was evaluated under the microscope. Differences between treatments were analyzed by one-way ANOVA. Single post-hoc comparisons were tested by the least significant difference (LSD) method. Only differences of $p \leq 0.05$ were considered significant. Results showed that doses of 5-HT $\geq 10^{-12}$M increased desmoneme discharge. Regarding to Ca^{2+}, our results showed that an increase on cytosolic calcium involving an extracellular influx is required. The treatments with inhibitors and blocking compounds suggest that the serotonin receptor would be a GPCR, coupled with a Gq protein. Its activation triggers the release of Ca^{2+} from the endoplasmic reticulum through an IP_3R and the activation of a phospholipase C. In both cases, polyps exposed to 5-HT or GSH simultaneously with AST-C, showed a significant decrease of the number of desmonemes discharged, suggesting that this neuropeptide regulates the response evoked by serotonin or by the food. Finally, the evidence presented here together with previous ones from our laboratory allow us to propose that 5-HT is associated with the cnidocyst discharge being involved on the feeding behavior of *Hydra* sp.

A30
MECHANO-SENSING AND PIEZO PROTEIN IN *HYDRA*

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Cells are exposed to different kind of stimuli coming from their environment. Mechanical stimuli such as osmotic changes, compression and elongation forces are detected, and transduced by mechano-sensing systems. In order to maintain the homeostasis, these effectors induce appropriated responses. Piezo proteins are a recently discover family of mechano-gating ion channels (MS) in which opening is trigger by mechanical changes of the plasma membrane, allowing the influx of cation to the cell (mainly Ca^{2+}). Piezo proteins are widly represented in Metazoa, acting in several physiological systems. *Hydra sp.* is a freshwater member of Cnidaria a phylum assumed as the sister group of Bilateria. Despite that the existence of Piezo protein was documented in Hydra, their physiological roles remain unknown. The aim of this work was to study the relevance of MS Piezo in *Hydra sp*., analyzing different physiological responses. To accomplish that, we studied the effects of different doses of Jedi1, a specific agonist of Piezo1 protein, on the contractile behavior, and the cnidocyst discharge, which are processes that can be activated by mechanical stimuli. Once the effective dose of Jedi1 was determined, similar assays were performed using this dose (200 µM) in the presence of GdCl3, an unspecific inhibitor of MS channels. Finally, we compared the effects caused by Jedi1, with those triggered by natural mechanical stimuli (i.e. osmotic changes of the incubation medium) or chemical compounds released by the prey (i.e. reduced gluthatione (GSH)) on cnidocyst discharge. The results showed that the Piezo agonist Jedi1 caused a dose-dependent increase of the contractile behavior. We observed a significant increase in the occurrence of a particular kind of retraction, named contraction burst, by which, the body of the hydroids acquire a sphere-shape, with the tentacles also retracted. The observed contractile patterns were similar to those exhibited when hydroids are disturbed by mechanical agitation, or by osmotic changes of the incubation medium. Regarding cnidocyst (desmonemes) discharge, Jedi1 also caused an increase on the level of discharge, being it similar to the one triggered by GSH. Moreover, the effects caused by Jedi1 on both, the contractile behavior, and the discharge of cnidocysts were avoided by GdCl3 treatment. Finally, when hydroids were challenged with natural mechanical stimuli as a diminution in the medium osmolarity, the observed contractile behavior was similar to that caused by jedi1, and it also resulted inhibited by GdCl3. In conclusion, these results confirm the presence, and relevance of Piezo protein in Cnidaria (*Hydra sp*.). This work represents the first report of the physiological roles of MS Piezo as part of the signaling systems that regulate the contractile behavior, and the discharge of cnidocysts in *Hydra sp*. Indeed, this new family of proteins would participate in several situations caused by mechanical forces such as mechanical stress, environmental osmotic changes, and/or the presence of the prey.

ENDOCRINOLOGY AND METABOLISM

A31

METFORMIN AS AN EARLY LIFE INTERVENTION IMPROVES OXIDATIVE STATUS IN TESTES OF MIDDLE-AGED UM-HET3 MICE FED A HIGH FAT DIET

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Metformin is a synthetic dimethyl biguanide used for 60 years as an oral anti-diabetic drug in patients with type 2 diabetes mellitus. However, literature concerning metformin effects in the testis is still controversial and sometimes contradictory. On the other hand, accumulating evidence suggests that early life interventions (ELIs) might have long-term effects on aging. UM-HET3 mice are an established genetically diverse population frequently used in aging intervention studies which limits the occurrence of strain-specific effects, thus providing a more realistic model of human populations. This study focused on investigating the effects of metformin as an ELI on testicular traits in male UM-HET3 mice that were fed a high fat diet (HFD, 60 Kcal% Fat from Research Diets *commonly used to induce obesity in rodents*) for 9 months starting at the end of metformin treatment. Saline (control group) or metformin (200mg/kg/day) were administered i.p. once-daily, for 40 days, starting at the age of 15 days. Young adult (2 months old) and middle-aged (11 months old) UM-HET3 mice fed a standard diet (SD) were also analysed. In SD-fed mice, body weight (BW) and testicular weight (TW) were unaffected by age. Instead, TW expressed as % of BW (gonadosomatic index) and *non-fasting* blood glucose were lower in 11 month-old than in 2 month-old mice. Furthermore, lipid peroxidation (evaluated by TBARS production) did not show age-dependent changes, whereas catalase expression (determined by immunoblotting) was significantly higher and catalase enzyme activity (quantified by H_2O_2 determination) showed a non-statistically significant increase. At the age of 6-8 months, HFD significant impaired glucose tolerance while metformin ELI increased baseline blood glucose. Feeding a HFD to 11 month-old mice showed a non-statistically significant trend towards higher blood glucose levels, significantly increased BW, unaffected TW, diminished gonadosomatic index, increased lipid peroxidation and markedly decreased catalase expression and activity. Interestingly, in middle aged mice that had been exposed to metformin ELI, there were no changes in BW, TW, the gonadosomatic index or blood glucose, but testicular oxidative status improved by decreasing lipid peroxidation and increasing catalase expression and activity, thus abolishing, *either partially or totally*, HFD-dependent changes. Overall, this study reveals that metformin ELI shows a beneficial effect on testicular oxidative status in genetically heterogeneous middle-aged mice fed a HFD. However, whether these results are relevant for animal-to-human translation and thus, metformin may have a long-term positive impact on testicular aging when is administered at early age to patients with metabolic syndrome and/or diabetes remains to be more thoroughly investigated.

A32
MENIN, p27 and pAKT PROLACTINOMA DEVELOPMENT ROLE. SEXUAL DIFFERENCES

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It is known that menin is an important factor that regulates cell cycle and proliferation mechanisms. Menin plays a key role in pituitary function regulating $p27^{Kip1}$ and the AKT pathway. We studied the menin function on lactotrophs and prolactinoma development in two mouse models of prolactinoma: the first one is an animal model that overexpresses the β-subunit of the human chorionic gonadotrophin (hCGβ+) and, the other one, is a type-II dopamine receptor *knock-out* (Drd2KO). Only the transgenic female mice (β+ and KO) develop lactotroph hyperplasia and hyperprolactinemia from 3 months of age onwards. We analyzed the gene expression by Real-Time PCR, and the protein expression specifically on lactotrophs by double immunofluorescence (co-localization).
In the hCGβ+ animal model, we observed higher levels of *MEN1* gene expression in male pituitaries vs females, without genotype differences. No differences between sexes or genotypes were observed in the percentage of lactotrophs menin+ in any animal model, but we found important changes in the subcellular localization of menin. In lactotrophs from males and WT females, menin is expressed in cytoplasm and nuclei. In contrast, nuclear localization is lost in female tumoral lactotrophs, with menin expressed mostly in the cytoplasm. The lack of nuclear menin was concomitant with a decrease in the percentage of lactotrophs p27+ and a sharp increase in pAKT expression, concomitant with lower *PTEN* expression, compared to WT females. Male pituitaries these animal models showed no differences between genotypes in the menin subcellular localization, *p27* expression levels, or percentage of lactotrophs p27+. Also, males´ pituitaries showed lower cytoplasmatic pAKT expression concomitant with higher *PTEN* expression levels than WT females, without genotype changes. The decreased nuclear menin expression and the lower *PTEN* expression in lactotrophs from transgenic females could contribute to decreased p27 expression and increased pAKT levels leading to prolactinoma development. On the other hand, stable menin nuclei expression and higher *PTEN* expression observed in male pituitaries could help to control the lactotroph proliferation rate in this sex, despite genotype, avoiding prolactinoma development.

A33
PROINSULIN INCREASES ITS LOCALIZATION IN AUTOPHAGIC VESICLES IN PANCREATIC BETA CELLS IN THE DOPAMINE D2 RECEPTOR *KNOCKOUT* MOUSE ($DRD2^{-/-}$)

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The dopamine D2 receptor (D2R) plays a pivotal role in regulating endocrine and metabolic functions. In the pancreas, it plays a key role in modulating insulin secretion, thereby ensuring optimal glucose homeostasis. In addition, autophagy represents a pivotal lysosomal degradation mechanism for the functionality of pancreatic beta cells. The rapid clearance of a substantial quantity of the insulin precursor after translation is mainly facilitated by the autophagy process. Preliminary results from our laboratory show that 7-month-old male D2R *knockout* mice ($Drd2^{-/-}$) present glucose intolerance and decreased insulin secretion compared to wild-type (WT) control mice. They also show a lower pancreatic insulin content and we recently demonstrated an increased autophagic flux in pancreatic islets compared to WT mice. Since D2R regulates autophagy in several tissues, we were interested in evaluating the role of autophagy in regulating the levels and intracellular localization of proinsulin (proINS) in pancreatic islet cells of $Drd2^{-/-}$ animals using immunofluorescence assays and confocal microscopy. No significant differences were observed in the number of proINS *puncta* per cell between $Drd2^{-/-}$ mice aged 7-8 months and WT mice (t-test, p=0.15). However, mutant mice showed a higher number of proINS *puncta* co-localizing with the autophagic vesicle marker LC3 (t-test, p=0.002) as well as a tendency towards a higher percentage of proINS *puncta* co-localizing with LC3 (t-test, p=0.086). These findings indicate a greater localization of proINS in autophagic vesicles and suggest an increase in its degradation by autophagy in $Drd2^{-/-}$ mice, which would decrease its residence in the secretory pathway and its conversion to insulin, resulting in lower secretion in response to glucose. Preliminary studies have not shown significant differences in the number of *puncta* of Lamp2, a marker of acidic vesicles (lysosomes, autolysosomes), between genotypes (t-test, p=0.1). We are currently conducting studies on the co-localization of proINS with Lamp2, to elucidate the progress of the insulin precursor along the autophagic pathway. In conclusion, D2R is necessary for the regulation of the insulin precursor, proINS, in part through the modulation of the autophagy process, thus being essential for correct synthesis and secretion of insulin, and allowing optimal glycemic control. This work was funded by CONICET, ANPCyT, Fundación René Barón, Fundación Williams, and Fundación Bigand.

A34
CHARACTERIZATION OF ADENOSINE RECEPTORS IN NORMAL PITUITARY AND PROLACTINOMAS

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Prolactinomas are the most common pituitary adenomas and are usually treated with dopamine agonists which inhibit lactotroph functions through the dopamine type 2 receptor (D2R). However, 20% of patients develop resistance to this treatment and must undergo intracranial or transepto-sphenoidal surgeries to remove the adenoma. The molecular mechanisms by which dopaminergic inhibition is evaded are not fully understood. D2R belongs to the family of the membrane G protein-coupled receptors (GPCRs). These receptors can heterodimerize, forming complexes that can alter the receptors signaling. It has been shown that adenosine levels and its receptors are elevated in various tumors. There are four types of adenosine receptors: A1, A2a, A2b, and A3. All of them are expressed in lactotrophs. Activation of A1 and A3 inhibits the synthesis and secretion of PRL while activation of A2a or A2b stimulates it. It has been described that, when D2R-A2aR heterodimerize in brain regions, A2a signaling predominates, and the D2R response is blocked. We postulate that dimerization of D2R with other GPCRs may be one of the causes of resistance to dopamine agonists. In the first place, we characterized the gene and protein expression of adenosine receptors in WT pituitary glands and prolactinomas derived from C57 mice that have knocked out the D2 receptor. In this D2RKO model, only transgenic females develop prolactinomas. The gene expression of the receptors was analyzed by RT-qPCR and the protein expression by double immunofluorescence to study the expression of the A1R and A2a receptors, especially in lactotrophs. The pituitary glands of males showed higher levels of gene expression of type 1 (*Adora1*) and type 2 (*Adora2a*) adenosine receptors. This could be due to the lower circulating levels of estrogen in this sex, since we later demonstrated, by acute in *vivo* assays, that estradiol negatively regulates pituitary gene expression of *Adora1* and *Adora2a*. On the other hand, in D2RKO animals of both sexes, *Adora1* expression levels were lower than those found in WT pituitary glands. However, *Adora2a* expression did not show significant differences between genotypes. Through *in vivo* assays, by acute treatments with dopaminergic agonists or antagonists, we corroborate that there is a positive regulation by dopamine on the pituitary *Adora1* expression in males, but without effect in females. Preliminary protein expression studies show that the expression of A1R and A2aR in lactotrophs (colocalization with PRL) tends to decrease in KO female pituitaries compared to WTs. We conclude that there are sexual differences in the regulation of the gene expression of adenosine receptors in the pituitary that they are under estrogenic and dopaminergic regulation, and that in addition the gene and protein expression of these receptors is altered in transgenic animals with loss of dopaminergic control.

A35
LIRAGLUTIDE AND THE BLOOD-TESTIS BARRIER: EFFECT OF TREATMENT DURING THE JUVENILE PERIOD AND ITS IMPACT IN ADULTHOOD

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Liraglutide (Lira) belongs to the family of GLP-1 receptor agonists and is widely used to treat obesity and Type 2 diabetes. Recently, it was approved as a treatment for weight control in patients over 12 years old with obesity. However, the literature regarding the impact of this drug on testicular function is scarce. One of the most important structures in the testis is the Blood-Testis Barrier (BTB), which isolates developing germ cells in the adluminal compartment. It has been demonstrated that different drugs and toxic agents can compromise BTB integrity. Therefore, we aimed to analyze the effect of Lira treatment on BTB function during the juvenile period and its potential later impact on testicular function in adulthood. Pups were randomly assigned to the following groups: control group (C), receiving saline solution, and Lira group receiving 0,2 mg/kg/day of Lira. Treatments were performed subcutaneously from postnatal day (Pnd) 20 to 33, period of life that is essential to complete a functional BTB. The evaluations were done on Pnd 34 or Pnd 90. On Pnd34, BTB permeability and tubular apoptosis were assessed. RT-qPCR was performed to evaluate expression levels of different proteins that comprise BTB junctions and the androgen receptor. On Pnd 90, BTB permeability, stages of the spermatogenesis cycle, and daily sperm production were evaluated. Results obtained on Pnd34 show a higher percentage of permeated tubules (5,5±1,4; 11,9±3,3*; n=8; *p<0.05) and more tubules with apoptotic cells in the Lira group (5,5±2,1; 12,7±4,7* vs. Control; n=6 *p<0.05). However, no significant differences were observed in the mRNA levels of intercellular junction proteins such as ZO-1, Connexin-43, or Occludin. Besides, Lira treatment does not affect intratesticular testosterone levels or androgen receptor expression. Animals treated during the juvenile stage on Pnd90 showed no differences in BTB permeability compared with control animals. Moreover, testicular morphology was preserved, and no changes were found in the stages of the spermatogenic cycle. In addition, daily sperm production was not affected in animals treated during the juvenile period. Based on the results obtained, we conclude that Lira treatment during the juvenile period alters BTB permeability. However, the findings in the adult stage suggest that this effect is reversible and may not affect spermatogenic capacity.

A36
REPRODUCTIVE EFFECTS OF MATERNAL TREATMENT WITH METFORMIN IN FEMALE OFFSPRING OF MICE

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Metformin is a hypoglycemic drug widely used to treat type 2 diabetes. However, reproductive effects of this drug, among others, have been reported. Since it can cross the placenta, its use during pregnancy is controversial. Most studies focus on the metabolic consequences in the offspring of women treated with metformin but lack information on the reproductive effects due to exposure during development. Our hypothesis was that exposure to metformin during gestation and lactation affects the fertility and ovarian function of the offspring, in addition to metabolism. The objective of our study was to evaluate ovarian alterations and fertility in the female offspring of mothers treated with metformin. To do so, we worked with C57BL/6 mice. Mothers were treated with metformin four weeks before mating, and treatment was maintained throughout gestation and lactation. After weaning, for seven weeks, female offspring were weighed weekly and then divided into two subsets. In the first subset, the animals were monitored for 14 days and then sacrificed, isolating adipose tissue, blood, and ovaries for subsequent immunohistochemistry and western blot techniques. In the second subset, females were mated with males of proven fertility, and various parameters were analyzed to study natural fertility. We observed that the offspring of mothers treated with metformin had lower birth weights but exhibited more gonadal adipose tissue in adulthood than animals not exposed to the drug. Follicular development and anti-Müllerian hormone expression were altered, as well as ovarian angiogenesis. However, the estrous cycle, hormone production, and fertility were not affected by exposure to metformin during development. Additionally, the second generation of mothers treated with the drug showed higher birth weights. We concluded that metformin treatment during gestation and lactation affects offspring in adulthood, with consequences even in the second generation. Metabolic and ovarian function parameters were altered, without affecting animal fertility. Given that pregnancy and lactation are critical periods capable of affecting offspring, further studies are needed to determine the use of metformin in pregnant women.

A37
OLIGONUCLEOTIDE IMT504 IMPROVES METABOLIC STATE IN HIGH-FAT DIET FED MICE

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Type 2 diabetes (T2D) is a complex metabolic disease, related to obesity. Obesity and diabetes are associated to a low and chronic inflammatory process in peripheral tissues that are involved in the establishment of insulin resistance (IR). We have observed that the immunomodulatory oligonucleotide IMT504 (IMT) improves glucose homeostasis, diminishes food intake and body weigh in metabolic syndrome (MS) and T2D murine model induced by high-fat diet. This work was designed to elucidate the mechanisms by which the IMT504 improves the metabolic state in this model. Male C57BL/6LP mice were fed either a standard diet (SD) or a high-fat diet (HFD: ResearchDiet, D12492, 60% fat content) for 12 weeks. HFD animals showed higher non-fasting glycemia and body weight. SD mice

received one daily dose of IMT504 for 16 consecutive days (SD-IMT: 6mg/kg, sc) or saline (SD-sal). HFD mice received one daily dose of IMT504 for 16 consecutive days (HFD-IMT: 6mg/kg, sc), a control polyC oligonucleotide (HFD-PolyC: 6mg/kg, sc, to evaluate the specificity of the IMT504 effects), or saline (HFD-sal). Intraperitoneal glucose tolerance test (ipGTT) and insulin secretion test (IST) were performed (day 10). On day 16, after 3 hours fasting, glycemia were recorded, mice sacrificed and blood, white adipose tissue (WAT) and liver samples collected. Serum insulin levels (by ELISA), insulin resistance (by HOMA-IR), beta-cell functionality (by HOMA β-cell), serum leptin levels (by ELISA) and cytokines (by ELISA) were analyzed. IMT induced a significant recovery on insulin secretion in response to the glucose overload [IST: ANOVA: interaction NS; MAIN EFFECT: treatment $p<0.005$, SD-sal, SD-IMT and HFD-IMT different from HFD-sal and HFD-PolyC, $p<0.005$]. Hyperinsulinemia, and HOMA β-cell, HOMA-IR and QUICKI indexes alterations were all reversed by IMT but not by PolyC treatment [ANOVA, $p<0.05$ or less for all] in HFD mice. Serum leptin increased in HFD animals and partially lowered only by IMT [ANOVA, $p<0.05$, SD-sal and SD-IMT different from HFD-sal and HFD-PolyC: $p<0.05$]. Beside, TNF-α and IL-1β pro-inflammatory cytokines levels in HFD mice WAT were higher compared to SD and partially diminished only by IMT treatment [ANOVA, $p<0.05$, SD-sal and SD-IMT different from HFD-sal and HFD-PolyC: $p<0.05$, for both cytokynes]. However, no differences in the levels of these cytokines were observed in liver tissues among treatments. These results show that IMT504 is effective in improving metabolic state in a T2D animal model; by improving hyperinsulinemia, insulin resistance and ameliorating serum leptin levels and WAT pro-inflammatory cytokines levels. Further research is needed to understand its mechanism of action. Funding: CONICET, ANPCYT, Sidus Arg, F. R Barón, F. Williams, H. Bigand.

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 2

A38
EFFECT OF ENERGY SUBSTRATES ON MURINE SPERM CAPACITATION AND ITS IMPLICATIONS FOR OXIDATIVE STATUS AND FERTILIZATION

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Mammalian sperm must undergo functional and structural changes to acquire the ability to fertilize an egg. This process, known as capacitation, takes place as sperm transit through the female reproductive tract and requires an efficient energy supply. In mice, the female tract contains glucose (G), pyruvate (P), and lactate (L), which are commonly included in in vitro capacitation media. Consistently, we observed that activation of both glycolysis and oxidative phosphorylation, driven by these substrates, is required for the acquisition of sperm fertilizing ability in mice. In this study, we further investigated the effects of different energy substrates on sperm oxidative status and functionality. First, we assessed reactive oxygen species (ROS) levels during capacitation in media containing different combinations of substrates. Sperm incubated with G alone, without P and L, showed a significant increase in ROS levels compared to control and the remaining combinations ($p<0.05$). This increase in ROS was associated with elevated sperm DNA fragmentation ($p<0.0001$). However, ATP content remained unchanged across all conditions tested ($p>0.05$). To explore the impact of oxidative damage on sperm fertilizing ability, we conducted in vitro fertilization assays, applying short incubation times—a challenging condition for sperm. Spermatozoa incubated with glucose alone exhibited delayed fertilization compared to control ($p<0.001$). Subsequent gamete fusion assays indicated no significant differences in sperm-egg fusion between conditions ($p>0.05$), suggesting that sperm incubated with G, but without P and L, experience difficulties in penetrating the zona pellucida. In conclusion, the use of different exogenous energy substrates differentially impacts sperm functionality. These findings raise the possibility that endogenous energy sources may also play a role. Moreover, the oxidative damage observed in spermatozoa could have implications for the quality and future performance of embryos generated under these conditions.

A39
DYSLIPIDEMIA AFFECTS THE PROTEOME OF EXTRACELLULAR VESICLES IN FOLLICULAR FLUID

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Dyslipidemias are alterations of the plasma lipid profile with high prevalence in Argentina. Previously, we reported that dyslipidemia adversely affects oocyte maturation and influences the lipid profile of follicular fluid (FF) compatible with "intrafollicular dyslipidemia". Extracellular vesicles (EVs) are important mediators of intercellular communication which transport different molecules and regulate lipid metabolism and protein expression. The impact of dyslipidemia on these key mediators in the follicle has not yet been explored. The objective of this study was to isolate and characterize EVs from the FF of women with and without dyslipidemia and to evaluate the effect of dyslipidemia on the protein profile of these EVs. Follicular fluid samples were collected from 109 women without reproductive pathologies, with dyslipidemia (n=57) and without dyslipidemia (n=52). A principal component analysis was conducted to define the extreme quartiles (Q1, without dyslipidemia; Q4, with dyslipidemia) and thus identify women with the most contrasting lipid profiles. Extracellular vesicles were isolated by differential centrifugation of FF from Q1 and Q4. The size distribution and concentration of the EVs were analyzed using Nanoparticle Tracking Analysis (NTA). For phenotypic characterization and identification of the EVs, the presence of CD63, ALIX, CD81, HSP70, TSG101, CD9, and CD40 was determined by Western blot and flow cytometry. Additionally, a proteomic analysis of the EVs was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Nanoparticle Tracking Analysis showed a similar nanoparticle concentration (Q1= $6.4x10^{-8} \pm 2.3x10^{-8}$ particles/mL; Q4= $5.4x10^{-8} \pm 2.6x10^{-8}$ particles/mL) and particle size between quartiles (Q1= 158.44 ± 15.49 nm; Q4= 161.24 ± 7.50 nm), which is consistent with the expected size of exosomes, a specific EV subtype. Accordingly, this finding was confirmed by the presence of specific exosome markers. The proteomic characterization of the EVs allowed identifying 32 proteins that were differentially expressed between the two quartiles (12 in Q1 and 20 in Q4). Gene Ontology enrichment analysis of the differential proteins revealed distinct representation patterns for both biological processes and molecular functions. Furthermore, statistically overrepresented proteins associated with the immune system and antioxidant proteins were only found in the Q4. Immune and antioxidant response proteins overrepresented in the exosome-enriched fraction of FF from women with dyslipidemia evidence an altered intrafollicular environment with negative implications for follicular function.

A40
METABOLIC PARAMETERS OF *IN VITRO* FERTILIZED PORCINE OOCYTES

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In vitro fertilization (IVF) is currently used in the *in vitro* production of porcine embryos and involves the activation of the oocyte after its fusion with the spermatozoa. It has been described in mice and cattle that during oocyte activation there is a variation in its metabolic activity from a quiescent state, at the completion of *in vitro* maturation (IVM), to an increase in mitochondrial parameters and a fluctuation in reactive oxygen species (ROS) production. The aim of this study was to evaluate variations in internal mitochondrial membrane potential (IMMP), ROS production and redox state, represented by NAD(P)H+H^+ and FAD coenzimes concentrations, at the finalization of oocyte-spermatozoa co-incubation. Cumulus-oocyte complexes (COCs) obtained by aspiration of antral follicles from ovaries of slaughtered gilts were selected and incubated in supplemented 199 medium at 39°C, 5% CO_2 y 100% humidity during 44h. At the end of IVM, IVF was performed using fresh semen washed and resuspended in modified Tris buffer medium with a final concentration of 5 10^5 sperm/ml. At 0h (control oocytes) and 3.5h after FIV, cohorts of COCs were extracted and denuded with a fine Pasteur pipette for its study. Using epifluorescence microscopy, IMMP was assessed with JC1-1 staining, ROS production by 2′,7′-dichlorodihydrofluorescein diacetate (DCH_2FDA) staining, FAD and NAD(P)H + H^+ concentrations were determined using green (excitation 473 nm, emission 490-590 nm) and blue (excitation 405 nm, emission 420-520 nm) filters, respectively. Digital microphotographs were obtained and processed using IMAGE J software to quantify oocyte fluorescence intensity. Results were analyzed by ANOVA followed by a Bonferroni test ($p<0.05$). A significant decrease in NAD(P)H+H^+/FAD ratio and an increase in IMMP was observed at 3.5h compared to 0h of IVF ($p<0.05$), while no significant differences were observed in ROS production between both determinations. In conclusion, oocyte-spermatozoa fusion after IVF triggers a series of modifications in the porcine oocyte which imply an increase in its metabolic rate and leads to zygote formation and subsequent embryonic development. These events could be linked to an immediate increase in IMMP associated with a decrease in the NAD(P)H+H^+/FAD ratio, which could precede possible ROS production fluctuations.

A41
MITOCHONDRIAL ACTIVITY CHANGES IN THE PRESENCE OF TROLOX OR RESVERATROL DURING *IN VITRO* MATURATION OF BOVINE OOCYTES

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During oocyte *in vitro* maturation (IVM), mitochondrial activity is an indicator of the gamete capacity to carry out nuclear and/or cytoplasmic maturation processes. The properties of the antioxidants Trolox (T), resveratrol (R) and their concentrations are being analysed in different cellular systems. The aim of this study was to determinate the effect of T and R during IVM of bovine oocytes on the number of active mitochondria, mitochondrial membrane potential (MMP), and redox state. Antral ovarian follicles were punctured and aspirated, and cumulus-oocyte complexes (COCs) were recovered. The COCs were matured in medium 199 with eCG for 22 hours at 39°C in a humidified atmosphere of 5% CO_2 in air, with the addition of T 0 µM (C_T), 25 µM (T_1), 50 µM (T_2) or 100 µM (T_3), or R 0 µM (C_R), 0.1 µM (R_1), 1 µM (R_2) or 5 µM (R_3), and then denuded with 0.1% (w/v) hyaluronidase. The number of active mitochondria was determined by incubating the oocytes for 30 minutes with MitoTracker Green FM, the MMP for 30 minutes with JC-1, and the redox state by the FAD/NAD(P)H ratio measuring the intensity of the endogenous autofluorescent compounds FAD and NAD(P)H. The individual luminosity of each oocyte was evaluated using IMAGE J. Oocyte nuclear maturation was assessed by the presence of metaphase II using Hoechst 33342. Quantitative variables were analysed by ANOVA, and qualitative variables by Chi-square ($p<0.05$). The number of active mitochondria in oocytes gradually decreased with T increasing concentrations. This result was significant with T_3 compared to C_T ($p<0.05$), and also decreased in the presence of R_1, R_2, and R_3 compared to C_R ($p<0.05$), with no significant differences between them. A gradual decrease in oocyte MMP was detected in the presence of T, with significant differences observed with T_2 and T_3 compared to C_T ($p<0.05$), and it also decreased with R_2 and R_3 compared to C_R ($p<0.05$). The FAD/NAD(P)H ratio in oocytes gradually decreased with T increasing concentrations, observing significant differences between T_3 and C_T ($p<0.05$); this ratio also decreased with R_1, R_2 and R_3 compared to C_R ($p<0.05$), with no significant differences between these groups. No significant differences were found in the percentage of nuclear maturation of the oocytes across the different treatments. We can conclude that Trolox or resveratrol supplementation to the IVM medium modulates oocyte mitochondrial activity, and these changes do not affect their nuclear maturation capacity. Further studies are required to evaluate their effects on oocyte cytoplasmic maturation.

A42
RELEVANCE OF CRISP1 AND CRISP3 PROTEINS FOR FEMALE FERTILITY AND EMBRYO DEVELOPMENT

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CRISP (Cysteine-Rich Secretory Proteins) family proteins mainly expressed in the male reproductive tract of mammals, play critical roles in different stages of the fertilization process and are relevant for male fertility. Moreover, CRISP1, CRISP2 and CRISP3 are also expressed in the female tract, including the cumulus cells that surround the egg where CRISP1 plays a chemoattractant role for sperm during fertilization. Although single knockout (KO) females for CRISP proteins are fertile, double KO (DKO) females for CRISP1 and CRISP3 exhibit subfertility suggesting the existence of compensatory mechanisms between homologous proteins. Based on this, the aim of the present work was to study the mechanisms leading to reduced fertility in the mutant females. First, we carried out *in vivo* fertilization assays in which estrous females were mated with control males overnight and the eggs were recovered from the ampulla the following day and incubated *in vitro* for additional 5 days. Results showed a significant decrease in the percentage of fertilized eggs reaching both the 2-cell stage (day 1) and the blastocyst stage (day 5). Since this decrease could be due to defects in sperm arrival to the eggs within the female tract, *in vitro* fertilization assays were performed by co-incubating cumulus-oocyte complexes and zona-free eggs from control and DKO females with capacitated control sperm. Whereas in both cases no differences in fertilization rates between groups were observed, embryos from DKO females again showed a significant decrease in the percentage reaching the blastocyst stage. Considering that meiosis resumption is a critical step for subsequent blastocyst development, we next analyzed maternal DNA immediately after gamete fusion. For this purpose, zona-free eggs were incubated with capacitated control sperm, and the DNA within the eggs was analyzed by Hoechst staining. Results showed no significant differences in the percentage or in the meiotic stage of fertilized eggs, suggesting that the absence of CRISP1 and CRISP3 proteins would be affecting subsequent post-fertilization events. Taken together, these results show the relevance of female CRISP family proteins not only for fertilization but also for early embryo development. We believe these results will contribute to a better understanding of the molecular mechanisms underlying embryogenesis as well as to the development of new methods of diagnosis and/or treatment of female infertility.

A43
EVALUATION OF SPERM ADHESION TO BOVINE OVIDUCTAL CELLS INFECTED WITH BOVINE VIRAL DIARRHEA VIRUS

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As in many mammalian species, bovine spermatozoa maintain their viability until fertilization by adhering to oviductal epithelial cells (OEC) through molecular interactions. Bovine viral diarrhea virus (BVDV) has tropism for reproductive tract cells, including OECs. Although it has been observed that OECs infected with non-cytopathic BVDV strains do not show macroscopic alterations in the oviduct, the influence of this viral infection on the sperm adhesion process has not been fully elucidated. The aim of this study was to evaluate the interaction of spermatozoa with BVDV-infected CEOs. CEOs were cultured in monolayers and explants, infected with a non-cytopathic BVDV genotype 1b strain. Monolayers were co-incubated with $1x10^6$ sperm/mL and explants with $2x10^6$ spermatozoa/mL for one hour. The number of adhered spermatozoa was quantified by image analysis (FIJI software), the results are expressed as adhered spermatozoa/0.15 mm². To quantify the released spermatozoa, both before and after release induction by addition of heparin (1 mg/mL), the spermatozoa adhered to the monolayer were first quantified. Spermatozoa released into the supernatant were then collected and quantified using a hemocytometer, with results expressed as spermatozoa/mL. The data were analyzed by ANOVA (RStudio 4.3.3), and were expressed as statistical means, with a significance level of 0.05. In infected CEO explants, the amount of attached sperm was significantly higher (131.6 sp/0.15 mm²) compared to uninfected controls (101 sp/0.15 mm²) while, in infected and control cell monolayers, the values of attached sperm (61.9 sp/0.15 mm² and 58.7 sp/0.15 mm², respectively) did not exhibit significant differences. A high proportion of the spermatozoa attached to both infected and control cells, 96% and 95% respectively, were released. No significant differences were found in the number of spermatozoa released from infected and control monolayers (181.545 sp/mL and 147.833 sp/mL, respectively). The preservation of the three-dimensional architecture of the oviductal epithelium of the explants favored the interaction of spermatozoa with ciliated cells. Likewise, infection with the non-cytopathic biotype of BVDV promoted adhesion, suggesting that the virus may influence cell viability and the regulation of surface molecules.

A44
AMINO ACIDS AND ENDOGENOUS LIPIDS CATABOLISM DURING BOVINE OOCYTE *IN VITRO* MATURATION

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Most of the metabolic studies carried out during oocyte in vitro maturation refers to carbohydrates metabolism, being few information about amino acids (AA) and endogenous lipids metabolism. Many of these studies were performed in complex and undefined routine maturation media yielding contradictory results. The aim of this work was to study the use of AA or endogenous lipids as unique oxidative substrates for bovine oocyte in vitro maturation. Cumulus-oocyte complexes (COCs) were obtained by aspiration of antral follicles from ovaries of slaughtered cows. COCs were matured in a defined media (SOFm) without oxidative substrates (negative control), supplemented with AA, AA+salicylate (oxidative deamination inhibitor), AA+ glucose (Glc), or Glc, to study AA catabolism; or supplemented with L-carnitine (fatty acid β-oxidation activator), etomoxir (fatty acid β-oxidation inhibitor), L-carnitine+Glc, or Glc, to study the endogenous lipid consumption. Nuclear maturation, ammonia production and oocyte lipid content were evaluated after 22 h of maturation, and cleavage and blastocyst rates were evaluated at 48 h and 7 days after fertilization, respectively. The study of AA catabolism showed that oocytes matured in media supplemented with AA or Glc had higher nuclear maturation rates respect to the negative control or AA+salicylate ($p<0,05$), and a synergic effect was observed in the group added with AA+Glc ($p<0.05$). An increase in ammonia production was observed in the medium added only with AA as oxidative substrates ($p<0.05$). The patterns of cleavage rates followed those of the nuclear maturation rates ($p<0.05$), but embryo development up to blastocyst stage was only observed in the group supplemented with AA+Glc ($p<0.05$). The study of endogen lipid catabolism showed that the medium supplemented with L-carnitine presented a higher nuclear maturation rate respect to the negative control or etomoxir ($p<0.05$), but media added with Glc or L-carnitine+Glc increased nuclear maturation with respect to the other groups ($p<0.05$). A higher lipid content was observed in the group supplement only with Glc respect to the negative control, L-carnitine or etomoxir ($p<0.05$). Media added with Glc or L-carnitine+Glc increased cleavage rates with respect to the other groups ($p<0.05$), but no group was able to reach up to the blastocyst stage. In conclusion, catabolism of AA, endogenous lipids or Glc alone can sustain bovine oocyte nuclear maturation, but they are insufficient for inducing

oocyte developmental competence. To obtain competent oocytes, maturation must be carried out in media supplemented with both AA+Glc.

A45
IMPACT OF SUBCLINICAL *ESCHERICCHIA COLI* LIPOPOLYSACCHARIDE INFECTIONS ON VASCULAR AND INFLAMMATORY MEDIATORS PROFILE ON IMPLANTATION SITES AND PLACENTAS IN RATS

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Subclinical infections cause dysregulations in the inflammatory response that would produce placental dysfunction and impact the development of pregnancy and progeny. Previously, we developed a model of subclinical infection in rats during early gestation with low doses of lipopolysaccharide (LPS) from *Escherichia coli*. Our results show that subclinical infections affect the uterus's vascular adaptations and the fetuses' intrauterine growth, decreasing the offspring's weight. We also observed that the pups exhibit poor neurological development. Therefore, we decided to investigate the effect of subclinical LPS infection during gestation on the architecture and the immune and vascular balance of the implantation sites and placentas. For this, pregnant Wistar rats received either vehicle (i.p., saline solution as control) or LPS (20 µg/kg on day 6 + 50 µg/kg on days 7, 8, and 9 of gestation). The animals were euthanized on days 10 or 15 of gestation. The implantation sites or the placentas were extracted and processed. The architecture of the day 10 implantation sites was conserved, without differences in the thickness of the muscle layers. No differences were found in the area of the mesometrial vessels. Furthermore, there was a trend towards an increase in the area of the mesometrial decidua and total areas of the sinusoids in the LPS-treated rat sites. Additionally, the expression of CD31, an endothelial marker, was increased in the LPS sites, suggesting a deficiency in vascular remodeling. On day 15, the placentas from mothers treated with LPS exhibited a dark purplish-red color and a more fragile structure. No differences were observed in the labyrinth, the junction or the basal decidua zone areas. When we investigated the levels of inflammatory and vascular mediators, we found that while the mRNA of PlGF and VEGF-A decreased in the LPS implantation sites, CXCL 10 and IL-6 were increased. In the placentas, the mRNA levels of PlGF and CXCL10 were increased. Surprisingly, IL-6 decreased compared to control placentas. The mRNA levels of TNF-α showed no differences in the implantation sites or placentas in control vs. LPS. Moreover, we evaluated the protein levels of COX-2 and iNOS at day 10 implantation sites and found no differences. In the placentas, there were no variations in COX-2 expression or PGE2 levels either. In summary, our subclinical infection model causes differential expression of crucial mediators related to vascular remodeling and inflammation at the implantation site and placenta. We, therefore, propose that these alterations during gestation explain the previously described effects on the macrovasculature, fetuses and offspring.

GENERAL BIOLOGY, MICROBIOLOGY AND IMMUNOLOGY

A46
BIOINFORMATICS ASSESSMENT OF GABAB RECEPTOR AS POTENTIAL THERAPEUTIC TARGET IN PANCREATIC DUCTAL ADENOCARCINOMA

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GABA is the main inhibitory neurotransmitter in the mammal central nervous system, synthesized by glutamic acid decarboxylase (GAD). It binds to GABAA/C and GABAB receptors (GABABR). Recent studies show that the GABAergic system is also present in peripheral tissues, including the pancreas, and is involved in tumorigenesis and tumor immunity. GAD1 is upregulated in tumors, contributing to cancer progression. In most cases, expression levels of GABA and its receptors changed in tumor tissues. However, it is unclear whether GABAergic signaling could have a positive or negative role in the regulation of cancer cell behavior because it depends on the origin of the tumor and the type of receptor involved. Pancreatic cancer (PC) is the most lethal malignant tumor with a five-year survival rate of about 5 %, with pancreatic ductal adenocarcinoma (PDAC) the most prevalent. It is imperative to find novel drugs to improve survival and side effects of classical therapies. The aim of this work was to study GABABR as a potential therapeutic target in PDAC. We performed a transcriptomic analysis of *Gad1* and GABABR subunits (*Gabb1r/Gabb2r*) using databases of patients with PDAC from The Cancer Genome Atlas (TCGA) and normal tissue (GTEX), using Gepia2 and UCSC XENA platforms. We evaluated the association of these parameters with patient prognosis and clinical attributes. Finally, we used an enrichment analysis to examine the molecular functions and biological pathways associated with upregulated genes. *Gad1* was significantly overexpressed in PDAC tissue compared to normal tissue ($p<0.05$), although there were no differences in survival of PDAC patients with low or high expression. Although *Gabbr1* was similar between PDAC and normal tissue, upregulated *Gabbr1* expression in PDAC tissue had a positive impact on overall survival ($p<0.01$) and disease free survival ($p<0.05$) compared to those with low levels of *Gabbr1*. Both *Gabbr1* and *Gabbr2* expression tended to low levels in advanced stages of the disease, although only *Gabbr2* showed significant differences between stage I

and II ($p<0.01$). The phenotype associated with high *Gabbr1* expression levels revealed downregulation of translation, post-translation modifications, vesicle-mediated protein transport and DNA replication pathways. In conclusion, upregulation of *Gabbr1* transcripts in tumors correlates with favorable clinical outcomes and decreases cancer progression associated pathways. This suggests that repurposing GABABR agonists could improve PDAC treatment. Our ongoing studies are evaluating baclofen, a selective GABABR agonist, in cell proliferation, migration and invasion in PDAC cell line models. Supported by CONICET-ANPCYT.

A47
CHARACTERIZATION OF CHIMERIC GAG PROTEINS OF FELINE IMMUNODEFICIENCY VIRUS (FIV) CONTAINING SEQUENCES OF THE NUCLEOCAPSID PROTEIN OF SIMIAN IMMUNODEFICIENCY VIRUS (SIV)

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Lentiviruses assemble at the plasma membrane of the infected cells as a result of Gag polyprotein multimerization into spherical particles that then bud into the extracellular medium. Following virion budding, Gag is processed by the viral protease to generate the mature virion proteins: matrix, capsid, and nucleocapsid (NC). During assembly, the viral genomic RNA is incorporated into particles by the interaction of the encapsidation signal present in the viral RNA and the two zinc-finger motifs of the NC domain of Gag. The FIV and SIV NC proteins share a similar organization: an amino-terminal region, a proximal zinc finger motif, a basic region, a distal zinc finger, and a carboxyl-terminal region. In the FIV Gag precursor, the NC domain is followed by the C-terminal p2 peptide. In contrast, in SIV Gag, the NC domain is followed in the carboxyl direction by the SP2 spacer peptide and the p6 C-terminal domain. To investigate the functional relationship between FIV and SIV NCs, we generated two chimeric FIV Gag proteins: (a) NC1, in which we replaced the proximal and distal zinc fingers of the FIV NC with those of SIV, and (b) NC2, in which we kept the zinc fingers of the SIV NC but replaced the C-terminal domain of the FIV NC with the SP2 peptide of SIV. We first examined the ability of the chimeric FIV Gag proteins to assemble into virus-like particles (VLPs) by transfecting feline cells with the plasmids carrying the wild-type and chimeric FIV *gag* genes and analyzing the production of extracellular VLPs by Western blot. These assays showed that NC1 is assembly-defective; in contrast, the NC2 Gag protein assembles into VLPs with the same efficiency as wild-type FIV Gag. Based on these results, we expressed the NC2 *gag* gene in the context of the FIV genomic DNA. We found that NC2 is expressed and processed as wild-type FIV. Moreover, NC2 produces mature virions at wild-type levels. Of note, NC2 FIV virions incorporate viral genomic RNA at levels representing 51 ± 5% (mean ± SD) of those of wild-type FIV. Our studies demonstrate that an FIV carrying the SIV NC-SP2 module in its Gag protein assembles into virions and packages FIV genomic RNA. These results provide relevant information regarding the functional homology between the NC domains of primate and non-primate lentiviruses.

A48
LOCALIZATION AND TRANSCRIPTIONAL ACTIVITY OF THE ANDROGEN RECEPTOR AND THE INFLUENCE OF NOTCH RECEPTORS IN PROSTATE CANCER

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Prostate cancer is the second most common cancer among men in Argentina. Testosterone acts through the Androgen Receptor (AR), which is in the cytoplasm in its basal state, and the binding to androgens activates it, promoting its translocation to the nucleus and, consequently, the expression of target genes such as PSA and TMPRSS2. The efficacy of antiandrogens like Enzalutamide (Enz) is insufficient in treatment. There is evidence that Notch signalling may be involved in the development of the prostate gland, but its role in this pathology is not well understood. Previously, we found AR and Notch expression *in vitro* in human PC3 prostate cancer cells, and that the combination of Enz and the Notch activation inhibitor DAPT reduced cell viability ($n = 3$; $p = 0.0043$). The present objective was to evaluate the reciprocal activation of the AR and Notch pathways in androgen-sensitive LNCaP cells. After AR modulation with testosterone (10^{-7} M, 48 h), flow cytometry (FC) showed decreased cytoplasmic AR expression, accompanied by increased expression of *PSA* and *TMPRSS2*, as well as the Notch pathway target gene *HES1*, and a trend towards reduced expression of the tumor suppressor *BTG2*, as assessed by RT-qPCR. Treatment with Enz (50 µM, 48 h) tended to increase AR expression in the cytoplasm and decreased the expression of *PSA*, *TMPRSS2*, and *HES1*; it also showed increased expression of *BTG2*. Interestingly, treatment with DAPT (30 µM, 48 h) increased cytoplasmic AR expression, reduced *PSA*, *TMPRSS2*, and *HES1* expression, and increased *BTG2* expression. Combined modulation with testosterone and DAPT revealed an interrelationship between the two signals, with predominance of AR activation, as evidenced by an increase in *PSA* and *TMPRSS2* expression, and a positive correlation between *HES1* and *TMPRSS2* expression ($R^2 = 0.47$; $p = 0.06$), as well as a negative and significant correlation between *PSA* and *BTG2* expression ($R^2 = 0.65$; $p = 0.02$). Our results show that Androgen Receptor activation through its target messengers correlates with Notch activation and suggest an interconnection

between the two signalling pathways in prostate cancer, which may involve interaction between the intracellular domain of Notch and AR.

A49
FORMULATION AND OPTIMIZATION OF A KOMBUCHA-LIKE BEVERAGE USING GREEN TEA AND CANNABIS INFUSIONS: IMPACT ON ANTIOXIDANT AND CANNABINOID CONTENT

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Fermentation is a method used to preserve both the nutritional value and quality of food. In recent years, the consumption of herbal probiotic beverages, such as kombucha, has increased due to their beneficial health effects, particularly as they are significant sources of bioactive antioxidant compounds. This study focuses on formulating and optimizing a fermented kombucha-like drink using green tea, cannabis tea, and their combinations. To achieve this, infusions were prepared from commercial green tea and two varieties of cannabis: (1) THC-dominant and (2) CBD-dominant strains. Each infusion was made by steeping 8 g of plant material in 1 liter of water for 20 minutes. Five types of infusions were prepared: green tea (GT), green tea with CBD (1:1), CBD, green tea with THC (1:1), and THC. After filtration, the infusions were transferred to 1.5 L jars, and sugar was added at a ratio of 100 g/L. Once the infusions reached room temperature, a kombucha starter culture (SCOBY), which is a cellulose-gelatinous mass composed of a symbiotic bacterial-fungal consortium, was introduced to initiate the fermentation process. Fermentation was conducted in an open system, without agitation, at 25ºC for 7 days. Throughout this process, the pH was monitored daily, and antioxidant capacity, flavonol, and polyphenol levels were measured at both the beginning and end of the fermentation. Additionally, cannabinoid levels were measured in the CBD and THC infusion fermentations. The initial pH values ranged from 3.87 to 5.59, while the final pH values dropped to between 3.40 and 2.73. The results indicated that the antioxidant capacity of the cannabis-infused teas was significantly lower than that of green tea and its combinations, with a decline observed across all samples by the end of fermentation. In contrast, flavonol content increased proportionally in all infusions, suggesting that the fermentation process encourages their production. The rise in polyphenol content was mainly attributed to the presence of green tea, as cannabis-infused teas did not contribute significant amounts. Furthermore, cannabinoids measured at the end of the fermentation process did not show detectable levels of THC or CBD.
In conclusion, this study demonstrates that while cannabis tea can complement green tea in kombucha production, its contribution to the overall functional properties is minimal.

A50
IN SILICO ANALYSIS OF THE EFFECT OF PHOSPHOLIPASES A_2 ISOLATED FROM *BOTHROPS DIPORUS* VENOM ON THE INHIBITION OF METASTATIC AND ANGIOGENIC POTENTIAL

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Snake venom contains proteins and polypeptides, with phospholipases A_2 (PLA_2s) being one of the most abundant components with pharmacological potential. Previous *in vitro* assays have demonstrated that two basic PLA_2 isoforms isolated from *Bothrops diporus* venom exhibit potential antimetastatic and antiangiogenic effects. The objective of this study was to evaluate, through an *in silico* approach, the potential mechanisms of action of these toxins in inhibiting tumor processes. To model the possible interactions between PLA_2s and integrins, the partial sequences of PLA_2-I and PLA_2-II were completed using the BLAST tool for homologous sequence searches and multiple alignments. Additionally, a reduced model of the αVβ3 integrin, comprising the ectodomain obtained from the Protein Data Bank, was used. For modeling protein-protein interactions, AlphaFold-Multimer (AF-Multimer) and its ColabFold version in Google Colab were employed, using the sequences of the integrin and both PLA_2s as input. Confidence in intra-chain predictions was assessed with the local distance difference test, while inter-chain arrays were evaluated using the prediction alignment error. Furthermore, PLA_2-integrin interaction models were generated with the ClusPro server by performing blind couplings on the model surfaces predicted by AF-Multimer. The 30 best models interacting with the integrin RGD site were selected for further analysis through molecular dynamics simulations. AF-Multimer indicated that both PLA_2s bind to the same integrin RGD site with high confidence, although there is less certainty regarding the PLA_2 residues at the interaction interface, potentially due to missing data or non-specific binding. To clarify this, protein-protein docking calculations were performed to predict alternative binding modes. The PLA_2 conformations obtained from ClusPro and AF-Multimer were subjected to molecular dynamics simulations. Only the best conformation of PLA_2-II exhibited a

binding strength that outperformed the tenth domain of fibronectin, with a stability increase of 11 kcal/mol. Basic residues of PLA_2-II, such as Arg 108, interact with the RGD site of integrin, suggesting that it may block fibronectin binding. PLA_2-II demonstrated stronger binding to integrin than PLA_2-I, possibly due to its higher basic character. Electrostatic potential maps indicate complementarity at the interaction interfaces, favoring PLA_2-II binding to αVβ3 integrin, which could be relevant for the inhibition of tumor processes.

A51
EVALUATION OF THE EFFECT OF AQUEOUS EXTRACTS OF YERBA MATE *(Ilex paraguariensis)* ON MURINE MAMMARY TUMOR CELLS

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Ilex paraguariensis (A.St.-Hil. 1822), popularly known as "yerba mate" (YM), is a native species of South America, with its natural distribution limited to Brazil, Paraguay, and Argentina. It is well-known for its antioxidant properties, which protect the body from oxidative damage and contribute to the prevention of various diseases, including cancer. Therefore, the aim of this study was to evaluate the potential cytotoxic effect of aqueous YM extracts on murine mammary tumor cells. The aqueous YM extracts were prepared from commercial "mate cocido" tea bags (Unión brand), by adding 3 g of *I. paraguariensis* to 200 mL of freshly boiled distilled water, allowing it to steep for 5 minutes. The infusion was filtered, lyophilized, and stored at -20°C. Cytotoxicity assays were performed on the LM3 cell line (CVCL_D269) derived from a malignant neoplasm of murine mammary glands. Cells were seeded in 96-well plates (2.5 x 10^4 cells/well) in DMEM-5% FBS medium. Upon reaching 80% confluence, different concentrations of YM extracts (0.05-1.5 mg/mL) were added in the same medium. After 24 h of incubation at 37°C and 5% CO_2, cell viability was assessed using crystal violet staining, compared to the controls (100% viability). In addition, cellular apoptosis was evaluated by detecting DNA fragmentation through hematoxylin/eosin (HE) staining and the TUNEL assay (Terminal deoxynucleotidyl transferase dUTP nick end labeling-POD-ROCHE). LM3 cells, seeded on coverslips (1x10^6 cells/coverslip), were treated with 0.25 mg/mL of YM extract for 24 h. Negative (PBS) and positive (DNase I grade) controls were included. After treatment, the Converter-POD reagent was added, followed by the substrate solution. Sections were observed under light microscopy, and morphological characteristics of apoptosis were evaluated. The apoptotic index (AI) was calculated using the following formula: AI = (Number of TUNEL-positive cells/Total number of cells) × 100. The results first showed an increase in cytoplasmic vacuolization, a morphological indicator of apoptosis, after HE staining. This finding was confirmed by the TUNEL assay results, where the AI reached 20% after treatments, compared to the control group, which exhibited an AI of less than 1%. In conclusion, these results suggest that one of the mechanisms by which YM extract may inhibit tumor cell proliferation is through the induction of apoptosis, which could indicate a potential selective cytotoxic effect on malignant cells. This effect may be related to the presence of antioxidant compounds in the extract. Future studies focusing on the expression of key molecules involved in programmed cell death will help advance this line of research.

A52
CHARACTERIZATION OF A HUMAN CHORIOCARCINOMA (JEG-3) CELL LINE AS A POSSIBLE MODEL FOR INVESTIGATING THE EFFECTS OF CANNABINOIDS ON TUMOR BIOLOGY

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The best-known and main pharmacologically active constituents of the *Cannabis sativa* plant (phytocannabinoids) are tetrahydrocannabinol (THC) and cannabidiol (CBD). There is huge evidence regarding the antiproliferative, proapoptotic and antimigratory effects of phytocannabinoids in both *in vitro* and *in vivo* tumour models. The human choriocarcinoma tumour line (JEG-3) is characterized by high proliferation and invasiveness rates. The aim of our work was to study the effects of CBD and a high CBD extract (CBD-HSC / CBD:THC ratio = 20:1) on the JEG-3 line. To carry out the study, the following were carried out: 1- Cell viability assay (MTT), to determine the IC50 and to be able to establish the effect on cell viability/death according to the different concentrations of CBD and CBD-HSC. Above 7.5 µM both compounds showed a proapoptotic effect. 2- Cell migration assay in three different culture conditions: a) control, b) CBD 1 µM and c) CBD-HSC 1 µM, for 24 hours. For this, the percentage of regenerated area after 24 hours was measured by digital morphometry, resulting in (60.30 ± 5.50) %; (39.90 ± 3.90) %; (41.60 ± 3.60) % for the control, CBD 1 µM and CBD-HSC 1 µM, respectively. 3- Immunocytochemistry, using Ac. Anti-Ki-67 (proliferation), Cas-3 (apoptosis) and MMP-9 (metalloproteinase) monoclonal antibodies were used to analyze the results observed in the migration assays. No significant changes were observed in the expression of Cas-3, but a significant decrease in cell proliferation (Ki67) and MMP-9 expression was observed when cannabinoid treatments and control were compared. 4- RT-PCR with primer sets for the endocannabinoid system receptors CB1,

CB2 and GPR55. Using this procedure, the expression of CB1 and CB2 could be determined, being negative for GPR55. For images capturing we used the Micrometrics LE program (NY-USA) and to analyze them, ImageJ (NIH-USA) software was used. The statistical analysis was performed using GraphPad Prism 8.0.2.263. Based on the results obtained, we demonstrated that the JEG-3 tumor line expresses CB1 and CB2 receptors, through which the cannabinoids supplied (1µM) would be exerting their effects. In agreement with other tumor lines, JEG-3 showed a decrease in cell proliferation and MMP-9 expression in response to CBD and CBD-HSC, which makes them an attractive model for the study of tumor biology associated with the endocannabinoid system.

A53
MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF HUMAN MAP KINASE PHOSPHATASES 3 (MKP-3) ISOFORMS

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MAP kinase phosphatases (MKP) constitute a regulatory network that controls the duration, intensity and spatiotemporal patterns of MAP kinase (MAPK) activities in response to various physiological and pathological signals. MKP-3 (or DUSP6) is a MKP induced mainly by proliferative stimuli and its most characterized substrate is phospho-ERK. The human MKP-3 gene generates the complete transcript (MKP-3L) and an alternative spliced product (MKP-3S). Our objective is to structurally and functionally characterize the MKP-3 isoforms using HEK293 cell model. Bioinformatic studies revealed significant differences between MKP-3 isoforms that would affect their functions and interactions. We showed, by immunocytochemical and Western blot analyses, that MKP-3L localization is exclusively cytoplasmic. In contrast, MKP-3S is distributed in both nuclear and cytoplasmic compartments. The results of the structural analysis of MKP-3S indicate a reduction in enzymatic activity and alterations in regulation in comparison to MKP-3L. Our results indicate that MKP-3S is unable to dephosphorylate ERK in HEK293 cells overexpressing this variant, compared to control cells. Also, we studied the role of MKP-3 isoforms on another described substrate, FOXO1. Fluorescence resonance energy transfer (FRET) analyses revealed that both isoforms are able to interact with the transcription factor FOXO1. By immunofluorescence analyses, we detected that MKP-3L, but not MKP-3S, is able to promote nuclear translocation of FOXO1. This differential regulation is related to the dephosphorylation ability of MKP-3L isoform, which promotes nuclear localization of FOXO1. Since p21 (an inhibitor of cyclin-dependent kinase) transcription is dependent on FOXO1 activation, we studied the effect of MKP-3 isoforms overexpression on p21 mRNA levels by qPCR. MKP-3L overexpression increases p21 mRNA levels compared to control cells. However, MKP-3S overexpression significantly decreased p21 expression, indicating that FOXO1 transcriptional activity is differentially modulated by each MKP-3 isoform. The results presented show the differences between MKP-3S and L in terms of both structural and functional characteristics, emphasizing the role of isoforms in cell signaling and transcriptional regulation.

A54
THREE-DIMENSIONAL MODEL OF MAMMARY ACINI FOR THE STUDY OF BIOACTIVE COMPOUNDS FROM TANNAT GRAPE

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According to Global Cancer Observatory, Breast Cancer (BCa) is the second most common neoplasm where the age of the patients is a prominent risk factor for aggressive BCa. Lifetime exposure to hyperglycemia, dicarbonyl stress, together with lifestyle promote advanced glycation end-products (AGEs) accumulation in multiple tissues, including breast. AGEs are the result of a non-enzymatic reaction between a reducing sugar and an amino group. During breast tissue aging collagen-IV is modified by advanced glycation end-products (AGEs), inducing the loss of regulatory functions of the basement membrane (BM) that surround glandular acini. These alterations in the BM generate aberrant mechano-signals that modify cell-cell and cell-BM adhesions, leading to cell proliferation and migration. To better understand how aging switches on or exacerbates BCa progression, we employed a reductionist noise-free model of age-dependent BM stiffness, caused by AGEs accumulation and non-enzymatic crosslinking of its major constituent collagen IV. MCF10A non-tumoral breast epithelial cells were seeded on this BM and on untreated BM as a control, and the formation of mammary acini in these 3D cultures were studied. Microscopy analysis revealed that acini circularity was affected by AGEs-crosslinked BM when compared with native 3D cultures, which also was reported for prostate acini. Moreover, we demonstrated that the addition to the culture media of the phenolic fraction obtained from Tannat grape skin (TGS) by hydroalcoholic-acid extraction, significantly restored acini circularity altered by the AGEs-crosslinked BM ($p < 0.05$). These results suggest that AGEs-crosslinking BM found in aged breast tissue

modified acini architecture and the phenolic fraction of TGS might protect from the impact of tissue aging and beginning of BCa. The mechanisms underlying this phenotypic restoration remain to be elucidated.

BIOCHEMISTRY, PHYSIOLOGY AND NEUROSCIENCES

A55
EFFECTS OF EARLY STEVIA CONSUMPTION ON BEHAVIOR ASSOCIATED WITH THE DOPAMINERGIC SYSTEM

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In recent years, childhood obesity has grown rapidly in Argentina, following the global trend. This phenomenon is associated with an increase in type 2 diabetes, hypertension and psychosocial problems. The main source of excess calories comes from sugary drinks that are widely available and at low cost. Therefore, artificially sweetened beverages have emerged as an alternative to provide sweet taste with few or no calories. Despite their increasing use, little is known about the impact of sweetener consumption, especially in children. Here we studied the effect of unlimited consumption of a decoction of Stevia Rebaudiana Bertoni (Stv) diluted in drinking water (4% V/V) in juvenile Sprague Dawley rats. Animals were exposed to Stv and water, or only water (control) during postnatal days 25 and 60. Motor coordination, balance and motor learning were assessed using the rota rod test. The coordination of the forelimbs and hindlimbs was also analyzed by measuring the performance of the rats on the horizontal ladder. While no differences were found between the Stv and control groups on the rota rod, differences were detected on the horizontal ladder: animals in the Stv group presented a greater number of deficits in both limbs, evidenced by an increase in the number of slips mainly of the hind legs when crossing the ladder (t-test, $p<0.01$). To explore the involvement of the dopaminergic system, the hedonic response of the animals to a 2% sucrose solution and the expression of the dopamine transporter (DAT) in the nucleus accumbens (nAcc) and the medial prefrontal cortex (mPFC) were studied. Animals in the Stv group presented anhedonia (insensitivity to reward, $p<0.05$) and a lower expression of the DAT in the mPFC, without significant changes being recorded in the nAcc. Taken together, these results demonstrate that early and chronic consumption of stevia produces alterations in the mesolimbic dopaminergic system and behaviors associated with this system. PICT-1695/2020.

A56
GENISTEIN (GEN) AMELIORATES NEUROINFLAMMATION AND COGNITIVE DEFICIT IN A RAT MODEL FOR METABOLIC SYNDROME (MS)

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MS is the medical term for the combination of at least three of the following metabolic risk factors: hyperlipidemia, hyperglycemia, insulin resistance, hypertension and central obesity. Our model, the spontaneous hypertensive rat (SHR) is an accepted model for the study of human MS that acquires all the features of the syndrome when fed high-fat and high-carbohydrate diets. In previous studies we have found increased reactivity of GFAP+ astrocytes and IBA1+ microglia in several brain regions, decreased neurogenesis in the hippocampus and decreased cognitive capabilities evidenced by several memory tests.
GEN is a phytoestrogen found in soybeans with known neuroprotective actions for several neurodegenerative diseases. This compound binds to several estrogenic receptors such as the classic receptors (ERα and ERβ) and the membrane receptor GPER without producing the carcinogenic and feminizing effects associated with classic estrogen hormones. We have previously found changes in the phenotypic distribution of microglias present in both the hippocampus and the prefrontal cortex of animals with MS, where a hypertrophic or activated phenotype was much more prevalent in animals with MS when compared to metabolically healthy animals. This phenotypic distribution is reverted when these animals are injected with a dose of 10mg/kg of GEN subcutaneously for 2 weeks.
In this study, we studied these IBA1+ microglia through fluorescent immunohistochemistry. We colocalized IBA1+ microglia with a proinflammatory microglia marker (TNFα) and with an anti-inflammatory microglia marker (Arg-1) in two different regions of the hippocampus (CA1 and the DG). We found an increased rate of TNFα+ microglia in the hippocampus of SM rats when compared to

metabolically healthy WKY rats which was reverted with GEN treatment. At the same time, we observed a decreased rate of Arg-1+ microglia in MS rats that increased with the same treatment.
Also, we continued characterizing the cognitive deficit in SM rats with a discontinuous YMAZE (d-YMAZE) and continue to analyze mRNA expression of pro and anti-inflammatory cytokines and transcription factors present in the hippocampus of these animals through RT-PCR.
Our results show that GEN has neuroprotective and anti-inflammatory effects for MS-associated encephalopathy. These results open an interesting possibility for proposing a neuroprotective therapy for MS based on this phytoestrogen.

A57
EFFECTS OF MODULATION OF MINERALOCORTICOID RECEPTOR (MR) IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) MICE

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Literature reports show evidence that the MR plays a role in innate and adaptive immune responses. The inflammatory profile is directly linked to MR activation not only in cardiovascular diseases but also in other pathologies, such as autoimmunity, chronic renal disease, and obesity. MR and GR bind corticosterone although with different affinity. In this study we explored if the pharmacological modulation of MR with the agonist deoxycorticosterone (DOCA) and the antagonist spironolactone (SPIRO) influences plasma corticosterone levels, neuroinflammation and neurodegeneration in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE) model of Multiple Sclerosis. Animals were treated from day 1 until sacrificed on day 17 post-induction and experimental groups were divided in: EAE+DOCA (0.75mg/kg s.c every 3 days), EAE+DOCA+SPIRO (Spironolactone: 25 mg/kg i.p daily injections), vehicle-treated EAE (EAE+VEH) and Control (CTRL). The MR antagonist (a) significantly decreased inflammatory parameters TLR4 ($p<0.05$), IL-1β ($p<0.01$) and microglial CD11b ($p<0.05$) mRNAs, the % of infiltrated area ($p<0.01$) and reactive gliosis (number of GFAP+ and IBA1+ cells ($p<0.05$ for both) vs EAE+DOCA (b) increased the neuronal marker NeuN+ area ($p<0.05$ vs EAE+DOCA and EAE+VEH) (c) improved functional performance in the rotarod test ($p<0.01$) and clinical signs ($p<0.05$) vs EAE+DOCA. Interestingly, plasma corticosterone was increased in EAE+VEH and EAE+DOCA vs CTRL ($p<0.05$) while SPIRO administration raised even more corticosterone levels vs EAE+DOCA ($p<0.05$). We hypothesized that MR blockage with SPIRO downregulated inflammation-related spinal cord pathology, whereas anti-inflammatory effects found in the EAE+DOCA+SPIRO group may be due to excess glucocorticoid exposure.

A58
ACTION OF *STEVIA REBAUDIANA* OVERCONSUMPTION ON REPRODUCTIVE INTEREST AND ANXIETY-LIKE BEHAVIOR

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Recently, sucrose in food and beverages has been replaced by non-caloric sweeteners. Among them is stevia, derived from the plant *Stevia rebaudiana* Bertoni (Asteraceae), which is native to the tropical region of South America. Although there is controversy surrounding its consumption, several studies suggest that stevia affects reproductive capacity, while others highlight its beneficial properties. In previous studies, we observed that stevia administration in female rats (F) induces changes in the estrous cycle, pregnancy capacity, litter size, and offspring survival. The aim of this study was to assess whether this sweetener affects reproductive interest and anxiety-like behavior. Female rats (SD) received water (CON group, n=8) or stevia-sweetened water (STE group, n=8) starting from PND21. Between PND 74-82, the reproductive interest test (RIT) and the elevated plus maze (EPM) were conducted. Activities were recorded, and the videos were analyzed using the ANY-Maze© software. During the RIT, the female rat (in proestrus) was placed for 10 minutes in a T-shaped device where, at each end and behind a metal mesh, there was an active male, a proestrus female, and an ovariectomized female. The percentage of preference (%P) was calculated as the time spent with the male divided by the total time spent in proximity to each stimulus animal. The EPM consisted of placing the female rat (in diestrus) for 5 minutes on an elevated cross-shaped maze with two open arms (OA) converging at a central zone (C) and two closed arms (CA). The number of entries (E), time spent (T), and distance traveled (D) in each zone were measured, along with grooming (G) and rearing (R) activities and risk-taking behavior (RTB). The STE group showed a reduced %P compared to CON ($p=0.0343$). In the CA of EPM, the STE group had fewer E (23.24%; $p=0.0410$), T (18.23%; $p=0.0485$), and D (24.83%; $p=0.0142$) than the CON group. No differences were observed in G and R. The STE group performed RTB more frequently than CON (109.71%; $p=0.0357$). These results indicate that stevia overconsumption in female rats may reduce reproductive interest, increase risk-taking behavior, and exert an anxiolytic effect. We consider these findings relevant for medical recommendations regarding *S. rebaudiana* consumption (PICT2019-623).

A59

STUDY OF THE TRANSCRIPTOMIC RESPONSE AFTER BLOOD INGESTION IN *RHODNIUS PROLIXUS*, VECTOR OF CHAGAS DISEASE

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Rhodnius prolixus is an obligate hematophagous insect capable of ingesting up to 10 times its body weight in blood within a short period of time. This process activates essential mechanisms to maintain homeostasis, affecting the ion and fluid composition in the hemolymph due to the rapid consumption of large volumes of blood. This phenomenon induces osmotic stress, necessitating a fast diuretic and antidiuretic response. These insects act as vectors of the parasite *Trypanosoma cruzi*, the causative agent of Chagas disease, through the deposition of feces/urine, which is eliminated after feeding. Thus, understanding diuresis as a key link in the transmission of this disease, and studying the factors involved in its regulation, is crucial for grasping this vital process. In this work, we present a transcriptomic study that investigates differential gene expression 13 hours after blood ingestion in various tissues of the insect. Fifth-instar nymphs of *R. prolixus* were fed, and the tissues of interest: anterior intestine, Malpighian tubules, and nervous system were dissected. Total RNA was subsequently extracted, and 24 libraries (n=4 for each condition/tissue) were sequenced, generating 150 bp reads using Illumina sequencing technology. The quality and completeness of the obtained data were satisfactory. Differential expression analysis revealed that, 13 hours post-feeding, over a hundred transcripts showed changes in their expression in the studied tissues compared to the control group (unfed). We observed that differentially expressed transcripts were related to the neuroendocrine system, which releases biogenic amines and neuropeptides acting as diuretic or antidiuretic hormones. These hormones interact with various G protein-coupled receptors linked to second messenger systems, influencing ion transporters and aquaporins, thereby regulating fluid secretion. Many findings from this study have been previously reported, supporting hypotheses about the role of these genes in feeding, diuresis, and excretion mechanisms. This research extends physiological and molecular knowledge, enabling better identification and understanding of the molecules that regulate diuresis and excretion. Moreover, it offers a promising source of targets for controlling other harmful species.

A60
GLIAL REACTIVITY IN THE VENTRAL HORN OF FEMALE NFR/WR MICE: A MODEL OF GENETIC SUSCEPTIBILITY TO MOTONEURON DEGENERATION

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motoneurons leading to significant disability. ALS is more prevalent in males, while females usually experience onset after menopause indicating a role of sex steroids in ALS susceptibility. The Wobbler (wr/wr) mouse is a well-established model for ALS. The mutation in the wr gene affects the vesicular protein sorting (Vps) 54 transport protein resulting in motoneuron disease in homozygous (wr/wr) mice. In contrast, heterozygous mice (NFR/wr) display a healthy phenotype. Here, we analyse if NFR/wr heterozygous female mice develop signs associated to neurodegeneration. Previous data showed that Wobbler glial cells present decreased glutamine synthetase (GS) immunoreactivity (IR) in the spinal cord. GS is necessary to detoxify glutamate into glutamine. To explore if this abnormality persisted in NFR/wr mice, we analyzed the number of glial fibrillary acidic protein (GFAP) + astrocytes and GS+ cells/unit area in the ventral horn. In 12-month-old NFR/wr mice, we found a 2-fold increase in the number of GFAP+ cells/area with a reduction of GS-IR cells/area ($p<0.05$ vs NFR/NFR). GS was also quantified in motor cortex (M1), internal capsule, hippocampus (CA1 and dentate gyrus-DG). However, a slight reduction was found only in the internal capsule. We also studied the motor performance on the accelerating rotarod and activity in the open field. We found that 12-month-old NFR/NFR mice showed better performance than NFR/wr ($p<0.05$) in the rotarod test. Similarly, activity in the open field was higher in 12-month-old NFR/NFR. Four-month-old female NFR/wr mice showed a significant increase of GFAP-IR cells/area with similar values of GS-IR cells/area in the ventral horn, but this change was associated to a better performance ($p<0.01$) in the rotarod. Our data support an age-dependent genetic susceptibility for a decreased GS-IR associated to GFAP+ astrogliosis- These changes occurred at both ages in female NFR/wr suggesting that mutation of a single allele could have pathological and behavioural consequences.

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A61
COMPARATIVE STUDY OF THE EFFECTS OF SPERM ENERGY RESTRICTION AND RECOVERY (SER) TREATMENT ON BOVINE AND EQUINE CRYOPRESERVED SPERM

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In vitro embryo production in farm animals has been progressively increasing in recent years. While conventional in vitro fertilization (IVF) is widely used in the cattle industry, this technique remains inefficient for equine species due to inadequate sperm capacitation in vitro. Conversely, intracytoplasmic sperm injection (ICSI) is effectively used in horses but is less optimal in bovine species. Developing new treatments to enhance reproductive biotechnologies such as IVF and ICSI, and to improve embryo quality, is crucial for both industries. Previous results from our group demonstrated that sperm energy restriction and recovery (SER) treatment improved sperm function, fertilization, and embryo development rates after IVF in mice, and enhanced fertilization and embryo development after ICSI in bovines. In this study, we compared the effects of starvation and recovery on sperm function and capacitation-related events in bovine and equine sperm. Cryopreserved sperm were incubated in the absence of pyruvate and lactate (bovine) or in the absence of pyruvate, lactate, and glucose (equine) (Starved-ST), followed by recovery in the respective complete medium (SER). We observed that equine sperm motility ceased after approximately 20 minutes of starvation, with total (TM%) and progressive motility (PM%) restored to control values (capacitating medium) upon reintroduction of energy sources. This recovery was accompanied by increased curvilinear velocity, lateral head displacement, and decreased straightness as measured by CASA. In contrast, bovine sperm continued moving for at least 240 minutes under starvation conditions, with TM% and PM% increasing after recovery at 60 minutes. Starvation reduced mitochondrial membrane potential and increased intracellular Ca^{2+} levels in equine sperm, while no effects were observed in bovine spermatozoa. Additionally, equine sperm showed a higher percentage of live acrosome-reacted sperm and better sperm viability compared to control, while no changes in acrosome integrity were noted in bovine sperm. No differences were observed in substrates phosphorylated by PKA and tyrosine phosphorylation in either species. These results indicate species-dependent differences in sperm behavior under energy restriction and recovery and suggest that bovine and equine sperm utilize different metabolic pathways to induce and maintain sperm function and capacitation associated events. Further research will focus on elucidating these differences, specifically the alternative metabolic pathways used by bovine sperm under starvation conditions, and the effect of SER treatment on improving IVF and ICSI outcomes in both species.

A62
EPIGENETIC REGULATION: EXPRESSION OF DNMT3A AND TET1 IN VITRIFIED IN VITRO PRODUCED BOVINE EMBRYOS

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Cryopreservation can influence the epigenetic regulation of the embryo, altering gene expression and impacting its quality of the embryo. Correct DNA methylation is an essential epigenetic modification for normal embryonic development. In bovines, DNMT3A and 3B are involved in de novo methylation processes during embryonic development, particularly from the morula to the blastocyst stage. On the other hand, TET proteins mediate DNA demethylation, mainly TET1, which is established in stages after of the embryonic genome activation This indicates active DNA that functions to promote the pluripotency of the inner cell mass in blastocysts. This study aimed was to characterize the effect of vitrification on the regulatory mechanisms involved in the epigenetic reprogramming of *in vitro* produced embryos. *In vitro* produced embryos were obtained from *in vitro* matured oocytes from ovaries sourced from a local slaughterhouse. *In vitro* fertilization was performed using frozen semen straws. To evaluate the expression of *dnmt3a* and *tet1* genes, day 5 (16-cell) and day 7 (blastocyst) embryos were stored in lysis buffer at -80°C for further study. Additionally, following the laboratory's protocol using the Cryotop device (Kitazato®) as support, blastocyst stage embryos were vitrified. After warming, embryos were recovered at 38,5°C, 5% CO_2, and 5% O_2 for 3 hours. Their re-expansion was assessed and stored in lysis buffer at -80°C. Total RNA extraction from embryos was performed using a specific kit for the extraction of few a cells (Pico-Pure®), and cDNA was synthesized using a kit (Roche). Through quantitative PCR with SYBR Green (amplification efficiency R2>0.99), the relative expression of *dnmt3a* and *tet1* genes was evaluated using *gapdh* as the endogenous gene. The *dnmt3a* gene of interest, normalized to the endogenous gene at day 7, showed overexpression compared to the group at day 5, while the *tet1* gene showed a decrease in expression. This same pattern was observed in vitrified blastocysts compared to fresh ones. These results indicate an increase in mechanisms associated with *de novo* methylation and a concomitant decrease in mechanism associated with demethylation during blastocyst stage development. Furthermore, vitrification was found to affect the methylation status in blastocysts by the expression of methylation and demethylation regulatory genes such as *dnmt3a* and *tet1*. The expression of these protein-coding genes can be proposed as markers of the effects of vitrification on *in vitro*-produced embryos.

A63
NOVEL ROLES FOR "CATSPER" CHANNEL IN CUMULUS PENETRATION AND GAMETE FUSION DURING FERTILIZATION

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CatSper (Cation channel of Sperm) is widely recognized as the principal Ca^{2+} channel in mammalian sperm, pivotal for the initiation and maintenance of hyperactivation, a vigorous type of sperm motility essential for enabling sperm to reach the ampulla and penetrate the zona pellucida (ZP) that surrounds the egg. The relevance of CatSper is further highlighted by the finding that deletions or mutations in this channel lead to male infertility in both mice and humans. Whereas the role of CatSper in ZP penetration is well established, its relevance in other stages of fertilization remains unclear. To address this gap, cumulus-oocyte complexes (COC), ZP-intact and ZP-free eggs were incubated *in vitro* with capacitated control and CatSper knockout (KO) sperm and the percentage of fertilization analyzed by staining the eggs with Hoechst. As already reported, we observed a complete failure in fertilization in both COC and ZP intact eggs that could not be overcome by softening the ZP with glutathione (GSH), indicating the severe effect of the lack of CatSper on sperm hyperactivation development. In order to evaluate the role of CatSper in cumulus penetration, COC were exposed to capacitated control or mutant sperm previously stained with Hoechst, and the number of sperm within the cumulus mass was determined 15 minutes later. In this case, results revealed a significant decrease in the ability of mutant sperm to penetrate the cumulus matrix compared to controls, revealing the relevance of CatSper for cumulus penetration. Notably, when the potential involvement of CatSper in gamete fusion was analyzed by *in vitro* fertilization of ZP-free eggs, we observed a severe to a complete decrease in fertilization depending on sperm concentration. These sperm-egg fusion defects could not be attributed to a failure in either the spontaneous or the induced acrosome reaction nor in Izumo1 relocalization known to be needed for gamete fusion, as no differences in these parameters between groups were observed. Taken together, our results show, for the first time, that CatSper is involved not only in ZP penetration but also in those stages preceding (i.e. cumulus penetration) and following (i.e gamete fusion) sperm-ZP interaction during fertilization. Whereas the lack of hyperactivation may explain failure in cumulus penetration by mutant sperm, the fact that hyperactivation is not required for gamete fusion supports the existence of additional capacitation-associated defects in CatSper KO sperm.

A64
EFFECT OF TROLOX AND/OR RESVERATROL IN VITRIFICATION-WARMING ON PORCINE OOCYTE NUCLEAR AND CYTOPLASMIC RECOVERY

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Reproductive biotechnologies in the porcine species are essential to improve reproductive efficiency and to develop biomedical research models. Oocyte vitrification could be essential for germplasm conservation, although it is not yet fully developed in this species. The aim of this research was to evaluate the effect of the antioxidants trolox and/or resveratrol supplementation in the vitrification and warming of mature porcine oocytes on their nuclear and cytoplasmic recovery. Cumulus-oocyte complexes recovered from slaughtered ovaries were matured *in vitro* in medium 199 at 39°C for 44 h and then oocytes were denuded with hyaluronidase and vitrified-warmed (V/W) by the Cryotech method. To evaluate the effect of the antioxidants, vitrification and warming solutions (control) were supplemented with resveratrol and/or trolox at 2 µM and 50 µM concentrations, respectively. After V/W oocytes were cultured for 3 h to allow their recovery. To evaluate nuclear recovery, a group of oocytes were stained with Hoechst 33342 (10 mg/l) to prove the presence of metaphase II (MII). To evaluate cytoplasmic recovery, a group of oocytes were inseminated with $5x10^5$ spermatozoa/ml for 3.5 h and then stained with Hoechst 33342 to observe fecundation at 18 h (decondensed sperm heads and/or pronuclei formation). Matured oocytes without V/W (fresh) were used as positive controls. Data were compared using a Chi-square analysis for non-parametric data, considering a value of $p<0.05$ as significantly different. The number of oocytes with MII was significantly lower in those groups that were V/W compared to fresh oocytes ($p<0.05$). Besides, in the group of oocytes that was V/W simultaneously with trolox and resveratrol MII recovery was significantly lower compared to the rest of the V/W oocyte groups ($p<0.05$). Regarding oocytes ability to be fertilized, no significant differences were found between any of the treatments studied. However, a higher polyspermy rate was found in the group of oocytes V/W with the combination of both antioxidants compared to the rest of the groups ($p<0.05$). So, the vitrification and warming of mature porcine oocytes would produce damage at the meiotic plate level that impede its complete nuclear recovery after the cryopreservation process, and the use of trolox and/or resveratrol would not mitigate this damage. Oocyte cytoplasmic recovery would not be affected in the early stages of the fertilization process by the vitrification and warming and the combination of both antioxidants in the vitrification and warming solutions would have an adverse effect compared to both compounds separately.

A65
EFFECT OF CHOLESTEROL ADDITION ON THE MEMBRANE LIPID ORDER AND THE VOLUME OF OSMOTICALLY ACTIVE WATER IN BOVINE OOCYTES

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Exposure of oocytes to anisotonic solutions during cryopreservation induces osmotic stress and abrupt changes in cellular volume. Additionally, temperature decrease leads to alterations in the cell membrane as a result of changes in its fluidity. Considering the role of cholesterol as a modulator of membrane lipid order, we modified the cholesterol level of bovine oocytes to enhance their resistant to cryopreservation. This study aimed to evaluate the biophysical state of the cell membrane in cholesterol-loaded (+chol) and non-loaded (ctr) oocytes, and determine whether these variations affect cellular volume and the volume of osmotically active water in the oocyte. Bovine *cumulus*-oocyte complexes obtained from slaughterhouse ovaries were loaded with cholesterol at the end of *in vitro* maturation for 45 min using 15 mM methyl-β-cyclodextrin/cholesterol. The biophysical state of the membrane was assessed post-maturation at decreasing temperatures (38.5°C to -15°C) through fluorescence spectroscopy using the probe Laurdan. Mathematical modeling of the osmotic response and exposure to solutions of known osmolality, in the absence of permeable solutes (Boyle-van't Hoff assays), were conducted to calculate the volume of osmotically active water in the oocyte from images recorded under initial and final conditions (10 min). Generalized polarization profiles of Laurdan indicated that +chol bovine oocytes exhibit a more fluid membrane compared to ctr at physiological temperature. In the temperature range of 20°C to -5°C, +chol oocytes showed a more ordered membrane state, whereas between -5°C and -15°C, this effect was reversed, revealing a more fluid state than that of the ctr group. Under isotonic conditions, no significant differences in oocyte volume were observed between the two groups (ctr: $V^0 = 0.70 \pm 0.05$ nL; +chol: $V^0 = 0.68 \pm 0.06$ nL; $p = 0.54$). The fraction of inactive cellular volume (b) was determined through exposure to various solutions of known osmolality (hypo-, iso-, and hyperosmotic). These estimates enabled the calculation of the volume of osmotically active water ($W = V^0 (1 – b)$). The addition of cholesterol did not significantly affect the W of the oocyte (ctr: $W = 0.46 \pm 0.06$ nL; +chol: $W = 0.48 \pm 0.07$ nL; $p = 0.61$). Although +chol bovine oocytes exhibit increased membrane fluidity at physiological temperature—an effect that may occur when the plasma membrane composition is originally highly ordered—this did not influence cell volume under isotonic conditions or the volume of osmotically active water in the oocytes. Furthermore, the incorporation of cholesterol increased membrane fluidity at very low temperatures, an effect aimed at preventing damage caused by cryopreservation.

A66
OVARIAN MITOCHONDRIAL ALTERATIONS IN POLYCYSTIC OVARY SYNDROME

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age, affecting between 5% and 10% of women. This syndrome is characterized by endocrine, metabolic, and reproductive alterations. PCOS is associated with excessive androgen production, amenorrhea or oligomenorrhea, reduced fertility, and hypertrophied polycystic ovaries. It can also be linked to metabolic conditions such as obesity, insulin resistance, or dyslipidemia. The aim of this study was to investigate potential ovarian mitochondrial alterations caused by elevated androgen concentrations. To do so, we used a PCOS model developed in rats and cultured rat granulosa cells. To establish the PCOS model, prepubertal Sprague Dawley rats (21 days old) were administered the androgen dehydroepiandrosterone (DHEA) subcutaneously at a dose of 6 mg/100g of body weight for 15 consecutive days. The control group received the same volume of corn oil. On day 16, the rats were sacrificed, and their ovaries were collected for analysis by Western blot and picrosirius red (PSR) staining. Rat granulosa cells were also isolated and stimulated with the androgen dihydrotestosterone (DHT). The cells were incubated for 24 hours, and proteins were collected for Western blot analysis. In the in vivo PCOS model, we observed a reduction in TOMM20, correlating with a lower number of mitochondria, a decrease in mitofusin 2 and DRP1, indicating mitochondrial dynamics alterations, and a reduction in the deacetylase sirtuin 1. We also found increased collagen fiber deposition in the PCOS ovaries compared to controls. When analyzing these protein levels in cultured granulosa cells stimulated with DHT, no significant differences were observed compared to unstimulated cells. These results suggest mitochondrial alterations in the rat PCOS model. Under the culture conditions used, we did not observe these alterations in granulosa cells incubated under excess androgen conditions. Further studies are needed to analyze the role of mitochondria in the pathophysiology of PCOS and to explore potential therapies aimed at improving mitochondrial function in patients with this syndrome.

BIOTECHNOLOGY AND GENETICS

A67
GENETICALLY EDITED VIABLE EWES BY SMGT AND FTAI

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Gene editing in farm animals has important applications to several animal industries, metabolic efficiency, customizing to the environment, disease resistance, bioreactors. Nevertheless, is in precarious development even with new tools as per edition by zygote microinjection by CRISPR Cas-sgRNA. This is due to the high cost-low efficiency of the usual methods. Zygote CRISPR Cas microinjection averaged 0,33-1,8 genetically modified viable animals (GMVA) even with high mosaicism grade. Conversely with IVF plus sperm mediated gene transfer (SMGT) results in 68,5% GM pig embryos; AI+SMGT gives 74% GM piglets, of 27 born. In this

paper we show GM viable-lambs pursued by fixed time artificial insemination (FTAI)+SMGT. Four ewes (1 control; 3 treated), was hormonally stimulated to accomplish FTAI. Fresh ejaculated semen from a selected ram were co-incubated with commercial *pEGFP N-1* encoding green fluorescent protein (GFP) to inseminate treated group, while fresh no co-incubated sperm were used in control group. Intra Oviductal insemination with 10^5 sperm/ewe, was performed by intra-abdominal laparoscopy at the end of clinic estrous. GFP expression was assessed postpartum, by live trans illumination with 420 nm UV light. To analyze GFP expression in peripheral blood mononuclear cells (PMNC), heparinized blood samples was suspended at RPMI 1640™ medium supplemented with 10% FCS. PMNCs was extracted by pre-layering blood onto Lymphoprep™ (Stem Cell Technologies ™) in a centrifuge tube. Samples were centrifuged 800 x g for 20 minutes at room temperature. The upper plasma layer was discarded without disturb the plasma Lymphoprep interface. PMNC layer was recovered without disturbing erythrocyte/granulocyte pellet, and washed once with medium. Samples of 100 microliters drop was dried at air over slide and stained with May-Grünwald solution for 5 min washed, let dry and analyzed by epifluorescence microscope. At parturition date six lambs were born, four of the three treated sheep (one gave birth to twins) and control ewe also gave birth to twins. Two lambs died within 48 h postpartum one control, one treated group. Four lambs from the group inseminated with treated sperm exhibit GFP expression on surface epidermis, the two lambs born from the ewe inseminated with no-treated sperm did not show fluorescence when trans illuminated with a UV lamp. At adult age, PMNCs was microscopically evaluated from one control and three treated sheep. One control sheep was PMNCs GFP negative, while treated ewes was GFP-PMNCs positive.

A68
FIRST DATA ON THE GENETIC VARIATION OF ARGENTINIAN POPULATIONS OF *ERYTHRODIPLAX ATROTERMINATA*

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The odonate genus *Erythrodiplax* (Brauer) includes 59 species predominantly of Neotropical distribution, and 22 of them have been recorded in Argentina. These species have wide distribution areas and, in particular, *E. melanorubra*, *E. atroterminata*, *E. fusca*, *E. connata*, *E. media*, and *E. nigricans* are common and abundant in various regions. The variability of coloration patterns of these species seems to be influenced by geographic clines, as is the case of *E. atroterminata* which populations present in Argentina a marked morphological variation in the size of the wing-spots, which seems to be influenced by environmental variables. This study aims to determine the genetic variation among populations of this species, using markers of the COI gene. DNA was obtained from the third pair of legs of specimens deposited in the collection of the Laboratory of Biodiversity and Environmental Genetics (BioGeA), from the provinces of Misiones (n=4), Corrientes (n=1) and Buenos Aires (n=2), and *Rhionaeschna* sp. was included as an external group. The protocols used were the Puriprep T kit (Inbio Highway, Tandil) and a kit of magnetic nanoparticles developed at the National University of La Plata. A 677bp fragment of the mitochondrial gene COI was amplified by PCR and sequenced by Sanger technique. Alignment and sequence analysis were performed with the MEGA v.11 and Arlequin v.3.5 programs. Twenty variable sites were found, including 17 transitions and 3 transversions. The AMOVA analysis in the comparison with *Rhionaeschna* resulted in a 35.9% variation, while within the species *E. atroterminata* a 7.69% variation was observed between provinces and 56.4% among individuals from the same province. The results obtained so far suggest that there are no significant differences between the populations analysed, probably due to a constant gene flow that prevents the formation of clusters or subgroups. In this sense, the morphological variations observed between the populations of *Erythrodiplax atroterminata* could be subject to considerable environmental influence.

A69
RELEVANCE OF VAMP721/2 ACTIVITY AND GENE DOSE UNDER CONDITIONS OF RAPID CELL EXPANSION IN *ARABIDOPSIS THALIANA*

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Plant growth and development involves cell division, expansion and differentiation throughout an individual's life with a large impact of biotic and abiotic environmental factors. VAMP721 is an indispensable R-SNARE protein for the last step of vesicle fusion that drives cell expansion and division. While loss of VAMP721 or VAMP722 does not lead to an apparent phenotype in *Arabidopsis thaliana*, the *VAMP721-/- VAMP722 -/-* double mutant genotype is lethal and its deletion shows severe cytokinesis defects in seedlings. The *VAMP721 +/- VAMP722 -/-* and *VAMP721 -/- VAMP722 +/-* mutants show a wild-type appearance under normal growth conditions, but are haploinsufficient in responses to biotic and abiotic stresses. Our results showed that lower gene dosage also altered hypocotyl elongation during growth in darkness (skotomorphogenesis). The slower elongation rate of hypocotyls responds to lower elongation at

the cellular level as hypocotyls possess a fixed number of cells and suggests that under conditions of high demand the gene dose of VAMP721/2 may be a limiting factor. The fusogenic activity of VAMP721/2 depends on its ability to interact with Q-SNAREs in the formation of fusogenic complexes. VAMP721/2 possesses an autoinhibition mechanism by the folding of its N-terminal longin domain which blocks its interaction with other SNAREs. The degree of autoinhibition is regulated by phosphorylation of the Y57 residue in the longin domain. Using different mutants of this residue we have observed that the lack of phosphorylation prevents the correct elongation of the hypocotyls, reflected in a slower elongation rate of the hypocotyls. Similarly, germination speed is affected. Our results show, broadly speaking, that both gene dosage and activation of VAMP721/2 by phosphorylation are important factors for hypocotyl elongation, a process that requires high secretion.

A70
DNA-BASED HAIR COLOR PREDICTION APPLYING MACHINE LEARNING TECHNIQUES

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Research on the human genome made it possible to identify genes involved in the determination of externally visible characteristics (EVC) such as eye, hair, skin color, and facial features of a person. It is possible to predict these EVCs by analyzing certain SNPs (single nucleotide polymorphisms), being of important application in Forensic, Health Sciences, and Biometrics. However, the accuracy of the predictions decreases when populations of mixed ancestry are analyzed. In this work we propose to improve hair color predictions by using models of Machine Learning (ML) on a genetic dataset from the province of Buenos Aires, Argentina. The dataset comes from 214 Buenos Aires residents aged between 18 and 58 years old. Thirty-eight SNPs of the MC1R, OCA2, ASIP, SLC45A2, TYR, TYRP1, and PIGU genes were evaluated. Hair color was determined using visual classification: 12 individuals with blond/light hair colors, 121 brown/medium, and 81 black/dark. Additional attributes such as gender, age, and place of birth of the volunteer, parents, and grandparents were added to the dataset. Two ML models were developed based on Extreme Learning Machine (ELM) and Random Forests (RF). The input attributes for the ELM model were SNPs rs1042602, rs1393350, rs1800407, rs28777, rs683, rs1312768986, and rs885479, age, sex, and birthplaces of the donor's grandparents. In turn, for the RF model the number of attributes was reduced - SNPs rs1042602, rs1393350, rs28777, and rs683, age and sex- which is why 30% additional synthetic data was generated using the SDV library based on Generative Artificial Intelligence. The Grid Search technique was applied to obtain the best hyper-parameters of both models, meanwhile, the Cross Validation technique was used to improve the RF model training. An accuracy value of 69% was achieved in the testing phase for the ELM model with an F1-Score between 67% (for the blond/light class), and 72% (brown/medium). Regarding the RF model, an accuracy value of 84% was obtained with an F1-Score between 67% (for blond/light), and 89% (brown/medium). These predictive models are competitive with respect to the current literature. This was possible through data analysis techniques, dimension reduction, hyper-parameter setting, intensive model training, and synthetic data generation.

A71
GFP-EXPRESSING RABBIT EMBRYOS ACHIEVED BY FTAI AND SMGT

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Animal models as bioreactors, or to the study of human diseases, has, in genetically modified (GM) rabbit an important tool. Paths of protein glycosylation are important concern when selecting tissues to produce molecules of pharmacological application in the human model. The rabbit glycosylation pattern is a lower risk model for generating oligosaccharides, associated with the target molecule, which can induce an undesirable human immune response. Moreover, generation gap and litter size could produce more transgenic events in less time interval than farm ruminants. Sperm mediated gene transfer (SMGT) associated with fixed time artificial insemination (FTAI) as natural transfection method, without use of invasive microscopic tools, that could produce cytoskeletal damage, is a key method to increase rates of healthy-viable GM events. Focusing on this we try to expand our expertise in SMGT in ruminants to *Oryctolagus cuniculus*. Spermatic fraction of rabbit seminal plasma is singularly associated with phospholipids (PL), mostly in forms of droplets. PLs induce early changes in spermatic membrane fluidity and sensibility to the exogenous environment. Droplets are similar in size and weight than sperm, thus, centrifugation does not completely separate PL from spermatic cells, this condition conspires against our usual method to membrane stabilization allowing DNA entry into sperm nucleus. To obtain DNA inclusion rates, similar to those observed in ruminant sperm, was necessary add to a washing by centrifugation with buffered saline solution, a PLs removal method. With this aim, sperm/collagenase and time/concentration incubation, were performed. Semen samples, stained with Hoechst 33342, were divided in three groups: control, labeled GFP co-incubated, and treated with collagenase then GFP co-incubated. Samples treated with collagenase showed DNA uptake rate greater than 60%, while untreated samples less than 20%. In five trials, 8 female rabbits were hormonal stimulated and synchronized to ovulate at fixed time. Four of them received vaginal FTAI with sperm treated with collagenase then, GFP co-incubated; other four, control group, with raw semen. 48 h after AI, a surgical ablation of the genital tract was performed to flush oviducts as well as uterine horns and collect embryonic structures (ES). 44 ES were collected then analyzed by fluorescence

microscopy. 29 of them were high quality morulae (15 treated sperm group, 14 from control group). 15 embryos from treated group were GFP positive in all blastomeres. 14 from control group and 15 no fertilized oocytes were negative to fluorescence.

PHARMACOLOGY, TOXICOLOGY AND ECOTOXICOLOGY

A72
EFFECTS OF THE IN-VITRO EXPOSURE TO BENZOPHENONE 2 OR 3 ON LHβ AND FSHβ GENE EXPRESSION IN LβT2 CELLS AND PITUITARY GLANDS

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Benzophenones (BP), endocrine-disrupting chemicals used as UV filters in plastics and personal care products, inhibit kisspeptin-10-induced GnRH gene expression GT1-7 cells. In this study, we evaluated the effects of the exposure to BP2 or BP3 on LHβ/FSHβ gene expression in LβT2 immortalized gonadotrophs and pituitary glands. Methods: LβT2 cells were exposed to BP2, BP3 ($1x10^{-7}$ or $1x10^{-9}$ M; BP2-7, BP2-9, BP3-7, BP3-9) or control (C: DMSO), for 6 or 24h, n=5. Pituitaries from adult male and female C57Bl/6 mice were cut in half, incubated with BP2, BP3 ($1x10^{-9}$M) or medium alone (C) for 8h, n=4. Total mRNA from cells and pituitaries was extracted, reverse-transcribed and gene expression evaluated by qPCR. Results were expressed as Media±SEM and analyzed by Repeated Measures One-way ANOVA or paired t-test. Results: In LβT2 cells, BP3-7 increased FSHβ after 24h treatment [C: 0.9±0.1, BP3-7: 1.3±0.2, BP3-9: 1.0±0.1;$p<0.05$]. BP3-9 decreased LHβ after 24h treatment vs C [C: 0.9±0.1, BP3-7: 1.0±0.1, BP3-9: 0.6±0.1;$p<0.05$]. BP2-7 decreased LHβ vs C after 6h incubation[C: 1.1±0.1, BP2-7: 0.5±0.2, BP2-9: 1.4±0.2; $p<0.05$]. Exposure to the BP2-7 for 24h significantly increased LHβ vs C [C: 0.9±0.1, BP2-7: 1.3±0.1, BP2-9: 1.2±0.3; $p<0.05$], without altering FSHβ. In isolated pituitary glands, BP2 decreased FSHβ (C: 1.6±0.2, BP2-9: 0.5±0.1, $p<0.05$) but increased LHβ (C: 1.1±0.2, BP2-9: 1.7±0.3, $p<0.05$). BP3 had the opposite effects, increasing FSHβ (C: 1.0±0.1, BP3-9: 1.6±0.1, $p<0.01$) and decreasing LHβ (C: 1.0±0.1, BP3-9: 0.2±0.1, $p<0.01$). Conclusions: Exposure to BP2 and BP3 alters gonadotropins gene expression in immortalized gonadotropes and in pituitary glands. More experiments are underway to further explore the effects observed. Supported by: CONICET, ANPCyT, Fund. R. Barón, Fund. Wiliiams, Fund. H. Bigand.

A73
LITHIUM BIOACCUMULATION IN *DISTICHLIS SPICATA* PLANTS

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Distichlis spicata is a halophytic herbaceous plant commonly found in the saline areas of La Pampa, Argentina. The ability of this species to absorb minerals from soil or aqueous substrates has been little explored. Therefore, the objective of this study is to evaluate the response of *D. spicata* to lithium exposure and to assess lithium bioaccumulation in its roots and aerial parts. Plants were collected from the Don Tomás Lagoon (Santa Rosa, Argentina) and placed in pots within a growth chamber set at 25ºC with a 16:8 light-dark photoperiod. The experimental design consisted of four randomized replicates across two treatments: (1) control plants without lithium application, and (2) a LiCl solution irrigated three times over a 60-day period, reaching a final lithium application of 30 mg Kg^{-1}. Twenty days after the final lithium application, the aerial parts and roots were collected for measurements of plant height, lipid peroxidation, antioxidant capacity, peroxidase activity, and lithium content via atomic absorption spectrometry. Although a significant increase in plant height was observed in the lithium-treated plants, no differences in lipid peroxidation, antioxidant capacity, or peroxidase activity were detected between the control and treatment groups, suggesting that lithium did not act as a stress factor. However, lithium content was significantly higher in the treated plants compared to the control, with accumulation occurring in both aerial parts and roots. A mean of 58 mg Kg^{-1} of lithium was accumulated in the aerial parts, while a mean of 164 mg Kg^{-1} was found in the roots. These results highlight the potential of *D. spicata* as a lithium phytoextractor, suggesting its possible use in phytomining.

A74
CHRONIC UNDERNUTRITION AND AIR POLLUTION: NON-GENETIC STRESSORS AND THEIR IMPACT ON EXCRETORY ORGANS

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Children, due to their physiological characteristics, are a highly vulnerable subpopulation to both air pollution (gases and particulate matter- PM) and undernutrition. Oxidative stress and inflammation are the main mechanisms by which PM negatively affects health. PM effects are evident not only locally in the lungs, but also in distant organs such as liver and kidneys, which are essential for xenobiotic detoxification. Therefore, the aim of this study was to evaluate the impact of subchronic exposure to Residual Oil Fly Ash (ROFA, a surrogate of air PM) on lungs, liver and kidneys of animals with chronic undernutrition. Weaning Wistar Rats were fed *ad libitum* (Control- C) or with a restricted diet of 20% C (Nutritional Growth Retardation-NGR) during 4 weeks and, were intranasally instilled with ROFA (0.17mg/kg body weight) or vehicle (PBS) 3 times per week, describing 4 experimental groups: C, ROFA, NGR, NGR+ROFA. Oxidative stress was espectrophotometricaly evaluated by determining the activity of antioxidant enzymes (Catalase-CAT and Sueproxide Dismutase-SOD) and lipoperoxidation (TBARS) in the 3 organs. The inflammatory process was evaluated analyzing interleukin expression (IL-6 and IL-10) by RT-PCR. The NGR group showed changes in antioxidant defense levels with respect to C. In the lungs, CAT was mobilized while SOD activity changed in all the organs evaluated ($p<0.05$). Additionally, in the lungs, ROFA caused a decrease in CAT activity in the C group (13%, $p<0.05$) and an increase in SOD in the liver of the NGR group (16%, $p<0.05$). Regardless these variations in antioxidant activity, ROFA exposure induced lipoperoxidation in the lungs of the NGR group (28%, $p<0.05$).The inflammatory state in the lungs and liver showed a similar pattern. Animals from the NGR group presented higher levels of IL-6 and IL-10 with respect to C. ROFA exposure induced an increase in ILs levels in the C group, whereas there was no response in NGR+ROFA. In the kidneys, only the ROFA group showed a decrease in IL-10. Our results emphasize the harmful effects of chronic PM exposure on the organs responsible for detoxifying the body. Chronic undernutrition can cause these organs to undergo functional changes, which may impair their ability to process and eliminate harmful substances, known as xenobiotics.

A75
EVALUATION OF THE ALEXITERIAL ACTIVITY OF *CROTON URUCURANA* LATEX IN THE TOPICAL TREATMENT OF LESIONS INDUCED BY *BOTHROPS DIPORUS* VENOM

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Croton urucurana latex, known for its traditional medicinal properties in South America, has been used for centuries to treat various ailments, including its topical application as a healing agent and its oral use as an antidiarrheal. Previous studies have shown that the latex of this plant has antibacterial, antifungal, antiviral and antitumor properties. Furthermore, it inhibits the hemolytic activity of bothropic venoms and has neutralizing proteolytic activity, making it a promising candidate for the treatment of venomous snakebites. On the other hand, the venom of *Bothrops diporus,* a snake known as the "Yarará chica", is highly toxic and causes severe tissue damage, such as necrosis, inflammation and cell destruction at the bite site. In these cases, immediate immunotherapy is essential, although ethnopharmacological treatments, such as the use of *C. urucurana* latex, could offer an alternative or complement to traditional treatment. This study focused on evaluating the alexitheric effect of *C. urucurana* latex against *B. diporus* venom using an in vivo experimental model in mice. The effects of a topical latex cream in preventing and mitigating venom-induced tissue damage were analyzed. The venom, known for its dermonecrotizing effect, causes severe destruction of the epidermis and dermis, accompanied by inflammatory infiltration and necrosis. In the experiment, four groups of mice were formed: one without treatment, one treated only with venom, one with only latex cream, and a fourth with venom and cream treatment. Histological techniques such as hematoxylin-eosin staining and Gomori trichrome were applied to assess the state of the tissues. The results showed that mice treated only with venom presented severe tissue damage, while mice treated with the latex cream after venom inoculation showed a significant reduction in signs of necrosis and damage. Collagen in the dermis was less disorganized and the tissue repair process was more evident compared to the group treated only with venom. This is the first study to demonstrate the alexitheric activity of *C. urucurana* latex against *B. diporus* venom. The results suggest that the latex has significant potential to be used as an alternative therapeutic treatment for venomous snakebites. This opens the possibility of bioprospecting the latex to develop new drugs based on its biological properties without local cytotoxic effects. In conclusion, *C. urucurana* latex could be a valuable resource in the treatment of snakebites.

A76
EFFECT OF L-CARNITINE ON OVARIAN FUNCTION IN A CHEMOTHERAPY-INDUCED PREMATURE OVARIAN FAILURE MODEL

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Advancements in early cancer diagnosis and treatment have improved survival rates; however, ovarian toxicity from chemotherapy and radiation remains a significant tissue in pediatric and young adult patients. This increases the risk of premature ovarian failure (POF),

early-onset menopause, ovarian endocrine disorders, and subfertility or infertility. Therefore, the side effects of oncological treatments and the long-term quality of life after illness have become increasingly relevant. This study aims to evaluate whether L-Carnitine (L-CAR) acts as a protective micronutrient for the ovaries in POF induced by the alkylating agent Cyclophosphamide (CTX) through the modulation of key biological mechanisms regulating ovarian function (morphology, apoptosis, steroidogenesis, and angiogenesis) in a rodent model. F1 mice (BALB/c x C57BL) aged 8-10 weeks (n=6/group) were used as the animal model. Four groups were established: the CTX group received i.p. CTX (75 mg/kg) and the control group received saline solution. The CTX+L-CAR group (200 mg/kg) received CTX on day 1 of treatment and 5 doses of L-CAR i.p. (200 mg/kg). Another group received only L-CAR (200 mg/kg). Sacrifice was performed on day 15. Ovaries were analyzed histologically and immunohistochemically for CD34 (endothelium) and α-actin (periendothelium). The expression of steroidogenic proteins (3β-HSD, aromatase, P450scc, and STAR), angiogenesis-related proteins (VEGF), and pro - and anti - apoptotic proteins (BAX and BCL-2) were also analyzed by Western blot. Additionally, serum progesterone levels were measured using ELISA kits. Statistical analysis was conducted using ANOVA followed by Tukey's test. CTX treatment reduced the percentage of primary, early antral, and mature follicles compared to the control group ($p<0.05$). However, L-CAR, with or without CTX, increased the percentage of these structures ($p<0.05$). While CTX increased the percentage of atretic follicles compared to the control group, L-CAR, with or without CTX, decreased it ($p<0.05$). No significant differences were observed in steroidogenic protein expression or progesterone levels among the groups. CTX reduced the endothelial and periendothelial area compared to the control group ($p<0.05$), whereas L-CAR was able to increase it in the presence of CTX ($p<0.05$). No significant differences were found in VEGF expression or the BAX/BCL-2 ratio among the experimental groups. These results suggest that L-CAR could act as a protector of ovarian function in POF, mitigating the adverse effects of CTX on follicular morphology and vasculature. These findings indicate that L-CAR represents a novel non-invasive and low-cost strategy to preserve fertility in Young women undergoing chemotherapy.

A77
EFFECTS OF THE IN-VITRO EXPOSURE TO BISPHENOL A, BENZOPHENONES 2 OR 3 ON PRO-INFLAMMATORY PROTEIN EXPRESSION IN HEMI-HYPOTHALAMI FROM ADULT C57BL/6 MICE

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According to a recent report from the United Nations Environment Programme and the Basel, Rotterdam and Stockholm conventions secretariat (1), around 13,000 chemicals are involved in the production of plastics. Among these chemicals, we find monomers, additives and non-intentionally added substances. Several chemicals used in plastics are endocrine disrupting chemicals or EDCs. Examples of EDCs are Bisphenol A (BPA), a monomer of polycarbonate plastics, and Benzophenones (BPs), UV-filters. Our hypothesis is that exposure to these EDCs causes brain inflammation. Previous results showed that the in-vitro exposure to BPA increased mRNA expression levels of Glial Fibrillary Acidic Protein (GFAP) in whole hypothalami from adult male mice. Here, protein expression of pro-inflammatory markers (Iba1 and GFAP) was evaluated in hemi-hypothalami from adult C57Bl/6 mice. Hemi-hypothalami were incubated in Krebs-Ringer medium in the presence or absence of BPA, BP2 or BP3 ($1x10^{-9}$ M) or medium alone (C) for eight hours. Tissues were lysed in RIPA buffer with protease inhibitors and protein concentration measured by Bradford. Proteins were separated in polyacrylamide gels and transferred to PVDF membranes. Iba1, GFAP and tubulin were detected using specific antibodies. Results were expressed as Media±SEM and analyzed by Paired t-test (Statistica). Exposure to all EDCs increased protein expression of Iba1 in hypothalamic of male mice (Iba1, C=1.0±0.1, BPA=1.5±0.1, Paired t-test $p<0.05$, n=7; C=0.9±0.1, BP2=1.1±0.1, Paired t-test $p<0.05$, n=7; C=0.9±0.1, BP3=1.3±0.2, Paired t-test $p<0.05$, n=7). Exposure to BP2 increased GFAP compared to C (C=1.0±0.1; BP2=1.3±0.1, Paired t-test $p<0.05$, n=7). Neither BPA nor BP3 changed GFAP protein expression. These results will help us to understand how exposure to EDCs affects brain inflammation. More studies are underway to further explore the observed effects. Supported by: CONICET, ANPCyT, Int. Soc. for Neurochemistry, Fund. René Barón, Fund. Williams, Fund. H. Bigand. Reference; 1. BRS. Global governance of plastics and associated chemicals. Secretariat of the Basel, Rotterdam and Stockholm Conventions, United Nations Environment Programme, Geneva. Raubenheimer K, Urho, N. 2023.