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"FROM BASIC RESEARCH TO PUBLIC HEALTH: A BACK-AND-FORTH WAY"

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CONFERENCE

A1
CONTROL OF ALTERNATIVE mRNA SPLICING BY CHROMATIN AND TRANSCRIPTION AND THE CURE OF A HEREDITARY DISEASE

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Evidence on the co-transcriptionality of pre-mRNA splicing and on a role for the transcription machinery on splice site selection produced a radical change in the view of regulatory mechanisms of splicing, originally conceived as a purely post-transcriptional event. We showed that alternative splicing (AS) is coupled to RNA polymerase II (RNAPII) transcription and envisioned two non-exclusive models: AS is affected by the recruitment of splicing factors to the transcription apparatus (recruitment coupling) or by the speed of RNAPII elongation (kinetic coupling). Transcription by a slow mutant of RNAPII promotes higher exon inclusion, by favoring the recruitment of splicing factors to the splice sites in the pre-mRNA. Slow elongation can also promote skipping of certain alternative exons by favoring the recruitment of negative splicing factors to their target sites in pre-mRNA. Changes in elongation can be elicited by changes in RNAPII CTD phosphorylation and/or by changes in chromatin structure. An example of the first mechanism is the regulation of AS by DNA damage caused by UV irradiation. As for the roles of histone marks and chromatin structure on splicing, a whole fascinating chapter of RNA biology is being written. Specific histone marks affecting chromatin structure were shown to regulate AS. This knowledge was recently exploited in our laboratory to devise a combined therapy for spinal muscular atrophy based on an approved splicing-correcting antisense oligonucleotide and histone deacetylase inhibitors (DOI: 10.1016/j.cell.2022.04.031).

EXPERT SCIENTISTS' SYMPOSIUM

A2
'CAPACITATION IN-VITRO MATURATION OF OOCYTES (CAPA-IVM)' FROM RESEARCH BENCH TO CLINICAL APPLICATION IN THE FERTILITY CLINIC

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Today, the IVM technique is less commonly practiced in human ART, for the main reason that pregnancy rates are still lower compared to regular (stimulated) ICSI. IVM might be developed more generally as a first-line option to treat infertile patients with a 'normal and high' ovarian reserve. Previous studies in animal models have demonstrated the necessity of applying specific pretreatment, retrieval technology, culture media, and growth factors for IVM to make it successful. Setting up pre-maturation (named "capacitation") culture and making use of physiological *in vitro* additives (follicular growth factors like C-type natriuretic peptide and Amphiregulin concentrations) can compensate for the 'immaturity' of the COC retrieved from small follicles between 2 and 8 mm. Our recent research on human small antral follicles has indicated key factors that could allow the immature oocyte to gain developmental competence in-vitro (DOI: 10.1093/humrep/dex262; DOI: 10.1007/s10815-019-01551-5). The presentation will cover the most recent scientific and clinical evidence that the in-vitro "capacitation" approach is efficacious, safe (DOI: 10.1093/humrep/dez121), and cost-effective. The follow-up of children born after CAPA-IVM does not demonstrate any alterations in psychomotor development (DOI: 10.1093/humrep/deac115). Hence it might be suggested as a first-line treatment for patients with a high ovarian follicle count or a high anti-Mullerian (AMH) hormone concentration in the blood.

A3
FROM BASIC RESEARCH TO THE DEVELOPMENT OF AN INNOVATIVE PRODUCT AGAINST HEMOLYTIC UREMIC SYNDROME

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Hemolytic uremic syndrome (HUS) is an orphan disease, for which there is currently no treatment available in the world. Given the magnitude of the social and economic problems caused by Shiga toxin-producing *Escherichia coli* (STEC) infections, there is an urgent need for specific therapies that prevent the development of this disease. One of the greatest challenges to solving the problem is to develop a safe treatment that has the capacity to neutralize the activity of Shiga toxin (Stx) to block the appearance of HUS in patients infected with STEC. We designed two new immunogens to present the Stx b Subunit efficiently (BLS-STX1 and BLS-STX2). From these, we have developed a hyperimmune serum with a high neutralizing titer against 8 Stx variants, called INM004. We have

successfully completed preclinical and Phase 1 trials for this product, which has been shown to be safe for use in subsequent stages of the research. During 2019, a Phase 2/3 clinical trial was started, to evaluate the efficacy of INM004 in preventing the development of HUS in STEC-positive patients. It had to be interrupted due to the SARS-CoV-2 pandemic. Capitalizing on the knowledge obtained in the development of this serum and using the same technological platform, we produced a medicine in record time capable of reducing mortality in severe COVID-19 patients. During the pandemic, more than 25,000 patients were treated with it, showing clinical improvement in 42% of the patients who received the full treatment.

A4
THE CHALLENGE OF TURNING SCIENTIFIC DISCOVERY INTO A BIOTECH STARTUP AND MAKING IT GROW: OUR EXPERIENCE AT RADBIO

Dewey RA

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RADBIO S.A.S. is a technology-based company or startup belonging to CONICET, founded in 2018 through investment from the Center for Technological, Business, and Social Innovation (CITES) belonging to the Sancor Seguros Group. RADBIO is a biotechnology company focused on the development of new diagnostic and treatment strategies for complex chronic diseases with unmet medical needs. Our EBT has two lines of development, a therapeutic one in which we are developing a biological drug for the treatment of pulmonary fibrosis, skin fibrosis, liver fibrosis, and cancer; and another diagnostic one in which we are validating a biomarker capable of measuring the activity of arthritis rheumatoid and potentially applicable to other inflammatory diseases. RADBIO's technology got its start with the discovery of a new molecule that has been shown to act as an inhibitor of the TGF-β pathway. TGF-β is a key cytokine involved in the pathogenesis of organ fibrosis, cancer, and more. Our discovery is a soluble isoform of TGF-β receptor II (TβRII-SE protein) produced endogenously by human cells. The intellectual property of the RADBIO technology belongs to CONICET and the "Fundación Articular" and has been developed at the Technological Institute of Chascomús (INTECH). It has been licensed to RADBIO for its exclusive use and commercial exploitation through a license agreement signed by the parties. The foundation of the developed technology, the steps taken until the formation of RADBIO, and the challenges of the startup to continue growing and thus bring technology closer to the patient's bedside will be presented. We wish to share our experience to stimulate the scientific community to convert scientific discoveries into technological developments that can benefit the community and also generate jobs and foreign exchange that contribute to the development of the country.

URUGUAYAN SOCIETY OF BIOSCIENCES SYMPOSIUM

A5
FROM THE BENCH TO THE FIRST IN HUMAN STUDIES: A PATH OF CHALLENGES AND NEW LEARNING

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In recent years, our research group has been working on the development of potential drugs for the treatment of chronic non-communicable diseases, with an emphasis on cardio-, immuno-, and metabolic diseases. In our region and worldwide, these diseases are the main cause of morbidity and mortality. Consequently, it is highly relevant to bend efforts to discover its causes, search for new molecular targets and study new therapeutic strategies to reduce its incidence. In the etiopathogenesis of these diseases, a chronic, sterile inflammatory process, developed at low noise at the systemic level, plays a central role. In this context, we have focused our efforts on the design of new structures that contain pharmacophores with recognized anti-inflammatory activity, taking into account the principles of green chemistry for their synthesis, and their physicochemical and biological characterization both *in vitro* and *in vivo* using different models of the main pathologies studied. This work has made it possible to have a chemical library of potential drugs protected by a portfolio of international patents and the consolidation of the interdisciplinary group. At the end of 2016, with the sublicensing of the patents and the creation of a tech-science startup, Eolo Pharma, we obtained seed funding from Cites (Center for Business and Social Technological Innovation, Sunchales, Argentina). Since then, we began the transfer process from the academy to the company. In the first three years, we studied the steps to follow, defined the lead molecule that could become a drug and its therapeutic indication, addressed multigram-scale synthesis, *in vitro* and *in vivo* efficacy and toxicity studies, as well as market and business plan. At the end of 2019, with a defined plan to take MVD1, our lead compound, to the first studies in humans, we closed an A round of private investment to finance the following stages. In particular, to complete the study of its mechanism of action, the production of the lead compound (MVD1) under GMP (CMC) standards, and all the preclinical studies requested by the Australian regulatory agency. Recently we closed a series A2 that will allow us to finance the first-in-human study (phase I) in that country (last quarter of 2022). In summary, this way of challenges and new learning has brought us to where we are today, achieving the milestone of developing the first-in-human study (phase 1) in Australia, with a drug designed and developed in our laboratories: MVD1.

A6
DIAGNOSIS OF RARE DISEASES THROUGH HIGH-THROUGHPUT SEQUENCING TECHNOLOGIES

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Seven percent of the population suffers from rare diseases. Although in isolation each pathology is very rare (1 in 2000), taken together they involve a large number of affected individuals. Most of them affect children, have a high impact on quality of life and life expectancy, most are chronic and progressive, and the vast majority of them have no specific treatment. Moreover, by their very nature of infrequency, they present a diagnostic challenge. In fact, it is generally referred to as a "diagnostic odyssey" that can last for years and involves dozens of consultations with specialists and studies (often invasive or requiring general anesthesia), with the consequent economic and emotional cost. The problems that these diagnostic delays entail, for the patient, his family, his physicians, the health system, and the research teams, are of enormous magnitude. The majority of these diseases, probably 70–80%, are of genetic cause. Therefore, the vast majority of efforts to improve diagnosis are focused on genome-wide techniques, especially high-throughput sequencing techniques. At the Institut Pasteur de Montevideo, together with the Faculty of Medicine (Department of Genetics) in the context of a project called URUGENOMES, we carry out genomic studies to contribute to the diagnosis of rare diseases. This allows us, on the one hand, to reach a molecular diagnosis, which is of great value for the family, and on the other hand, to understand a little more about the underlying mechanisms of the diseases. In this opportunity, I will present some of the work carried out in URUGENOMES.

A7
FROM THE LAB TO THE CLINIC: GUT MICROBIOTA ANALYSIS FROM URUGUAY

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The path of four scientists who left the laboratory and decided to take their research to society in a way that was not basic science. The uncertainty and challenges of communicating what we do, applying it and offering services that adapt to the needs of a potential client. How to apply, how to communicate, how to sell our knowledge. What is important when transforming our science into a potential company with international impact? Enteria is a company dedicated to the analysis of gut microbiota that was born in Uruguay and offers services throughout Latin America. It grew as a way to apply our knowledge and take it directly to a health professional's office. Undoubtedly, it has been a difficult path, full of successes and failures that we want to transmit to those who want to walk the same path: the path of leaving the laboratory and taking science to the doctor's office.

BIOLOGY SOCIETIES SYMPOSIUM

A8
USES OF DROPLET DIGITAL PCR IN PUBLIC HEALTH

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Detection of SARS-CoV-2 has created an enormous workload for laboratories worldwide resulting in a restriction at the time of massive testing. Pool testing is a strategy that reduces time and costs. However, beyond the detection of infectious diseases in blood banks, this approach is rarely implemented in routine laboratories. Therefore, what was learned from the SARS-CoV-2 pool testing should represent an opportunity to increase diagnostic capabilities. The present work carried out in the context of a diagnostic laboratory of a public hospital during the COVID-19 pandemic represents a contribution to this end. The main limitation of pool testing is the risk of false negatives that could have been identified by individual tests. These limitations are the dilution of samples with a low virus load during pooling and the integrity of the sample may be affected by the quality of the sample collection. Fortunately, both limitations coincide with the main strengths of droplet digital PCR (ddPCR). ddPCR is a third-generation PCR that splits the amplification into thousands of droplets that work in parallel, increasing sensitivity and resistance to inhibitors. Therefore, ddPCR is particularly useful for pool testing. Here we show how to factor between test sensitivity and savings in test time and resources. We have identified and optimized critical parameters for pool testing. The present study, which analyzed 1000 nasopharyngeal samples, showed that pool testing could detect even a single positive sample with a CT value of up to 30 in pools of 34 samples. This test was performed using three different standard extraction methods, the simplest being heating only, which resulted in substantial savings of extraction reagents in addition to PCR reagents. Moreover, we show that pooling can be extended to use saliva, which is less invasive and allows self-collection, reducing the risk for health personnel. Using a similar strategy, we recently set up the determination of SARS-CoV-2 variants by ddPCR in pooled samples (see poster). Currently, we are working on 3 more projects based on ddPCR. One is the development of a non-invasive screening

platform for colon cancer from liquid biopsies (peripheral blood and feces), the other is non-invasive monitoring of bladder samples of patients with recurrent Non-Muscle invasive Bladder Cancer, and the third is *Legionella* sp. in drinking water testing in our Hospital.

A9
TEST TUBE BABY AND INFERTILITY: A STORY AT THE SERVICE OF REPRODUCTIVE HEALTH

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Forty-four years after the first successful *in vitro* fertilization (IVF), it is estimated that, worldwide, approximately 9,000,000 babies were born through this technique. Areas such as pharmacology, cell biology, microbiology, immunology, genetics, and gynecology have worked together in order to generate highly controlled conditions that favor reproductive results. Basic sciences and work with experimental animals served as a platform to give rise to several milestones in assisted human reproduction (AHR), such as intracytoplasmic sperm injection, the study of cell apoptosis, knowledge of DNA integrity, cryopreservation of gametes and embryos, etc. Although the great advances in RHA techniques have made it possible to significantly improve the treatment of couples with reproductive problems, the rate of infertility continues to grow. This represents a challenge for researchers, who focus their work on the search and detection of biochemical markers capable of predicting the quality of gametes and embryos generated in the laboratory. Among the causes leading to failure in IVF are premature oocyte activations, abortive activations, alterations in the blocking of polyspermy, gamete fusion failure, sperm DNA damage, and arrest of embryonic development. Also, changes in the redox state of seminal plasma and follicular fluid favored the generation of low-quality embryos, poor implantation capacity, and recurrent abortions. Knowledge of the pathogenesis of sterility-infertility and its importance in clinical biology would favor the development of technologies aimed at improving reproductive outcomes as well as the application of personalized treatments.

YOUNG SCIENTISTS SYMPOSIUM

A10
THE CROSSTALK BETWEEN BIOMEDICAL RESEARCH AND THE HEALTH SYSTEM. RECEPTORS AND LIGANDS OF THE NORTHWEST OF THE PROVINCE OF BUENOS AIRES

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Junín has an Interzonal Hospital (HIGA A. Piñeyro), and it is the center of the health region (RSIII); however, it has no biochemical or pathological anatomy laboratories of high complexity technology. In this context, the research at a medical science center of the University and CONICET in the interior of the province is pulled and feedbacked by the health local needs. They are shown by: (1) The generation of High-Level Technological Services (STANs) that managed to structure responses to the city and region needs in cancer and infection diagnostic. (2) At the beginning of 2020, when the Covid-19 pandemic was declared, the National Ministry of Health designated our center with a team of researchers and doctoral students from CIBA, together with biochemists from HIGA, as members of the network of PBA laboratories for the diagnosis of Covid by real time-PCR to the entire RSIII and sometimes, also to the neighboring regions. The joint work between RSIII, HIGA, and CIBA made it possible to get the molecular diagnosis for the entire population in a timely manner. This had a great impact on local socio-sanitary decisions during the emergency. The achieved results demonstrated the strength of integrating the health and academic–scientific system in common objectives to benefit society. (3) Currently, HIGA Biochemistry Residency performs a rotation in molecular biology in our center, complementing the training of residents with techniques that the hospital can't cover at the moment. Also, the Ministry of Health in PBA has started a training program in health research at HIGA in which the coordination with CIBA and UNNOBA research secretariat is crucial for training health professionals who work at the hospital. (4) We advanced VPH diagnostic strategies in our region together with RSIII, the local government, and the Provincial Cancer Institute under the Ministry of Health, integrating a multidisciplinary team with engineers, industrial designers, gynecologists, and clinicians. (5) Finally, our research in pituitary tumors and our sustained work with the health system, have given rise to a national multicenter project of clinical and development research coordinated by our group from Junín. It includes the Services of Neurosurgery and laboratory of more than ten public hospitals and private clinics in the country.

A11
IMPACT OF SEROTONINERGIC ANTIDEPRESSANTS IN THE DEVELOPMENT OF EMOTIONAL PREFRONTAL CIRCUITS

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Serotonin-related antidepressant drugs like fluoxetine have their main pharmacological action by blocking the serotonin transporter, SERT, which mediates their therapeutic effects. Early-life application of fluoxetine during the first postnatal weeks in rodents produces neural and emotional alterations in adult life. Here, we show evidence that transient SERT expression in glutamate neurons of the prefrontal cortex during neurodevelopment contributes to these alterations, controlling the synaptic maturation of descending prefrontal circuits involved in emotional responses. This is the case of the circuit connecting the prefrontal cortex to the dorsal raphe nucleus, the main source of forebrain serotonin. Our study demonstrates the vulnerability of specific cortical circuits that can act as targets of serotoninergic antidepressants during early postnatal life, highlighting that alterations upon these circuits could increase the risk to develop mental disorders such as anxiety or depression later in life.

A12
STUDY OF THE VIROME AND IDENTIFICATION OF NOVEL VIRUSES IN BATS FROM ARGENTINA

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Bats provide important ecosystem services, but they have also been considered natural reservoirs of viruses, many of which cause severe diseases in humans. Characterizing viruses of bats inhabiting different geographical regions is important for understanding their viral diversity and for detecting viral spillovers between animal species. Using a metagenomic approach, the viral diversity of five species of insectivorous bats from Argentina inhabiting the center of Rosario (*Tadarida brasiliensis* maternal colony) and the Parque Villarino (Zavalla, Santa Fe) was investigated. Briefly, selected fecal samples were processed and pooled according to species and time or collection site; viral nucleic acids were extracted, enriched, and sequenced (MiSeq and NextSeq, Illumina). Viral sequences were subjected to quality analysis and assembled *de novo* using several bioinformatics tools. Assembler contigs longer than 500 bp were classified using viral databases such as BLASTn, Centrifuge, and DIAMOND. The results of viral taxonomic classification were further summarized to the taxonomic level of the family using MEGAN and Pavian. Thirty-five novel DNA viruses were characterized and classified in *Genomoviridae*, *Circoviridae*, *Smacoviridae*, *Papillomaviridae*, and *Anelloviridae* viral families. Samples from the *T. brasiliensis* colony exhibited lower viral DNA diversity than bat samples from Parque Villarino, showing a possible influence of habitat on virome composition. On the other hand, sequences classified into the *Coronaviridae* family were identified, corresponding to 3 complete genomes and 2 partial genomes of novel Alphacoronaviruses. These viruses would represent novel species, sharing 60 to 80% amino acid sequence identity within ORF1ab with respect to other coronaviruses. This study provides relevant data for the surveillance of zoonotic viruses infecting bats living in close contact with humans and contributes to determining their role as possible reservoirs of pathogens. *[Grant: ANPCyT PICT 2019-01790.]*

SHORT COMMUNICATIONS

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 1

A13
USE OF AMINO ACIDS AS OXIDATIVE SUBSTRATES DURING *IN VITRO* MATURATION OF BOVINE OOCYTES

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During *in vitro* maturation (IVM) of cumulus oocyte complexes (COCs) glucose (G) represents the main oxidative substrate present in culture media. Nevertheless, very little is known about the role of amino acids (Aa) during this process. The main aim of this work was to study the nuclear and cytoplasmic maturation and the subsequent embryo development in a defined media supplemented only with Aa. Cumulus-oocyte complex were obtained by aspiration of antral follicles from ovaries from slaughter cows. Those COCs with dense

and compact cumulus were selected under microscope. IVM was carried out in mSOF (without pyruvate and lactate) supplemented with FSH, LH, EGF, insulin, PVA y gentamicin, under mineral oil at 39ºC, 5% CO_2 in humidified air for 22 h. COCs were randomly divided in five groups: (a) without oxidative substrates, (b) Aa, (c) Aa + salicylate (an inhibitor of glutamate dehydrogenase), (d) Aa + G, and (e) G. Nuclear maturation was evaluated using Hoechst 33342 fluorescent staining, observing the presence of metaphase plate II. Cytoplasmic maturation was assessed by *in vitro* fertilization (FIV), which was performed in IVF-mSOF at 39ºC, 5% CO_2 in humidified air for 20 h, and subsequent embryo development was carried out in IVC-mSOF in humidified air with 5% O_2:5% CO_2:90% N_2. IVF rates and blastocyst rates were evaluated at 48 h and 7 days after FIV, respectively. To study the use of Aa as oxidative substrates by COCs, the production of ammonia at the end of maturation was determined in the IVM medium by spectrophotometry. Ammonia production by COCs was analyzed by ANOVA and nuclear maturation, IVF and blastocyst rates were analyzed by Chi-square ($P < 0.05$). A higher maturation rate was observed in the media supplemented with Aa than in the media without oxidative substrates or Aa + salicylate ($P < 0.05$), not observing differences with G. However, a higher maturation rate was observed in Aa + G than in Aa ($P < 0.05$). A significative increase in ammonia production by COCs was registered in COCs matured only with Aa supplementation ($P < 0.05$). In IVF, a lower cleavage rate was observed in Aa respect to G or Aa + G ($P < 0.05$), being the latter group significantly higher than G ($P < 0.05$). In media supplemented with Aa + salicylate cleavage was lower than in the other groups ($P < 0.05$). It was observed that only in the group Aa + G embryo development reach blastocyst stage ($P < 0.05$). From these results it turns that Aa hold in part *in vitro* maturation, they are deaminated to obtain carbon skeletons as an energy source. The combination of Aa + G as oxidative substrates is necessary for bovine embryo development *in vitro*.

A14
THE PROTEIN KINASE D FAMILY REGULATE SPERMATOZOA CAPACITATION

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The protein kinase D family (PKDs) are serine/threonine kinases named PKD1, PKD2, and PKD3. These isoenzymes are involved in fundamental biological process regulation, including signal transduction, cytoskeleton remodeling, and oxidative stress, among others. Sperm are specialized and transcriptionally inactive cells dependent on post-translational modifications, such as protein phosphorylation, to carry out their functions. These changes occur during the process called sperm capacitation. The presence and functions of PKDs in the male gamete have not been described. Our aim was to characterize the presence and function(s) of PKDs in these cells. Through indirect immunofluorescence (IIF) and western blot (WB), we observed that PKD1/2 is present and active (p-PKD) in bovine, equine, mouse, and human sperm in not capacitating (NC) and capacitating conditions (C). The PKD's localization was similar in all the species studied: head and flagellum. Interestingly, the p-PKD distribution pattern varies between species. In humans, it is in the middle piece, and, in mice, it is in the main piece and head. Regarding PKD3, it was present and mainly located in the middle and connecting piece. Moreover, we evaluated whether PKD would be involved in the sperm-capacitation process. When mouse-spermatozoa were incubated under capacitating conditions with specific PKD inhibitors (Kb142-70 [5 µM] and CRT0066101 [2.5 µM]), an increase in events associated with capacitation such us tyrosine-phosphorylation and phospho-PKA substrates proteins was observed (WB). Also, an increase in motile ($P < 0.01$), progressive ($P < 0.05$), and hyperactivated ($P < 0.01$; N = 6) sperm population was observed (SCA, Microptic system). Additionally, the induction of the acrosomal reaction with progesterone [30 µM] –another event associated with capacitation– was evaluated using Acrosin-eGFP transgenic mouse sperm by flow cytometry. The inhibition of PKD increased the percentage of reacted spermatozoa ($P < 0.05$; N = 7). These findings allow us to conclude for the first time that PKDs are present and active in mammalian sperm and would be involved in the regulation of the sperm capacitation process.

A15
EFFECT OF MORIN ON PROGESTERONE PRODUCTION DURING *IN VITRO* MATURATION OF PORCINE OOCYTE CUMULUS COMPLEXES

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Morin (3,5,7,2',4' pentahydroxyflavone) is a flavonoid used in our laboratory on *in vitro* maturation tests (IVM) of porcine *cumulus*-oocyte complexes (COC). It reduces peroxide levels in oocytes and affects nuclear maturation, at high doses. Flavonoids not only have an antioxidant effect but also inhibit steroidogenesis in *cumulus* cells. Progesterone induces germinal vesicular rupture, and its inhibition reduces oocyte maturation. This work aimed to determine if Morin added to the IVM medium affects progesterone concentrations during IVM of porcine COC. The COC were obtained by follicular aspiration from slaughter ovaries and were matured *in vitro* at a rate of 50 COC per well, containing 500 µL of medium 199 supplemented, with the addition of 5, 10, 50, and 100 µM of Morin or without Morin (control group), in a 5% CO_2 atmosphere, saturated with humidity, at 39°C. At 22 and 44 h of IVM, the culture medium was collected and cryopreserved at −20ºC until use. The progesterone present in the culture medium was evaluated by the chemiluminescent macroparticle immunoassay (CMIA) technique, and three replicates were performed for each measurement of each treatment. Data were statistically analyzed using the Kruskal–Wallis test, considering a statistically significant difference with $p \leq 0.05$ and expressed as mean ± SEM. In all cases, the progesterone concentration (ng/mL) increased significantly between 22 and 44 h, and, in the 100 µM Morin

group, it was significantly lower (28.75 ± 0.91 at 22 h and 64.43 ± 6.9 at 44 h) than in the other groups. The 50 µM Morin group (40.77 ± 3.9 at 22 h and 90.53 ± 10.45 at 44 h) also showed a tendency towards a decrease in progesterone concentration to the other groups (control = 61.48 ± 10.18 at 22 h and 128.18 ± 33.77 at 44 h; 5 µM Morin = 63.74 ± 7.62 at 22 h and 215.94 ± 42.5 at 44 h; 10 µM Morin = 60.37 ± 7.23 at 22 h and 194.92 ± 28.66 at 44 h). In conclusion, the *cumulus* cells actively produce progesterone in our IVM system. The decrease in progesterone production in the groups treated with Morin at higher concentrations could explain the decrease in nuclear maturation indices found at concentrations of 50 and 100 µM previously reported by our work group. In the future, work will continue with the lowest concentrations of 5 and 10 µM of Morin to determine the convenience of its addition during IVM in the porcine species.

A16
EFFECTS OF SOLUBLE AND MEMBRANE ADENYLATE CYCLASE INHIBITION IN HEPARIN CAPACITATION OF BOVINE SPERMATOZOA

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In heparin-induced sperm capacitation, intracellular signals are activated with a specific binding of the inducer with its membrane receptor, involving the participation of different key enzymes such as adenylate cyclase, producing a respiratory burst in the mitochondria. Soluble and membrane isoenzymes of adenylate cyclase could have a different degree of participation in the intracellular signaling mechanism during this process. The aim of this work was to determine the participation of adenylate cyclase isoenzymes in the signal transduction mechanism induced by *in vitro* heparin capacitation in cryopreserved bovine spermatozoa. Heparin was used as a capacitation inducer, LRE-1 as a soluble adenylate cyclase inhibitor, and 2,5-dideoxy adenosine (2,5-D) as a membrane adenylate cyclase inhibitor. Five treatments were performed with thawed semen in TALP medium at 38°C: control, heparin, heparin/LRE-1, heparin/2,5-D, and heparin/LRE-1/2,5-D. Capacitation was evaluated by the chlortetracycline epifluorescent technique and membrane viability and integrity by trypan blue vital staining with differential interferential contrast. Sperm motility was evaluated by microscopy and analyzed with the ISAS-Prosier software. Mitochondrial membrane potential was measured using the fluorochrome JC-1. Data were analyzed by ANOVA and Tukey's test ($P < 0.05$). LRE-1 and 2,5-D inhibitors, individually or in combination, inhibited capacitation by 75% ($P < 0.05$). Mitochondrial activity in heparin-capacitated samples was 63.67 ± 5.57%, while in treatments with inhibitors, it decreased significantly (heparin/LRE-1 31.11 ± 4.15%, heparin/2,5-D 41.50 ± 8.85%, heparin/LRE-1/2,5-D 31.25 ± 8.61%) ($P < 0.05$). Total and progressive motilities also decreased in inhibitor treatments compared to heparin-capacitated samples ($P < 0.05$). Both adenylate cyclase isoenzymes participate significantly in the signal transduction mechanism triggered by heparin during *in vitro* capacitation of bovine spermatozoa, which is evidenced in oxidative metabolism and sperm motility.

A17
ROLE OF AUTOPHAGY IN THE TESTICULAR AGING PROCESS IN THE GOLDEN HAMSTER

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Our group has previously described that, in the Golden hamster (*Mesocricetus auratus*), aging is associated with a significant increase in the testicular levels of inflammatory markers expression (NLRP3 inflammasome, proinflammatory cytokine IL1β) and indicators of oxidative stress (thiobarbituric acid reactive substances –TBARS– and antioxidant enzymes), two closely interconnected processes. Autophagy is an evolutionarily conserved catabolic process that is activated in response to different stressors (reactive oxygen species, DNA damage) in order to maintain cellular homeostasis through the degradation of damaged organelles and long-lived and/or poorly folded proteins. A decrease in autophagy has been associated with accelerated aging and age-dependent diseases, whereas optimal autophagy would partially protect cells from the natural aging process. Taking this into account, the objective of the present work has been to investigate the impact of (1) the inhibition of the NLRP3 inflammasome pathway and (2) the activation of autophagy, in the physiological testicular aging process in the Golden hamster. To this aim, testicular fragments from young adult (5 months old) and aged (22 months old) Golden hamsters maintained in a normal photoperiod were incubated *in vitro* in the presence or absence of activators/inhibitors of the NLRP3 inflammasome pathway and of autophagy, followed by the determination of the degree of lipid peroxidation through the TBARS assay. Testicular levels of TBARS were significantly higher in aged hamsters than in young animals ($P < 0.05$). However, when aged hamster testes were incubated in the presence of an NLRP3 inhibitor (MCC950) or autophagy activators (rapamycin or metformin), lipid peroxidation levels were similar to those detected in gonads from young animals. Next, in young adult hamster testes, the NLRP3 inflammasome pathway was activated (either by ATP, which increases K^+ efflux through the activation of the purinergic receptor P2X7, or an infectious agent –LPS–) or, alternatively, autophagy was inhibited (using bafilomycin A1), observing, in all cases, a significant increase in the generation of TBARS ($P < 0.05$). However, pre-incubation with the autophagy activators (rapamycin or metformin) blocked the increase in lipid peroxidation in response to ATP and LPS, respectively. In summary, our results suggest that the activation of the autophagy process would exert a protective effect against chronic inflammation and oxidative stress in the aged Golden hamster testis.

A18

RELEVANCE OF METABOLIC SYNDROME FOR MALE FERTILITY: CHARACTERIZATION OF MURINE GONADAL FAT

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The prevalence of metabolic syndrome (MS) has increased in alarming proportions in recent years, coinciding with reproductive age and becoming a risk factor for fertility disorders. For this reason, our group studies the possible relationship between MS and male fertility. To achieve this, C57BL/6J male mice are fed a high-fat diet (35%) for 30 weeks, while the control group receives a standard diet (6% fat). Although treated mice develop MS, we do not find defects in their fertility or sperm parameters. Moreover, the large increase in the gonadal fat weight in these animals, contrary to what is expected by the literature, does not affect those reproductive parameters. Thus, the present work aimed to characterize the gonadal fat of these animals. To this end, we isolated the two subregions of this tissue: proximal fat (PF) to the adipose pad adjacent to the cauda epididymis, where sperm are stored, and distal fat (DF) to the rest of the tissue. We found a significant increase of DF in animals with MS compared to controls ($P < 0.005$), and no differences in PF weight between groups. From histological analysis, we quantified the size of adipocytes as well as the presence of Crown-like structures, markers of inflammatory processes. On one hand, a significant increase in the adipocyte size of DF was detected in animals with MS with respect to controls ($P < 0.05$), which was not observed in PF of animals with or without MS. On the other hand, a higher incidence of Crown-like structures was found in DF of animals with MS compared to controls ($P < 0.05$), whereas these structures were practically not observed in PF regardless of the diet. As a first approach to the molecular characterization of these tissues, the expression levels of ATGL (triglyceride lipase, which plays a key role in lipolysis) and UCP1 (a marker of brown fat) were analyzed by Western blot. We observed a significant decrease in the level of ATGL in DF of animals with MS compared to controls ($P < 0.05$), which was not found in the PF. Regarding UCP1, surprisingly, its presence was observed only in the PF, also finding a decrease in animals with MS compared to controls ($P < 0.05$). Overall, we conclude that there are differences in the characteristics of the gonadal fat proximal and distal to the epididymal cauda, with the proximal pad being less susceptible to metabolic injury and thus generating a possible protective microenvironment around sperm.

A19

EFFECT OF RESVERATROL AND TROLOX SUPPLEMENTATION ON ACTIVE MITOCHONDRIA OF VITRIFIED AND WARMED PORCINE OOCYTES

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The increase in the use of reproductive biotechnologies has generated the need to develop reliable techniques for the conservation of gametes. Porcine oocytes present a major challenge to the success of these techniques due to their high susceptibility to cold injury. Mitochondria are one of the most metabolically involved organelles in this process. One of the alternatives to increase the efficiency of cryopreservation techniques is the use of antioxidants. The aim of this research was to evaluate the effect of the antioxidants Trolox and/or resveratrol on active mitochondria of vitrified and warmed porcine oocytes. Immature cumulus–oocyte complexes (COCs) were matured *in vitro* in medium 199 supplemented with porcine follicular fluid (FFP), cysteine, FSH and LH, at a 39°C, 5% CO_2 in humidified air for 44 h. Then, the COCs were incubated with hyaluronidase for 5 min at 37°C and denuded with a fine Pasteur pipette. The oocytes were vitrified and warmed by the Cryotech® minimum volume method. To evaluate the effect of the antioxidants, the vitrification and warming solutions were supplemented, or not, with resveratrol and/or Trolox at a concentration of 2 µM and 50 µM, respectively. After warming, oocytes were cultured in medium 199 + FFP for 3 h to allow their recovery. After this time, the evaluation of active mitochondria was carried out by staining the oocytes with 0.5 µM of MitoTracker Green for 45 min in the dark. Then, they were washed three times in PBS-PVA medium and placed on a slide for their observation under an epifluorescence microscope. The exhibited fluorescence by each oocyte was analyzed using the ImageJ software from the images obtained. Data were analyzed by ANOVA ($P < 0.05$). It was found that the vitrification and warming process generates a significant increase in active mitochondria compared with fresh oocytes ($P < 0.05$). This increase was also evident in the group of oocytes vitrified in the presence of Trolox, which did not differ from the group vitrified without antioxidant supplementation, but it was significantly higher than the group of fresh oocytes ($P < 0.05$). On the other hand, the addition of resveratrol to the vitrification and warming medium, either alone or together with Trolox, allowed to partially restore the level of mitochondrial activity, being these two groups significantly lower than those vitrified in the absence of antioxidants or in the presence of just Trolox, but significantly higher than the group of fresh oocytes ($P < 0.05$). These results suggest that the addition of resveratrol to the vitrification and warming media may be an alternative to partially control the metabolic changes that this cryopreservation procedure generates in porcine oocytes. However, the effect of these antioxidants on the control of reactive oxygen species production and the ability of oocytes to be fertilized *in vitro* remains to be known.

A20
EFFECT OF MATERNAL STRESS ON VASCULAR PARAMETERS OF THE UTERUS AND PLACENTA

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Severe stress negatively affects health and has been associated with the development of various pathologies. In particular, maternal stress has been correlated with molecular and physiological changes in the placenta that alter its correct formation. It is postulated that these alterations are related to obstetric pathologies and have a negative impact on fetal development. However, the consequences of chronic, mild, and daily stress on reproductive health are less known. The objective of this work was to analyze the effect of mild chronic stress on pregnancy and in particular the vascular adaptations of the maternal–fetal interface. For this, female BALB/c mice were housed in cages with a third of litter material than the control group. In addition, every other day females from the stressed group were placed on a lid that was shaken for one minute. Serum levels of glucose, triglycerides, and cholesterol were measured. The major vessels irrigating the uterine horns were ligated, photographed, and the cross-sectional lengths of the uterine and arcuate arteries were measured. Fetuses and placentas were extracted, weighed, and measured. Protein levels of inducible nitric oxide synthase (iNOS) and vascular endothelial growth factor (VEGF) were analyzed in the placenta. Also, the activity of NOS in the placenta was quantified. Data were analyzed by one-way ANOVA, followed by Tukey's test. In the cases of non-parametric data, the Kruskal–Wallis test was performed. Differences were considered significant when the *P*-value was less than or equal to 0.05. First of all, we observed that the females exposed to the stress protocol presented higher levels of total cholesterol and triglycerides in serum. For their part, blood glucose levels did not change with treatment. The cross-sectional lengths of the uterine and arcuate arteries were lower in stressed females. The fetuses of these females presented lower weights than the controls. However, the weight and size of the placentas did not vary between groups. On the other hand, lower protein levels of iNOS and VEGF were detected in the placentas of the stressed group. In addition, these placentas presented lower total NOS activity than those of the control group. Taken together, these results suggest that mild chronic stress alters maternal physiology and modulates the vascular physiology of the uterus and placenta. Alterations in the formation of the uterine vascular bed could affect the development of the placenta and therefore the delivery of oxygen and nutrients to the growing embryo.

A21
INVOLVEMENT OF CRISP PROTEINS IN EARLY EMBRYO DEVELOPMENT

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Cysteine-Rich Secretory Proteins (CRISP) are mainly expressed in the male reproductive tract and have important roles in mammalian fertilization. Interestingly, CRISP1/CRISP3 double KO (DKO1/3) males are subfertile due to embryo development defects. Based on this, the aim of the present work was to elucidate the mechanisms leading to this reproductive phenotype. To investigate whether embryo development defects could be due to delayed fertilization mediated by an affected sperm transport within the female tract, superovulated females were mated with DKO1/3 or control males, and the percentages of fertilized eggs in the *ampulla* were analyzed only 4 h after mating. Under these conditions, fertilization rates were not significantly different between groups but those fertilized eggs corresponding to the mutant group exhibited once again clear deficiencies to reach the blastocyst stage *in vitro* compared to controls (12.5 ± 6.3 *vs*. 41.50 ± 14.0; N = 5, $P < 0.05$). As another approach to investigate whether sperm transport defects could be responsible for the mutant male phenotype, superovulated females were mated with GFP (Green Fluorescent Protein)-DKO1/3 or GFP-control males and sperm migration within the oviduct analyzed by fluorescence microscopy. Results confirmed that mutant sperm exhibited no defects to enter the lower isthmus nor to migrate toward the *ampulla* compared to controls. Finally, to analyze whether the embryo development defects observed for mutant DKO1/3 occurred at or after epididymal maturation, mutant and control epididymal sperm were inseminated into the uterus of superovulated females and the percentages of fertilized eggs both recovered from the *ampulla* and becoming blastocysts *in vitro* were determined. Although no differences in *in vivo* fertilization rates between groups were detected, the percentage of blastocysts was significantly lower for mutant males than controls (26.3 ± 12.7 *vs*. 59.1 ± 18.1; N = 5, $P < 0.05$), revealing that sperm defects leading to embryo development deficiencies in DKO1/3 males would be already present at the epididymal level. Together, these observations support a key role for CRISP1 and CRISP3 during epididymal maturation with subsequent impact on early embryo development.

BIOCHEMISTRY, PHYSIOLOGY AND METABOLISM

A22
IMPACT OF D2R DELETION FROM NEURONS ON AUTOPHAGY AND UPR GENE EXPRESSION IN LIVER

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Growth hormone (GH) secretion is sexually dimorphic in many species, including rodents and humans, and impacts hepatic gene expression differentially in females and males. Previous results from our laboratory showed that central disruption of the dopamine D2 receptor (D2R) in neuroDrd2KO mice decreases the growth axis and modifies the expression levels of various sexually dependent genes in the liver, thereby altering hepatic sexual dimorphism. On the other hand, autophagy constitutes a mechanism for maintaining cellular homeostasis through the constant elimination of proteins with aberrant folding and damaged organelles. Previous studies show sexual differences in the levels of autophagy, which are implicated in various types of diseases that affect one or the other sex differentially. Likewise, it is known that dysfunctional autophagy generates endoplasmic reticulum stress and promotes the unfolded protein response (UPR) tending to reverse this situation by inducing the transcription of autophagy genes. Therefore, it is of our interest to study the effect of central D2R depletion on sexually dimorphic autophagy and UPR gene expression in liver. To this end, liver tissue was collected from adult neuroDrd2KO mice of both sexes and their controls $Drd2^{loxP/loxP}$, and real-time PCR assays were performed to determine the expression of the sexually dimorphic autophagy genes *LC3*, *SQSTM1/p62*, *Beclin1*, and *Bnip3*, and *UPR* genes *Bip*, *Chop*, and *Xbp1* pre- and post-splicing (*usXbp1* and *sXbp1*, respectively). Our results showed that in control mice, *LC3* gene expression levels were higher in males (*t*-test, $P = 0.033$, N = 6), thus confirming previous data from the literature. However, no significant differences were observed for the other autophagy or UPR genes studied, although a tendency towards higher expression in males was observed for *SQSTM1/p62*, *Bip*, *Chop*, *usXbp1*, and *sXbp1*, and a tendency towards higher expression in females for *Bnip3*. On the other hand, CNS D2R depletion did not modify the expression of autophagy genes *LC3*, *SQSTM1/p62*, *Beclin1*, and *Bnip3* in either sex (ANOVA ns, N = 6, 3, 5–6, and 6, respectively), although a trend towards increased expression was observed for all autophagy genes analyzed in neuroDrd2KO females, as well as decreased *Beclin1* expression in neuroDrd2KO males relative to their controls. The UPR genes *Bip, Chop, sXbp1* and *usXbp1* did not show significant differences between genotypes (ANOVA ns, N = 6, 6, 6, and 3, respectively), but they also evidenced a tendency to higher expression in neuroDrd2KO females for *sXbp1* and *usXbp1* in relation to control females, and lower expression in neuroDrd2KO males for *Chop* and *usXbp1* in relation to control males. These results confirm the existence of a hepatic sexual dimorphism in mice for the key autophagy gene *LC3* and show a possible alteration in the expression pattern of all analyzed genes, except *Bip*, promoted by the imbalance of the GH axis produced by the absence of the central D2R. *[This work was funded by CONICET, ANPCyT, Fundación René Barón and Fundación Williams.]*

A23
ONTOGENY OF THE PITUITARY ACTIVIN–INHIBIN SYSTEM. PARTICIPATION IN THE CONTROL OF PROLACTIN. SEX DIFFERENCES

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Serum prolactin levels increase progressively from birth to adulthood in female and male rats, being higher in females from birth. Although this gradual increase was associated with concomitant maturation of prolactin-releasing and -inhibiting factors, these processes do not fully explain some sex differences observed. In *in vitro* studies, using lactotrophs in culture, from rats of different ages, in the absence of hypothalamic control, it was shown that the secretion of prolactin to the medium increases with age during the first weeks of life. These data suggest the involvement of intra-pituitary factors. In the present work, the participation of pituitary activins, as paracrine factors, in the regulation of prolactin secretion during postnatal development was studied. Sex differences in this mechanism were also evaluated. Male and female Sprague Dawley rats aged 11, 23, and 45 days were used. Pituitary gene expression of activin subunits (*Inhba* and *Inhbb*) and activin receptors (*ActRI*, *ActRIIA*, and *ACTRIIB*) was evaluated by RT-qPCR. On day 11, the female pituitary showed the highest expression of *Inhba* and *Inhbb*, being even significantly higher than that observed in males. The gene expression of activin subunits decreases with age in females, with sexual differences disappearing at 23 days. In contrast, the expression of *Inhbb* increases strongly at 45 days only in the pituitary glands of males, being the predominant activin subunit in this sex in adulthood. Gene expression of activin receptors (*ActRIB*, *ActRIIA*, and *ACTRIIB*) also decreases in females during postnatal development, while remaining relatively stable in males. We also evaluated the protein expression of *ActRIB* and its co-location with PRL. The proportion of lactotrophs (PRL^+) that express the receptor ($ActRIB^+$) is highest in the pituitary glands of 11-day-old females and decreases with age. Activins have been described to inhibit PRL synthesis by activating phospho-p38 which inhibits the transcription factor *Pit-1* (or Pou1f1). We evaluated the gene expression of *Pit-1* as a measure of the biological activity of activins on lactotrophs. We found that, in both sexes, *Pit-1* increases during postnatal development. Finally, since dopamine and estradiol are the main factors that regulate lactotroph function, we evaluated *in vivo* the effect of these factors on the regulation of the gene expression of activins and their receptors. We found a positive regulation of dopamine on the expression of *Inhba* and activin receptors in the 11-day pituitary of both sexes. On the other hand, *in vivo* treatment with estradiol increased pituitary expression of both activin subunits and of the ActRIB and *ACTRIIB* receptors in both sexes. Our results show a higher expression and activity of activins and their receptors in 11-day-old females that decreases during postnatal development. This is not observed in male pituitary glands, on the contrary, in male pituitaries, the expression

of activins increases during development. The results obtained in this work contribute to the knowledge of the intra-pituitary regulation of PRL during postnatal development, and to understanding the mechanisms involved in the sex differences observed in the serum levels of PRL during early life. Our study suggests an important sex-specific role of pituitary activins in PRL inhibition at early ages.

A24
AGE-RELATED SEX DIFFERENCES IN METABOLIC ALTERATIONS IN MICE LACKING GABAB RECEPTORS IN *KISS1* CELLS

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Kisspeptin, encoded by the *Kiss1* gene, and GABA are key factors in the regulation of reproduction but also of metabolism, as they are expressed in the liver, pancreas, and adipose tissue. We developed a unique mouse lacking GABAB receptors (GABABR) exclusively from *Kiss1* cells/neurons (*Kiss1*-GABAB1KO or KO) to study the impact on reproduction/metabolism. In contrast to reproduction, we found clear alterations in metabolic parameters. 3-month-old (3M) KO females showed increased body weight (BW), non-fasted glycemia (NFG), insulin secretion and HOMA-beta-cell index, and reduced insulin sensitivity. 3M KO males showed normal BW and NFG, higher fasted glycemia (FG), serum insulin, and HOMA-IR index, altered response to glucose overload, and lower insulin sensitivity compared to control males. Here we determined whether these metabolic alterations persisted or worsened with age in 9-month-old (9M) KO and control mice. Interestingly, 9M KO males had higher BW and increased total white adipose tissue (WAT) mass and also WAT/BW. Although NFG and FG were similar between genotypes, KO males showed increased fasted serum insulin and pancreatic insulin content. Furthermore, HOMA-beta-cell and HOMA-IR indexes were increased in KO males. These alterations were not due to differences between genotypes in kisspeptin content in the medial basal hypothalamus. We did not find differences between genotypes either in serum kisspeptin, cholesterol (CH), and triglycerides (TRI) levels or in WAT, brown adipose tissue (BAT), and hepatic kisspeptin content. However, kisspeptin levels were decreased by 35% in the pancreas of the KO males, and this decrease could be leading to the increased serum and pancreas insulin levels observed at this age. In contrast, 9M KO females showed similar BW, NFG, FG, fasted insulin levels, HOMA indexes, and serum CH and TRI levels compared to controls. Although serum kisspeptin levels and kisspeptin content in the liver, pancreas, and BAT were similar between genotypes, 9M KO females had increased WAT kisspeptin content that could be leading to the decrease in WAT mass, and normalization of BW, insulin secretion, and insulin sensitivity. In sum, the lack of GABABR specifically in *Kiss1* cells has a clear sex difference in metabolic alterations that becomes apparent with age. Peripheral response to insulin and also pancreas function worsened in males by aging, possibly due to altered autocrine/paracrine regulation of the pancreatic islet. Interestingly, KO females showed a reversion in their phenotype with age. Our results highlight the impact of GABABR in the regulation of the peripheral pancreas kisspeptin system in males and the WAT kisspeptin system in females, which will be further studied. *[Supported by CONICET, ANPCYT, ISN-CAEN.]*

A25
OLIGONUCLEOTIDE IMT504 IMPROVES INSULIN RESISTANCE AND VISCERAL ADIPOSE TISSUE STATE IN A METABOLIC SYNDROME AND TYPE 2 DIABETES MURINE MODEL INDUCED BY HIGH-FAT DIET

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We have observed that the immunomodulatory oligonucleotide IMT504 induced a dose-dependent improvement in glucose homeostasis, food intake, and body weight in a metabolic syndrome (MS) and type 2 diabetes (D2T) murine model induced by a high-fat diet. Here we evaluated the effects of IMT504 on insulin secretion, tissue insulin resistance, and visceral adipose tissue (VAT) in this model. C57BL/6LP male mice were fed either a standard diet (SD) or a high-fat diet (HFD: ResearchDiet, D12492) for 12 weeks. HFD animals showed higher non-fasting glycemia (Gly: $P < 0.01$) and body weight ($P < 0.01$). HFD mice received one daily dose of IMT504 for 12 consecutive days (ip IMT: 20 mg/kg/day, 6 mg/kg/day, or 2 mg/kg/day) or saline. Insulin secretion tests (IST, day 10) were performed. On day 12, after 3 h fasting, Gly was recorded, mice were sacrificed, and blood and VAT samples were collected. Serum insulin levels (by ELISA), insulin resistance (by HOMA-IR), and key genes expression in VAT (by qPCR) were analyzed. Fasted insulinemia was higher in HFD mice and it significantly decreased with IMT treatment [ANOVA: $P < 0.01$; SD, IMT6 and IMT20 different from HFD, $P < 0.01$]. HFD animals showed insulin resistance, which was also improved with IMT treatment [IST: insulin (ng/mL): repeated measures ANOVA: interaction, NS, main effect: treatment, $P < 0.01$; HFD different from SD, IMT6, and IMT20: $P < 0.02$], [HOMA-IR: ANOVA, $P < 0.01$; HFD different from SD, IMT2, IMT6: $P < 0.02$]. VAT lipoprotein lipase gene expression showed a significant decrease in all HFD animals regardless of IMT treatment [ANOVA, $P < 0.001$, SD different from all: $P < 0.001$]. Leptin expression increased in HFD mice compared to SD and it was partially reversed by IMT6 and IMT20 treatments [ANOVA, $P < 0.05$, SD different from HFD: $P < 0.05$]. However, both IMT doses normalized the increased expression of the macrophage marker F480 observed in HFD mice [ANOVA: $P < 0.01$, HFD different from SD, IMT6, and IMT20, $P < 0.01$]. These results show that IMT504 treatment promotes a significant, dose-dependent, amelioration in diabetic condition by improving insulin resistance and VAT characteristics associated with MS and D2T in HFD mice. Further investigation is needed to understand its mechanism of action. *[Funding: CONICET, ANPCYT, F. R Barón, F. Williams, Johnson & Johnson.]*

A26
EFFECT OF DOPAMINERGIC AGONIST CABERGOLINE ON AUTOPHAGY IN BETA PANCREATIC CELLS *IN VITRO*

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Autophagy is a mechanism that enables cellular homeostasis through the constant removal of potentially toxic ubiquitinated proteins and damaged organelles. Its dysregulation in pancreatic beta cells contributes to the development of diabetes, making autophagy modulators potential therapeutic agents for the treatment of human diabetes. Previous data indicate that activation of the dopamine D2 receptor (D2R) can alter autophagy in several cell types. Therefore, the aim of this study was to determine the effect of D2R activation by its agonist Cabergoline (CAB) on the autophagic process in pancreatic beta cells. For this purpose, we stimulated cultures of the murine pancreatic beta cell line MIN6B1 with CAB 10^{-5} M for 1, 6, and 24 h to evaluate the kinetics of autophagic vesicle formation, and alternatively with CAB 10^{-5} M in the presence or absence of Chloroquine (CQ), an inhibitor of late stages of the autophagy process, for 1, 6, and 24 h in order to study the autophagic flux. Using Western Blot and immunofluorescence and confocal microscopy, the autophagy markers LC3 (autophagic vesicle marker) and p62/SQSTM1 (autophagy cargo receptor and also degradation substrate) were analyzed. CAB caused an increase in LC3 nucleation as a function of stimulation time, showing significant differences relative to the control after 6h (one-way repeated measures ANOVA, $P = 0.0038$; Tukey: Control *vs*. CAB6h, $P < 0.05$; Control *vs*. CAB24h, $P < 0.01$). In addition, there was a significant increase in p62/SQSTM1 levels at 24 h of CAB stimulation (one-way repeated measures ANOVA: $P = 0.0157$; Tukey: Control *vs*. CAB24h, $P < 0.05$). When studying the effect of CAB on the autophagic flux, we observed that CQ significantly increased LC3 and p62 levels at 24 h of incubation (two-way repeated measures ANOVA (CQ and CAB): for LC3: interaction $P < 0.05$, Tukey: CQ *vs.* Control, $P < 0.0001$; for p62/SQSTM1: CQ pretreatment effect $P < 0.05$), as expected. Interestingly, CAB decreased the accumulation of LC3 (Test-T Delta (CQ-Control) *vs*. Delta (CQCAB-CAB): $P = 0.04$) and p62/SQSTM1 (Test-T Delta (CQ-Control) *vs*. Delta (CQCAB-CAB): $P = 0.01$) caused by CQ at 24 h. Therefore, we conclude that CAB is able to increase autophagic vesicle formation and, in addition, to decrease autophagic flux after 24 h of stimulation in the MIN6B1 pancreatic beta cell line. *[This work was funded by CONICET, ANPCyT, Fundación René Barón, and Fundación Williams.]*

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 2

A27
USE OF ENDOGENOUS LIPIDS DURING *IN VITRO* CAPACITATION AND ACROSOME REACTION OF REFRIGERATED BOAR SPERMATOZOA

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Boar spermatozoa use a wide variety of energetic substrates to maintain their metabolic functions. However, mechanisms of production, control, and use of different oxidative substrates to generate the energy they need for successful fertilization, are not fully elucidated. The aim of this work was to study the role of endogenous lipids in the *in vitro* capacitation and acrosome reaction (AR) of refrigerated boar spermatozoa. Samples (N = 6) were fractionated and incubated at 37°C in TBM under different conditions: (a) with glucose and pyruvate, (b) without glucose and pyruvate, (c) with glucose and pyruvate + bicarbonate (as a capacitation inductor), (d) without glucose and pyruvate + bicarbonate, (e) without glucose and pyruvate + bicarbonate + etomoxir (beta-oxidation of fatty acids inhibitor), and (f) without glucose and pyruvate + bicarbonate + L-carnitine (beta-oxidation of fatty acids activator). After capacitation and AR (induced by 30% follicular fluid), motility (optical microscopy), viability (eosin-nigrosine technique), capacitation (fluorescent chlortetracycline technique), and true AR (differential interferential contrast with trypan-blue) were evaluated. Data were statistically analyzed by ANOVA and compared with the Bonferroni test ($P < 0.05$). No significant differences were observed between treatments for motility and viability. In TBM without oxidative substrates (d), spermatozoa did not respond to capacitation or AR induction, unlike TBM with glucose and pyruvate (c, $P < 0.05$). L-carnitine (f) produced a significant increase in the percentage of capacitated and acrosome-reacted spermatozoa (*vs.* d, $P < 0.05$), without reaching the percentages obtained in TBM with oxidative substrates (c, $P < 0.05$). These results suggest that boar spermatozoa, incubated in a capacitating medium without oxidative substrates, can use endogenous lipids as an energy source to perform sperm capacitation and AR. The energy obtained by the oxidation of fatty acids would not be essential for the maintenance of sperm motility, since in the presence of etomoxir this parameter was not affected. Future studies on the use of other oxidative substrates will complement these results and would help to elucidate the metabolic pathways used by boar spermatozoa to obtain the energy required for these processes.

A28

LIPOPOLYSACCHARIDE FROM *Escherichia coli* ALTERS FETAL GROWTH IN A RAT MODEL OF SUBCLINICAL INFECTION

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Subclinical infections cause dysregulation of immune homeostasis that could have serious consequences for pregnancy. It has been postulated that alterations in the inflammatory response of the maternal–fetal interface might cause placental dysfunction impacting the development of the offspring. Previous results from our laboratory show that the administration of lipopolysaccharide from *Escherichia coli* (LPS) to rats during early gestation is associated with an increase in the diameter of the uterine and arcuate arteries on day 9 of pregnancy. The aim of this study was to investigate the effect of subclinical infection on placentas and fetuses on day 15 of gestation and on the offspring. For this, pregnant rats of the Wistar strain received vehicle (saline, control) or intraperitoneal LPS as an infectious stimulus (20 µg/mg on day 6 + 50 µg/mg on days 7, 8, and 9 of gestation). The animals were euthanized on day 15 of gestation or were allowed to deliver. Several measurements were made on fetuses, placentas, mothers, and the offspring. Data were analyzed using one-factor ANOVA and Student's *t*-test. Differences were considered significant when $P < 0.05$. We observed that treatment with LPS did not produce macroscopic symptoms of infection in pregnant rats: no piloerection, increased body temperature, decreased intake, or lack of movement, were observed. Leukocyte infiltrates were not observed in organs such as the kidney, lung, and liver. On the other hand, no differences were found in the number of viable fetuses or the percentage of embryonic resorption between control females and those injected with LPS. However, LPS decreased the weight of fetuses without changing the weight of placentas. In addition, rats injected with LPS had a more intense and violet coloration of the blood contained in the uterine and arcuate arteries. The offspring of LPS-treated rats were born at term on day 22 of gestation and the treatment did not modify litter size or pup weight on postnatal days 1 and 4. Our results show that the doses of LPS administered affect the growth of intra-uterine fetuses without modifying the number of them or of the offspring, as well as the weight of placentas and the percentage of embryonic resorption. In addition, it does not produce visible symptoms of infection. Therefore, we propose that a subclinical infection might affect the structure and/or function of placentas which would cause a decrease in fetal weight.

A29

RELATIONSHIP BETWEEN MURINE SPERM PERFORMANCE AND THE COMPOSITION OF CAPACITATION MEDIA

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In order to fertilize the egg, mammalian sperm undergo functional and structural changes, which occur in the female tract, known as capacitation. This process can be carried out *in vitro* and, in the case of mice, different culture media that allow capacitation have been described in the literature. Based on this, the present work aimed to determine the most appropriate culture medium for the standard parameters used to evaluate sperm capacitation and fertilizing capacity. For this, sperm was recovered from the cauda epididymis of C57BL/6 x BALB/c adult males and capacitated in the following media: Fraser and Drury (FD), Toyoda–Yokoyama–Hosi TYH (TYH), TYH modified with HEPES (TYH–HEPES), or Human Tubal Fluid (HTF). We demonstrated by Western blot from capacitated sperm extracts that phosphorylations in PKA substrates and tyrosine residues, both events that occur during capacitation, were similar among the different media. On the other hand, we found subtle differences in total motility among the conditions, with the lowest percentages obtained with TYH–HEPES ($P < 0.05$), suggesting that HEPES may have a negative effect on sperm motility. The percentage of spontaneous acrosome reaction was higher in sperm capacitated in HTF or TYH–HEPES ($P < 0.05$) whereas the progesterone-induced acrosome reaction was higher only in HTF ($P < 0.05$). To analyze the sperm fertilizing capacity, *in vitro* fertilization and embryo development tests were carried out by inseminating eggs without zona pellucida (ZP) and eggs with ZP surrounded or not by the cumulus. Regarding eggs without ZP, the highest fertilization rate was obtained when sperm were capacitated in FD or HTF ($P < 0.05$). This could be due to the fact that these two culture media contain glucose, lactate, and pyruvate as carbon sources, compared to both TYH media which only have glucose and pyruvate. Moreover, in the case of eggs with ZP, capacitation in FD, which has the highest concentration of the three carbon sources, sustained the best fertilization rate ($P < 0.05$). Furthermore, when all the embryos were cultured in a medium that supports their development, the percentage of blastocysts decreased when sperm had been capacitated in TYH ($P < 0.05$). On the other hand, when the cumulus was present, no differences in the fertilization and embryo development rates were observed, confirming its beneficial effect on the fertilization process. In summary, although the analyzed sperm parameters were subtly modified in the different culture media, we showed that FD would be the most suitable medium for *in vitro* fertilization tests. Moreover, these results reinforce the idea that standard sperm parameters do not unequivocally reflect sperm fertilizing capacity.

A30

EFFECT OF RESVERATROL SUPPLEMENTATION ON A POOL OF BOAR REFRIGERATED SEMEN

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The porcine species represents a greater challenge for gametes preservation due to the spermatozoa's sensibility to cold damage, being the mitochondria one of the most affected organelles during this process. An alternative to improve preservation success is the use of antioxidants. The aim of this research was to evaluate the effect of resveratrol supplementation on pools of refrigerated boar semen samples in a long-term commercial medium, with greater interest focused on mitochondrial metabolism. Pools of proven fertility boars' refrigerated semen were supplemented with 50 µM resveratrol once they arrived at the laboratory and were conserved at 17°C for 7 days. Five pools of refrigerated semen were analyzed. For each treatment, a 6-mL aliquot was supplemented, or not, with resveratrol during the first 24 h of refrigeration, which means the moment the sample arrived at the laboratory. On days 0, 3, and 7 of refrigeration, 1-mL aliquots from each group were warmed at 37°C, washed, and resuspended on TALP medium. For each aliquot, it was evaluated the motility and plasmatic membrane functionality (hypo-osmotic test) using an optical microscope. Mitochondrial membrane potential and spermatic pre-capacitation level were evaluated using JC-1 and chlortetracycline fluorochromes, respectively, through an epifluorescence microscope. Viability and acrosome integrity were evaluated by the trypan blue stain, and acrosomal patron using a differential interference contrast microscope, respectively. Data were analyzed using ANOVA ($P < 0.05$). On day 0, the samples presented 76.4% live spermatozoa with intact acrosome, with 84.6% high mitochondrial membrane potential and 60.0%, 66.3%, and 1.3% progressive motility, membrane functionality, and pre-capacitated spermatozoa, respectively. No significant differences were observed for the percentage of pre-capacitation caused by preservation time or the addition of resveratrol. The percentages of live spermatozoa with intact acrosome, progressive motility, and functional plasma membranes decreased over preservation time ($P < 0.05$), regardless of the presence of resveratrol in the conservation medium. On the other hand, the percentage of sperm with high mitochondrial membrane potential decreased both due to storage time ($P < 0.05$) and the presence of resveratrol ($P < 0.05$). This significant decrease in the percentage of spermatozoa with high mitochondrial membrane potential could indicate that refrigeration of spermatozoa with resveratrol would allow for maintaining a favorable oxidation–reduction state to preserve mitochondrial metabolism and may contribute to maintaining the fertilizing capacity of refrigerated spermatozoa. To test this hypothesis, we are evaluating the effect of the addition of resveratrol on the production of mitochondrial superoxide anion and on the fertilizing capacity of semen through *in vitro* fertilization.

A31

EARLY DECIDUAL VASCULARIZATION IN A MURINE MODEL OF PERIGESTATIONAL ALCOHOL INTAKE: ENDOTHELIAL ALTERATION AND EXTRACELLULAR MATRIX REMODELING AND CHANGES IN THE EXPRESSION PATTERN OF VEGF-MMP-9 AND UNK

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Decidual vascularization involves processes of proliferation, differentiation, migration, tubulogenesis, luminal expansion, and remodeling of tissues and the extracellular matrix (ECM), through molecular mechanisms among which the VEGF/KDR-MMPs system stands out, where uNKs cells play a crucial role in the control of these processes. Previously, we demonstrated that peri-gestational alcohol consumption in the mouse up to day 10 of gestation produces alterations in angiogenesis/decidual vascularization of implantation sites (IS), associated with decreased expression of VEGF and uNKs population. Our aim was to determine the early origin of these alterations during D7–8–8.5 gestation and to evaluate potential changes in angiogenic mechanisms. Ethanol (10%) was administered in drinking water to female mice (TF) for 17 days before and up to days 7, 8, or 8.5 of gestation to obtain IS. Free-ethanol water was administered to control females (CF). TF IS-D7-8.5 showed reduced luminal expansion area ($P < 0.01$ and $P < 0.05$) (HyE-PAS, ImageJ) and endothelial proliferation index (Nr. $PCNA^+$ cells/mm^2) *vs*. SI-CF ($P < 0.05$). TF IS-D8-8.5 showed histological alterations and lower ECM deposition (PAS histology), which was accompanied by increased MMP-9 immunoexpression *vs*. IS-CF ($P < 0.05$). While the population of uNKs (Lectin-DBA) was increased in TF IS-D7-8.5 *vs*. CF ($P < 0.05$), the Nr. $VEGF^+$ cells/mm^2 in the vascular decidua ($P < 0.001$, $P < 0.05$) and the Nr. KDR^+ cells/mm^2 of stroma and endothelium ($P < 0.05$) increased in IS-D8-8.5 of TF *vs*. CF. These results suggest significant dysregulation of cellular processes and angiogenic mechanisms controlling early decidual vascularization induced by peri-gestational alcohol exposure in the murine model.

A32
STUDY OF CRISP PROTEINS AS TARGETS FOR CANINE CONTRACEPTIVE VACCINE

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The uncontrolled growth of dog populations causes a negative impact on both public health and animal welfare. Given that Cysteine Rich Secretory Proteins (CRISP) are evolutionarily conserved and relevant for fertilization in different species, and that at least one canine CRISP has been identified in our laboratory, the aim of this work has been to characterize the canine CRISP as a possible target for the development of a contraceptive vaccine for dogs. In this regard, the epididymis is considered an excellent male contraceptive target as it does not affect testicular function and because of the permeability of the blood–epididymal barrier. To study the epididymal origin of the identified canine CRISP protein, testes, and epididymis from surgical sterilizations of 8 dogs were analyzed by Western Blot, using the same anti-human CRISP3 antibody used to identify the canine CRISP. The results showed the presence of canine CRISP in all the epididymal extracts analyzed and its absence in the corresponding testicular extracts, confirming the epididymal origin of the canine CRISP. To investigate whether this CRISP is present in sperm and remains there even after capacitation, ejaculated canine sperm were incubated for 4 h at 37ºC and 5% CO_2 in two different capacitation media. The results obtained confirmed the ability of both media to capacitate canine sperm as judged by the levels of protein tyrosine phosphorylation, progressive motility, acrosomal reaction, and hyperactivity. On the other hand, indirect immunofluorescence studies using anti-human CRISP3 revealed the presence of canine CRISP on the surface of the acrosomal region of fresh sperm and its relocation towards the equatorial segment of capacitated sperm, indicating the accessibility of the canine CRISP to the antibodies to be generated after immunization. Furthermore, since the equatorial segment is the region through which gamete fusion occurs, these results support the possible involvement of canine CRISP in the gamete fusion step during fertilization. Together, these results indicating the epididymal origin of the canine CRISP protein and its presence on the surface of fresh and capacitated spermatozoa strongly support its use as a target for the development of a contraceptive vaccine for dogs of both sexes.

A33
OVERCONSUMPTION OF *Stevia rebaudiana* AND ITS EFFECTS ON THE ESTRAL CYCLE, REPRODUCTION AND THE PROGENY OF RATS

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Sucrose overconsumption during youth is detrimental to health. An alternative to sucrose is the non-caloric sweetener stevia, which comes from the plant *Stevia rebaudiana* Bertoni (Asteraceae), native to tropical South America. The administration of stevia produced effects at the gonadal level in male rats (M), inducing alterations in reproductive capacity. In a previous study, we observed that the administration of stevia to female rats (H) from DPN21 produced changes in the estrous cycle and in the amount of pregnant H. The objective of this work was to validate the previous results and to evaluate changes in the progeny. H (SD) rats received water (CON group, N = 16) or water sweetened with stevia (STE group, N = 16) from DPN21. The estrous cycle was evaluated between DPN50-71 through daily vaginal exudates. At DPN72, half of the H from each group was sacrificed at an equal stage, and body and uterine weights were recorded. The rest of the H mated with sexually active M between DPN72-76 (2:1). Day 1 of pregnancy was determined by observation of spermatozoa in the vaginal exudate. Pregnancy success (H pregnant/H total) and the duration of pregnancy were evaluated. In the litters, the quantity, sex ratio, survival (alive pups/total pups), and pup weights were studied. The STE and CON dams were sacrificed after weaning the pups (DPN21) at the same estrous stage, recording body and uterine weight. As observed in the previous trial, STE had fewer cycles (15.20%), normal cycles (25.73%), and proestrus (18.03%) than CON ($P < 0.05$). There were no differences between the body weights of STE and CON; however, STE presented a lower uterine weight (24.40%; $P < 0.05$). In mated STE, a 25% reduction in pregnancy success was observed. No differences in the duration of pregnancy were observed, but one of the six STE rats showed a longer period (24 days). STE dams gave birth to fewer pups than CON (42.75%; $P < 0.01$), with no differences in the sex ratio. The STE progeny had a lower survival rate than the CON progeny (71.43% for H and 74.07% for M; $P < 0.05$). The STE dams presented lower weights than the CON dams (11.73%; $P < 0.05$), but no differences were observed in uterine weights. These results support those obtained previously and indicate that the overconsumption of stevia in H rats affects the reproductive capacity and the survival of the progeny. *[Funding: CONICET-PIP00243 and PICT2019-623.]*

A34
CALTRIN – SPERM INTERACTION, RIDERS OF THE LOST RECEPTOR

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Caltrin is a protein secreted in the seminal vesicle of mammals, which binds to sperm at the time of ejaculation and regulates the entry of calcium into these cells during the transit in the female reproductive tract. In rats, caltrin binds exclusively to the acrosomal region of the sperm membrane. This distribution, observed by Indirect Immunofluorescence (IIF), suggests the presence of a receptor. The goal of this work is to study the mechanism of interaction of caltrin with murine sperm. In the first instance, we observed, by affinity chromatography, the interaction of caltrin with HongrES1, a soluble protein secreted in the cauda region of the rat epididymis. Protein-

protein interaction simulations were performed using the HADDOCK server, where different regions of affinity between caltrin and HongrES1 were identified. Then, it was determined if this interaction occurs *in vivo*, and for this purpose, cauda spermatozoa were treated with solutions of increasing ionic strength (NaCl 0, 0.25, 0.5, and 1 M) to remove HongrES1; after that, sperms were incubated with caltrin and their presence in spermatozoa was determined by IIF. The results show a 50% decrease in caltrin-labeled cells from 0.5M NaCl treatment. Cells washed with 1M NaCl were incubated with *cauda* lumen content (CLC, obtained by retroflushing) and then treated with caltrin. This treatment recovered percentages of caltrin-labeled cells similar to the no-wash control. On the other hand, spermatozoa from the *caput* region (which have a different degree of maturation than those from the *cauda*) were treated with CLC and caltrin. No significant differences were observed with respect to the samples without CLC treatment. Taken together, these preliminary results suggest that caltrin would bind to sperm through HongrES1, which would interact with the sperm membrane depending on its lipid composition.

A35
ENDOSULFAN ALTERS THE SIGNALING PATHWAYS THAT REGULATE OVIDUCTAL ADENOGENESIS IN THE BROAD-SNOUTED CAIMAN (*Caiman latirostris*) AND MODIFIES THE TEMPORAL PATTERN OF HISTOMORPHOLOGICAL CHANGES

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Adenogenesis is a critical process in the differentiation of the broad-snouted caiman oviduct. Regulation of adenogenesis depends on several molecular pathways, which, in turn, are modulated by sex steroid hormones and can be affected by exposure to endocrine-disrupting compounds (EDCs). Endosulfan (END) is a persistent organic pollutant classified as an EDC with xenoestrogenic action. Our aims were (1) to characterize changes in the oviduct epithelium and subepithelium during adenogenesis; (2) to evaluate the effects of *in-ovo* exposure to END (20 ppm) on these processes; and (3) to determine the circulating levels of E_2 and T. In the oviduct of prepubertal juvenile female caimans (VEH and END), we established a histofunctional score and the organization of subepithelial collagen fibers, and we evaluated the gene expression of proteins involved in subepithelium remodeling (metalloproteinases and its inhibitors), intracellular adherence (CTNNB1, the gene that encodes β-catenin, and the protein itself), and induction of epithelial invagination (Wnt-7a). The histofunctional score was evaluated through the frequency of observation of tissular and cellular features indicative of oviductal differentiation. Collagen organization was quantified as the percentage of the total subepithelial width occupied by poorly organized fibers or by highly organized fibers. Gene expression was assessed by qPCR, using L8 as a housekeeping gene. Protein expression was revealed by immunohistochemistry and quantified in digitalized images. *In-ovo* exposure to END advanced oviductal adenogenesis, as evidenced by increased histofunctional score (VEH 14.5 ± 2.38 *vs.* END 19.63 ± 1.40) and supported by increased gene expression of MMP2 (VEH 3.20 ± 0.98 *vs.* END 6.63 ± 2.26), MMP9 (VEH 2.36 ± 0.47 *vs.* END 3.65 ± 0.95), TIMP1 (VEH 1.74 ± 0.17 *vs.* END 2.61 ± 0.24), and CTNNB1 (VEH 1.23 ± 0.35 *vs.* END 1.96 ± 0.53). No changes were observed in the circulating levels of E_2 and T. Results (mean ± SEM) show that the molecular pathways that regulate adenogenesis and the temporal pattern of histomorphological changes of the oviduct are altered by *in ovo* exposure to END, which could lead to alterations in fertility.

VETERINARY AND ANIMAL BIOLOGY

A36
TISSUE REMODELING IN THE OVARY OF THE PLAINS VISCACHA

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During follicular dynamics and ovulation, there is tissue remodeling. Enzymes participate in it, among which metalloproteases 2 and 9 (MMP-2 and MMP-9) stand out, which degrade components of the extracellular matrix (ECM) such as type IV collagen and fibronectin. The plains viscacha (*Lagostomus maximus*) was used as a model to analyze this process because it has the highest poly-ovulation rate known to date among mammals. The objective of the work was to determine and compare the expression of MMP-2 and MMP-9 by immunohistochemistry and to quantify collagen in females with different reproductive states. Viscacha ovaries were used at different stages of pregnancy (early and middle) and non-pregnant (N = 15), captured at the ECAS (Ministry of Agroindustry, Province of Bs. As.), which were processed for inclusion in paraffin and cut every 3-µm thick. Antibodies anti-MMP-2 and anti-MMP-9 were used to perform the indirect immunohistochemical technique. Labeling was classified as: negative, weak, moderate, and intense. On the other hand, using the Picrosirius technique, ECM collagen was identified for subsequent observation using polarized light microscopy and quantification with Image-Pro Plus software. The immunostaining in oocytes at the different follicular stages in all females was moderate for MMP-2 and 9 in the cytoplasm. The same pattern was observed in follicular cells of primordial and primary follicles, and granulosa cells in secondary and tertiary. MMP-9 labeling was moderate in the internal and external thecae of secondary and tertiary follicles and the ECM surrounding these structures. MMP-2 was moderate in the theca interna and weak in the theca externa. MMP-9 labeling in corpus luteum (CL) ranged from negative, moderate, and strong in the cytoplasm of luteal cells. It was negative in peripheral connective tissue in early pregnancy and weak in mid-pregnancy. MMP-2 showed a characteristic mark in the center of the CL of pregnant females,

decreasing in areas near the periphery. On the contrary, that pattern was not observed in CL from non-pregnant females. The blood vessels of these structures were negative for both antibodies. In addition, MMP-9 was inconsistently positive in the nuclei of granulosa cells at different follicular stages. In all females analyzed, the superficial epithelium was marked intensely, while the tunica albuginea was moderate and the medullar connective tissue weak for both antibodies. In the tunica media of the medullary arterial vessels, labeling for MMPs 2 and 9 was intense. Furthermore, MMP-9 weakly labeled some endothelia of these vessels in mid-pregnancy. Both antibodies did not label the venous and lymphatic vessels. The total amount of collagen did not show significant differences among the analyzed groups. The presence of MMPs in the ovarian structures would correlate with the need for remodeling and the availability of greater space in the ECM that accompanies follicular development and the subsequent formation of CLs. Future studies are required to deepen the understanding of tissue remodeling during folliculogenesis and pregnancy.

A37
SEASONAL CHANGES IN THE OVARY OF *Myiopsitta* (PSITTACIFORMES, AVES)

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The aplasia of the right ovary and oviduct during embryological development is one of many adaptations associated with body weight loss and flight in birds. The appearance of the remaining left ovary in adults may change based on reproductive seasonality. The monk parakeet *Myiopsitta monachus* is one of the few species of Psittaciformes considered an agricultural pest. It has spread in its native range in Argentina and throughout the world. We aim to study the morpho-histology of the ovary of *Myiopsitta* during breeding (October–December) and non-breeding (January–August) seasons. Four adult specimens (two from each season) were analyzed. Ovaries were dissected and fixed in 4% formalin and preserved in 70% alcohol solutions. They were measured with a caliper (accuracy: 0.01 mm) and a weighing scale (accuracy: 0.001 g) and processed for histological techniques: hematoxylin–eosin, Gomori trichrome, periodic acid Schiff/hematoxylin (PAS), and orcein. In the non-breeding season, the ovaries are 6.31 mm long by 5.78 mm wide, and they weigh 0.087 g on average. They are triangular and have poorly defined follicles separated by slight furrows. While in the breeding season, the ovaries increase their size. They reach to measure 8.87 mm long by 6.35mm wide, and they weigh up to 0.113 g on average. Their appearance resembles a bunch of grapes, with follicles protruding from the surface and delimited by deep furrows. Histologically, a single cuboidal epithelium surrounds the ovaries. They have two distinct zones: the outer cortex with follicles at different developmental stages, separated by a thin connective tissue network with collagen fibers and a few elastic fibers; and the inner stroma composed of well-vascularized and innervated connective tissue. Growing follicles show PAS positivity in the basal membrane and the follicular epithelium. The external and internal thecas have collagen fibers arranged in concentric layers. Non-breeding season ovaries have primordial, primary, growing (previtellogenic), and non-bursting atretic (lipoidal and lipoglandular-invasive) follicles. While breeding season ovaries show the highest follicular development having several growing (previtellogenic) and vitellogenic follicles. They also have few preovulatory, postovulatory, non-bursting (lipoglandular-invasive), and bursting atretic follicles. The ovary of *Myiopsitta* is similar to that of other bird species with marked reproductive seasonality. This study represents the first of a series expected to set the baseline for future studies useful in veterinary medicine and pest control fields.

A38
MORPHOMETRIC STUDY OF THE PLACENTAL VESSELS OF THE CAT (*Felis catus*)

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The maternal–fetal interface of the feline placenta is endotheliochorial. The ability of the placenta to face the increased fetal demand for nutrients and oxygen depends on the expansion of the placental vascular networks. Our objective was to determine, through a morphometric study, changes that occur in these networks during the cat's placentation. Fourteen placental samples from cats at different gestational times were collected by ovariohysterectomy, required and authorized by the animals' owners. They were then processed using the routine histological technique. Three zones of the placenta were analyzed (Z1: fetal zone; Z2: laminar labyrinth; Z3: junctional zone). Ten images of each zone and each sample were obtained, then they were analyzed with the AxioVision Release 4.6.3 software to determine the following variables: number of vessels, vascular area, and vascular density. The data were statistically analyzed with the Infostat 2020 software. Our results demonstrated significant differences in the number of vessels among early, middle, and late placentas, being the blood vessels more numerous in those from advanced gestations ($P < 0.05$). Likewise, both vascular area and density were higher in these placentas compared to those from early and mid-gestation; however, there were no significant differences between the latter two ($P > 0.05$). Concerning the study of the vessels by zones, significant differences were only found between the three stages in the vessel number in Z2. It is possible to infer that the greater number of vessels recorded in mid-gestation placentas compared to earlier ones corresponds to a process of angiogenesis through branching since the vascular area does not change. The increase registered for all the variables in the last third of gestation coincides with a stage of significant fetal growth and, therefore, greater nutritional demands.

A39

EXPRESSION OF VEGF-A AND VEGFR-2 IN THE OVARY OF THE PLAINS VISCACHA *(Lagostomus maximus)*

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The plains viscacha, *Lagostomus maximus*, is the mammal with the highest ovulatory rate known to date (200–800 oocytes/estrus). Some processes related to poly-ovulation in this species, including angiogenesis, have not yet been studied in depth. This process is regulated by numerous factors, including vascular endothelial growth factor A (VEGF-A), which interacts with its receptors VEGFR-1 and VEGFR-2. This work aimed to determine and compare the expression of angiogenesis markers in viscacha´s ovaries at different reproductive stages. From material from animals captured at the Wild Animal Breeding Station of the Ministry of Agroindustry of the Province of Buenos Aires, ovaries were obtained from 9 females at different stages of pregnancy (early and mid-pregnancy) and non-pregnancy, which were processed for inclusion in paraffin. Sections were cut every 3 µm thick, and the indirect immunohistochemical technique was used with anti-VEGF-A antibodies and its receptor VEGFR-2. The following qualitative scale was used to determine the intensity of the marking observed: negative, weak, moderate, and intense. VEGF-A labeling was moderate in the cytoplasm of luteal, granulosa, and theca interna cells of both secondary and tertiary follicles. The external theca of these structures had markings that varied between weak and moderate. VEGFR-2 expression was observed in the same structures but with greater intensity than VEGF-A in granulosa cells. In the periphery of the primordial follicles, the immunostaining of most of the samples was positive, with a weak intensity, although in some samples, it was negative. The marking of the surface epithelium was intense for both antibodies in all stages. Both in the medulla and the ovarian parenchyma, mainly in the vicinity of follicles in different stages of development, the marking of the tunica media of the arteries was intense for both antibodies, being negative at the endothelial cells. Venous vessels of different calibers and lymphatics were negative in all their layers. The ligand's expression pattern and its receptor were similar in the different stages of follicular, luteal, and vascular structures. These results may be related to the need for angiogenesis activation that accompanies the follicle's development and the persistence of the corpus luteum during pregnancy.

A40

ESOPHAGUS AND CROP HISTOLOGY OF *Myiopsitta* (PSITTACIFORMES, AVES)

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The monk parakeet *Myiopsitta monachus* is a worldwide distributed species found in urban environments. It is considered an agricultural pest causing economic problems due to its mainly granivorous diet. One study from 1983 is known about the cranial portion of the digestive system of *Myiopsitta*, but it only analyzes specimens of unknown sex and from breeding seasons. The general structure of the digestive system is similar between birds, but it may have several specializations mainly linked to the different diets. For example, the crop (*i.e.,* an expansible portion of the middle esophagus in which food is temporarily stored) may be present in some species. We aim to study the esophagus and crop of female adult specimens of *Myiopsitta* by comparing our results with those available for the species. We also aim to find possible differences in the organs between the non-breeding and breeding seasons. Four adult specimens (two from each season) were analyzed. Esophagus and crops were dissected and fixed in 4% formalin and preserved in 70% alcohol solutions. Samples were processed for the histological techniques, hematoxylin–eosin and Gomori trichrome. The esophagus has three of the four usual layers of the digestive system: mucosa, muscularis, and adventitia (the submucosa layer is absent). The mucosa layer has a stratified squamous keratinized epithelium. In the crop, the keratinization thickens, and cellular band desquamation is observed. Deep to the epithelium is a thin connective tissue layer without glands, except in the thoracic esophagus where simple branched alveolar glands are present. The muscularis layer is thick and the two smooth muscle layers are separated by loose connective tissue with several nerve plexus and blood vessels. Finally, the adventitia layer has collagen fibers and is highly vascularized and innervated. Two noticeable results emerge from our observations: (1) the absence of muscle in the mucosa, described as being present in previous studies; and (2) the arrangement of the layers in the muscularis: an inner longitudinal muscle layer that is lost in the crop, and a thicker outer circular muscle layer. Compared to *Myiopsitta*, the arrangement of the muscle layers of the muscularis is reversed in most birds. Future studies on the embryonic development of the digestive tube could help clarify both aspects. Neither accumulate desquamation of the crop epithelium associated with the ‘crop milk’, nor relevant differences between specimens of the non-breeding and breeding seasons are observed. This suggests that females of this species do not produce the crop milk as reported for some Psittaciformes. Future immunohistochemical studies with anti-prolactin markers (a hormone linked to crop milk production) will help to reaffirm this hypothesis.

A41

MORFOHISTOLOGY AND GLYCOCONJUGATES EXPRESSION IN THE OVIDUCTAL GLAND OF A FISHERY RESOURCE OF COMMERCIAL IMPORTANCE: *Callorhinchus callorhynchus*

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Callorhinchus callorhynchus (Linnaeus, 1758) (cockfish) inhabits South American waters, distributed from the southwestern Atlantic to the southeastern Pacific. The cockfish represents the only Holocephalan species reported for the Argentine Sea and is commercially exploited throughout its distribution range. It is an oviparous species and lays its eggs in a complex and resistant capsule, secreted by an exclusive organ of Chondrichthyans: the oviductal gland (OG). The OG structure is closely related to the egg capsule morphology and the reproductive mode. In some chondrichthyans, the OG may serve as a sperm storage site. In this study, histochemical and lectin-histochemical techniques were used to analyze the histological structure of the OG, determine variations in the glycoconjugates expression in the different zones of the gland, and if there is sperm storage in the species. Specimens were collected from landings of the commercial fleet operating in "San Matías Gulf". For histological analysis and lectins treatment, mature females were selected. OGs were fixed in formalin or Bouin´s solution in seawater and processed according to routine histological techniques. The OG exhibits three tunics: mucosa, muscular, and serosa. The four characteristic zones were observed: from cranial to caudal, Club (CZ), Papillary (PZ), Baffle (BZ), and Terminal (TZ). The CZ presents lamellae extended apically and narrow at their base, separated by grooves in which simple tubular mucosal glands PAS and AB pH 2.5 (+) open. The PZ has papillae-shaped lamellae, separated by grooves in which the mucous glandular tubules PAS and AB pH 2.5 (+) open. In this region, caudal adenomers showed a greater reaction to AB (pH 2.5). The CZ and PZ are responsible for secreting the "jelly" that covers the egg. The BZ is the most developed zone and has tubular glands that produce serous granules, PAS and AB pH 2.5 (−), which will form the egg capsule. The TZ is not organized into lamellae and contains simple glandular tubules, with different reactivity to PAS and AB pH 2.5, depending on their height. In this area, the presence of sperm storage was confirmed. The four OG zone were positive for each of the lectins used but presented variations in intensity according to the lectin type and zones. This study provides the first histological and histochemical information on OG and the first record of sperm storage in the species. PGI 24/B286.

A42

EFFECTS OF GESTATIONAL ENVIRONMENTAL ENRICHMENT ON BEHAVIORAL PARAMETERS IN ADOLESCENT MALE AND FEMALE RAT OFFSPRING

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The perinatal environment is important for the neurophysiological and behavioral development of offspring. There is evidence that both positive stimuli such as maternal Environmental Enrichment (EE) and negative stimuli such as exposure to postnatal stressors generate neurostructural and behavioral changes in rodents. On the other hand, the brain is a sexually dimorphic structure and differential effects of EE on neuroendocrine and behavioral responses in animals have been described. In this work, we analyzed whether gestational EE could influence social and emotional behavior in male and female adolescent rats and whether it could modulate behavioral responses to early-life stress. Two-month-old virgin Wistar rats were mated and then housed during gestation (from day 1 to day 20) in EE cages (larger size, toys, running wheels, tunnels, ramps, etc.) in groups of 8 pregnant females or in standard cages in groups of 2. On postnatal days (PND) 1 to 21, one of the following stress conditions was applied: the presence of intruder male (IM), maternal separation (MS), or no stress. After weaning at PND 21, the offspring were separated by sex in standard cages. Between PND 45–50 offspring were assessed in Open Field (OF), Elevated Plus Maze (EPM), and Social Preference (SP). It was observed that females, regardless of gestational or postnatal condition, present a higher number of line-crosses and rearings, and spend more time in the center of the OF and in the open arms of the EPM compared to males. Nevertheless, males have a higher SP index. Interestingly, in animals subjected to early stress, an increase in the number of line-crossings, number of entries to the center, and a higher SP index were observed. On the other hand, the offspring of EE mothers presented a higher number of entries and time spent in open arms and a higher SP index. These results suggest that perinatal stimuli have differential effects on the behavior of adolescent male and female rats; females show fewer anxiety-like and social behaviors, but more exploratory behaviors than males. In turn, gestational EE prevents some of the deleterious effects of early exposure to stress. These results indicate that gestational EE may be a promising strategy for the future well-being of adolescents.

A43

RODLET CELLS AND GRANULAR EOSINOPHILIC CELLS, COMPONENTS PRESENT IN THE INFLAMMATORY RESPONSE TO METACERCARIASES OF *Ascocotyle* sp.

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In South America, the genus *Ascocotyle* (Digenea, Heterophyidae) is widely distributed and is of zoonotic importance. Trematodes of this genus are common parasites of fish-eating birds and mammals and their metacercariae are encysted in various fish tissues. These agents produce an extensive inflammatory response, with rodlet cells (RCs), a single cell type, with a capsule and distinctive cytoplasmic granule, being found only in a limited number of teleost fish. Granular eosinophilic cells (GECs) are also involved in the inflammatory response to parasitic agents and have cytoplasmic granules with various mediators and inflammatory factors. In a parasitological survey carried out in the "Río de la Plata" river, specifically in "Punta Lara", metacercariae cysts of the genus *Ascocotyle* sp. were found in the aortic bulb and gills of adult specimens of *Jenynsia lineata*. The objective of this work was to describe the cellular components present in the inflammatory response. For this purpose, gills from four specimens and two hearts with cysts fixed in buffered formalin 10% were processed by the traditional histological technique of wax embedding. Five-µm sections were subsequently stained with hematoxylin–eosin, Masson's trichrome, PAS, and acridine orange. Histological sections showed that the metacercariae were surrounded by a thin connective tissue capsule and were encysted in the gill filaments, secondary lamellae, and aortic bulb. The cystic areas of the gill filaments, a shortening of the length of the secondary lamellae, dilatation and congestion of the lamellar capillaries, and cellular desquamation were observed. In addition, at the base of the parasitized gill filaments and in the aortic bulb, an abundant inflammatory infiltrate composed primarily of lymphocytes, GECs, and RCs was seen. The PAS technique revealed the presence of neutral glycoproteins in the capsule of the RCs, while acridine orange identified the presence of heparin in the GEC granules. The impact of the inflammatory response produced by these cell types could be related to alterations in different physiological functions of the infected organs.

BIOTECHNOLOGY, GENETICS AND MICROBIOLOGY

A44

SPERM-MEDIATED GENE TRANSFER (SMGT) IN ARTIFICIAL INSEMINATION IN CATTLE (*Bos taurus*)

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Genetic engineering in farm animals is in precarious development, due to the high cost, low efficiency, and high biological risk of the usual methods. We seek to standardize methods that, using the spermatozoon as a vector of heterologous gene constructions, produce transgenesis in the act of fertilization in different species, either by In-Vitro Fertilization (IVF) or by Artificial Insemination (AI). In this work, we set out to achieve bovine (*Bos taurus*) embryos, genetically modified by AI method. Two semen donor bulls from the Las Lilas Genetic Center were evaluated for their ability to internalize DNA in properly treated sperm (SPZ), using two concentrations of the commercial plasmid (*pEGFP N-1 4.7kb*), labeled with Cy3. Aliquots of treated and untreated sperm suspensions were analyzed under a laser capture microscope (Nikon TE 2000). One of the bulls showed better uptake rates. The bull and plasmid concentration chosen were used to treat a sufficient quantity of SPZs to inseminate a total of four adult cows, hormonally stimulated (TSOV) to achieve ovarian superovulation on heat day. The TSOV was performed by insert a progesterone vaginal device (DIB of 1 g) on day 0, for seven days, associated with a sequence of four double/dose day of porcine follicle-stimulating hormone (FSHp) starting on day 4, plus a double dose of PGF2α on day 6, and a dose of GnRH on day 8, day of estrous. Days 8 and 9 from the start of TSOV treatment, eight doses of 5×10^6 SPZs, two untreated and six treated, were used for a deep intrauterine insemination, in double doses, half at right, half at left horn, with twelve interval hours between dose, in each experimental animal. On day 15 from the beginning of the TSOV, a transcervical, non-surgical, embryo collection was performed. A total of 24 embryonic structures (ES) were collected from the four inseminated cows, treated and control group. From the treated group, 18 embryos (E) and 6 unfertilized eggs (UO) were obtained, 15 E from 3 cows in this group, showed green (GF) and red (RF) fluorescence, 3 E from the control group were negative for GF and RF, as well as 3 UO obtained from both categories. Positive transfection efficiency from sperm to ova, were showed, since all embryos obtained from the treated group, in the three cows, show green and red fluorescence, in some, or all the blastomeres, while, the embryos obtained from the control cow, and the unfertilized oocytes did not show fluorescence.

A45

EVALUATION OF AN EXPERIMENTAL BOTROPIC ANTIVENOM PRODUCED WITH VENOM BLOCKED WITH Na_2-EDTA

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Snake envenoming is a serious medical problem in tropical developing countries and antivenoms are the only effective therapy. Antisera are produced by immunizing horses with snake venom and adjuvant, usually Freund's adjuvant. Botropic venoms contain metalloproteinases (SVMPs), responsible for the local effects of envenoming, such as hemorrhage, edema, and myotoxicity as well as systemic bleeding. SVMPs represent around 34.2% of the protein composition from *B. diporus* venom and cause lesions during the immunization of animals. In search of new processes, focused on animal welfare for the production of immunobiologicals, and taking into account that the toxic action of SVMPs is inhibited by Na_2-EDTA, in the present work, the immune response of animals inoculated with *B. diporus* venom blocked with Na_2-EDTA was evaluated. For this proposal, the *B. diporus* venom (1.9 mg/mL) was blocked by Na_2-EDTA (200 mM, B.dV/ Na_2-EDTA) and used as an antigen. Previously to the inoculation, the excess chelate was removed by molecular exclusion chromatography (Sephadex G-25). Likewise, the venom without inhibitor (B.dV) received the same treatment, and in both cases, the effective neutralization of SVMPs using azocasein as substrate was determined. Groups of five CF-1 mice were immunized subcutaneously on days 0, 15, and 30, with B.dV or B.dV/ Na_2-EDTA (7–30 µg or 14–60 µg) emulsified with complete Freund's adjuvant and incomplete (booster). On days 14, 29, and 37, blood samples were collected from the tip of the animal tails, and, on day 45, the final bleeding and the separation of the different sera were performed. These were destined for immunoassays and neutralization assays for proteolytic, indirect hemolytic, and coagulant activity. The results of the enzyme-linked immunosorbent assay showed that both anti-B.dV and anti-B.dV/EDTA sera had high antibody titers (1/74.850 – 1/186.150) at the end of the immunization protocol. Regarding the Western Blot, the anti-B.dV/EDTA serum recognized the main bands, corresponding to the venom proteins, in a similar way as the anti-B.dV. Additionally, the experimental sera produced showed a neutralizing capacity of the main toxic activities tested *in vitro*. This result shows that Na_2-EDTA does not affect the immunogenicity of proteins since animals immunized with B.dV/Na_2-EDTA respond to *B. diporus* venom in a similar way to animals immunized with venom without the inhibitor.

A46

PARTICIPATION OF *Bifidobacterium animalis* subsp. *lactis* INL1 IN THE MODULATION OF THE BEHAVIOR OF INTESTINAL TUMOR CELLS AND THE TUMOR MICROENVIRONMENT

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Parathyroid hormone-related peptide (PTHrP) promotes the epithelial–mesenchymal transition program (EMT) and other events associated with the aggressive phenotype in the HCT116 line from colorectal cancer (CRC). Based on the importance of the microbiota in intestinal epithelial homeostasis, herein the goal was to evaluate whether the strain *Bifidobacterium animalis* subsp. *lactis* INL1 (*B. lactis* INL1), considered a new probiotic, is capable of modulating CRC progression events. This strain was provided by Dr. Vinderola through a transfer agreement between UNS and UNL (file No REC-1092496-21). Initially, an *in-silico* analysis was carried out, which then guided us in the *in vitro* tests. Using the Gene Expression Omnibus (GEO) database and the GEO2R program, we obtained differentially expressed genes (DEGs) from the GSE15636 microarray dataset. DEGs resulted in 1289 overexpressed genes (Or) and 1420 downregulated genes (Dr) in the Caco-2 line from CRC exposed to cell-free supernatant (CFS) of *Bifidobacterium animalis* subsp. *lactis* 420 (B420). Functional enrichment by EnrichR showed that these Or genes are involved in the regulation of cell migration, cadherin binding, and cell-substrate binding. Furthermore, we observed that the relevant epithelial markers E-cadherin and ZO-1 increase their expressions in Caco-2 cells due to the CFS action of B420. Based on this analysis, we evaluated whether the CFS of *B. lactis* INL1 modulates TEM-associated changes. Through morphological studies, we observed in HCT116 cells that the pre-incubation for 4 h with this CFS inhibits the typical cell elongation of the mesenchymal state and induced by PTHrP. On the other hand, we recently reported that strains with recognized probiotic action decrease the expression of genes that are overexpressed in CRC clinical samples, including the macrophage inhibitory cytokine/GDF-15. For this reason, we evaluated whether the CFS of *B. lactis* INL1 is also capable of directly modulating macrophage migration, a phenomenon associated with antitumor surveillance of the tumor microenvironment. Using Transwell migration assays, we observed that the CFS of *B. lactis* INL1 increases the migratory capacity of RAW264.7 macrophages at 2 and 3 h of treatment. Taken together, these findings suggest that *B. lactis* INL1 modulates the behavior of tumor cells and their microenvironment and support the importance of analyzing the role of this strain in communication within the tumor niche.

A47

EVALUATION OF THE PREVALENCE OF ANTIBODIES ASSOCIATED WITH CELIAC DISEASE

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Celiac disease (CD) is a chronic autoimmune enteropathy based on permanent intolerance to a set of proteins called prolamines, which are present in wheat, barley, rye, and oats, in genetically susceptible individuals. According to the Ministry of Health, it is estimated that more than 400,000 people suffer from CD in Argentina; however, given that most do not present symptoms, it is inferred that it could be even higher. The titer of anti-tissue transglutaminase antibodies, isotype IgA (a-tTgA), is the serological marker of choice for diagnostic support. The objective of the work was to carry out a study of the prevalence of antibodies related to CD in the town of Chivilcoy, Buenos Aires. For this, serum samples were taken from 537 volunteers. Each volunteer signed an informed consent and completed a questionnaire on demographic and anthropometric characteristics, environmental factors, diet, physical activity, medication, family history, symptoms associated with CD, etc. The titer of a-tTgA and a-GliA was determined in all the samples using ELISA kits developed in the "Laboratorio de Inmunología" of the Universidad Nacional de Luján. Recombinant human type 2 tTg produced in *E. coli* and commercial gliadin were immobilized in 96-well plate. Subsequently, the serum samples of the volunteers were incubated. In order to detect the presence of specific IgA antibodies, the corresponding a-IgA antibody conjugated with peroxidase was added as a second step. Once the substrate was added, the reading was performed at 450 nm in a plate reader. Finally, the analysis of the results obtained was carried out, comparing the presence of antibodies with the information collected in the questionnaires. 7.26% of the volunteers had a diagnosis of CD (7.54% women and 6.35% men, respectively). In addition, 57.7% declared having other diseases pathologies, with a higher proportion of women than men (67.9% and 24.6%, respectively). 78.8% of women and 55.5% of men reported gastrointestinal symptoms associated with CD. Regarding the antibodies evaluated, the proportion of volunteers with positive a-tTgA was 9.89% and a-GliA 32.5%. The kits developed in our laboratory allowed us to carry out the analysis of a-tTgA and a-GliA antibodies in the population studied. In the present study, a high population incidence of the antibodies evaluated was observed. In many cases, the high levels of antibodies corresponded to the presence of an associated clinical manifestation, although a large number of volunteers did not manifest themselves as celiac in the interrogation.

A48

MARE OF THE QUARTER MILE BREED (*Equus ferus caballus*) (MAMMALIA: EQUIDAE) WITH FAILURES IN THE REPRODUCTIVE BEHAVIOR

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The mare Lock Lady Car of the Quarter Mile Horse breed from "Haras El Remanso" (Corrientes province, Argentina) was bought at the age of six months, and at 24 months of age, she got into a taming and training program for short-distance races (375 meters). Before the age of four, the mare was withdrawn from training due to her poor performance and undesirable and dangerous behavior. At 10 years old, the clinical evaluation indicates a normal body condition (between 4 and 5 according to the Henneke horse body condition scale), but behavioral analysis shows the absence of estrus or scarce and/or abnormal estrus, low libido, and poor acceptance or rejection of the stallion during the different reproductive seasons, sometimes triggering estrus behavior in other females. To determine the possible origin of the failures in the reproductive behavior of the mare Lock Lady Car, the following studies were performed: clinical examination, ultrasound scan of the reproductive organs, and cytogenetic analysis of mitotic chromosome preparations by conventional staining and chromosome bandings, obtained from peripheral blood lymphocyte cultures. The direct visual inspection of the external and internal organs revealed no abnormalities. The ultrasound scan of the internal reproductive organs showed gonadal hypoplasia and a low number of follicles smaller than 10 mm in diameter. The veterinary diagnosis ruled out the treatment carried out during the taming and training program as a possible cause of the failures in reproductive behavior. The cytogenetic analysis of 200 metaphase cells revealed that the mare has a predominant somatic chromosome number of 2n = 64 (98 cells/49%), in concordance with the normal chromosome number for *Equus ferus caballus*, and a chromosome number of 2n = 62 (62 cells/ 31%). Besides, 40 cells with different aneuploidies (20%) were detected. In the literature, it is observed that cytogenetic studies in *Equus* have allowed the identification of chromosomal mutations associated with congenital anomalies, decreased or total absence of production of functional gametes, and/or embryonic loss. Based on the clinical examination and the karyological characterization of the mare Lock Lady Car, the presence of a blood chromosome mosaicism is evidenced which as the first presumptive diagnosis could correspond to a decrease in her reproductive performance. In *Equus*, mosaicism is often associated with reproductive tract abnormalities and the degree of mosaicism can modulate the extent of reproductive abnormalities observed.

A49
GENERATION OF NANOBODIES AS A BIOTECHNOLOGICAL TOOL FOR APPLICATION IN DIFFERENT AREAS OF BIOLOGY

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Camelids naturally generate IgG antibodies formed only by heavy chain. The variable region of these antibodies that recognizes the antigen is called nanobodies (Nbs). These single domain antibody fragments have a series of properties among which stand out their small size, easy production, high specificity, and affinity for the antigens they recognize; they can penetrate cellular tissue, recognize hidden epitopes, and are soluble and stable under critical conditions of temperature and pH. Through the application of different methodologies, Nbs can be obtained recombinantly and given their intrinsic properties are applied in areas such as diagnostics, therapeutics, imaging, immunotherapy, among others. Several Nbs are in phase I and II clinical trials, especially against cardiovascular, neurodegenerative, and autoimmune diseases. In the present work, we describe the pipeline we have put in place to obtain Nbs against different antigens which are mostly involved in diseases of local, regional, and global relevance. The strategy includes the use of phage display technology to select Nbs that recognize the antigen under study. Subsequently, we employ the ELISA or nickel plate technique, which allows spatial orientation of the target protein, and as a result hundreds of Nbs can be characterized according to their specificity. The selected Nbs are expressed as recombinant protein and also characterized by their affinity. From this robust, versatile, and simple platform we are able to obtain Nbs and use them as a biotechnological tool to apply them in different areas of biology such as electron microscopy, crystallographic studies, optogenetics studies and diagnostic assays.

A50
PRODUCTION OF LYSOLECITHINS WITH IMMOBILIZED ENZYME FROM RATTLESNAKE VENOM

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Enzymes are highly effective catalysts produced by living things and are required in very low concentrations. These characteristics make them suitable for use in industrial, food, cosmetic, and pharmaceutical applications, among others. The NEA are found species that are of interest for offering a secretion rich in enzymes that may have industrial applicability, such is the particular case of the rattlesnake (*Crotalus durissus terrificus*), which is characterized by its venom having a high content of phospholipase A_2 (PLA_2). These enzymes act on glycerophospholipids, release the fatty acid from the 2-position of glycerol, and thus lead to the formation of lysophospholipids. These molecules are excellent emulsifiers and surfactants suitable for use in many industries. Taking into account that the catalysts must remain in the reaction medium when the catalysis products are removed, the objective of this work was to immobilize *Crotalus durissus terrificus* venom (rich in PLA_2) and evaluate its use on a source rich in lecithins for the production of lysolecithins. The venom was immobilized on CNBr-activated Sepharose TM 4B at room temperature, with magnetic stirring for 24 h. The amount of venom retained was assessed by absorbance measurement (UV-280 nm). The PLA_2 activity of the venom was determined by colorimetric assay with phenol red. The crude lecithin extract (CLE) was obtained from egg yolk (20 g) by solvent extractions: first step with ethanol (96%), and then, the soluble fraction was treated with acetone; finally, the precipitate was dried in an oven at 37ºC. Lysolecithins were obtained by means of an enzymatic reaction under batch type system with ELs and PLA_2 previously immobilized in 10 mM phosphate buffer, for 2 h under agitation at 37ºC. The initial sample (ELs) as the liquid phase after the contact of the ELs with the immobilized enzyme was subjected to TLC on silica gel 60 F254 using petroleum ether/ethyl ether/acetic acid (90:10:1 v/v/v) and chloroform/methanol/water (65:35:3 v/v/v) as mobile phases. Developed with Dragendorff's reagent. The stains obtained were analyzed by densitometry (ImageJ software), and from the areas of each peak, the relative percentage content was estimated of lecithins and lysolecithins present in the different samples. It was immobilized to 99.64% of the protein content present in the initial solution of the venom. On the other hand, although lysolecithins are present in a small proportion in the initial lecithin sample, treatment with immobilized PLA_2 raised lysophospholipids by 20% compared to the initial sample. These preliminary findings will contribute to the use of matrices with immobilized enzymes for the production of lysolecithins with potential biotechnological use.

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A51
ROLE OF CRISP1 IN EPIDIDYMAL EPITHELIUM FUNCTION

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Epididymal proteins CRISP1 and CRISP4 associate with the sperm surface during epididymal maturation and participate in the fertilization process. Double knockout (KO) males for these proteins are subfertile and show clear defects in the differentiation of the epididymal epithelium as well as an alteration in luminal pH acidification critical for sperm storage in the organ. Based on this, the aim of the present work has been to investigate the role of CRISP proteins in epididymal epithelium function. For this purpose, we used immortalized epididymal epithelial cell lines from different regions of the epididymis called PC1 and DC2. Initially, we analyzed the expression of CRISP1 and CRISP4 by RT-PCR in the cells, observing the expression of *Crisp1* messenger in both lines and the absence of that corresponding to *Crisp4*. The presence of CRISP1 protein was neither detected in cell extracts nor in culture supernatants, indicating that *Crisp1* messenger was not being expressed in the cells in culture. Based on this, the following studies were carried out by adding CRISP1 to the cells in culture, mimicking what occurs *in vivo*. Considering that CRISP1 KO sperm show alterations in the cAMP-PKA signaling cascade, we decided to evaluate whether CRISP1 protein was also involved in this cascade at the epididymal epithelial level. Purified native CRISP1 was then added to the cells in culture and the levels of phosphorylation in PKA substrates were evaluated by Western Blot. Results showed an increase in phosphorylation levels similar to that observed when the cells were exposed to cAMP and not detected in the presence of a PKA inhibitor. On the other hand, no significant differences were observed in the phosphorylation of PKC or Src substrates. Given that one of the targets of PKA phosphorylation, the CFTR channel, is involved in ATP release to the epididymal lumen, and that extracellular ATP is essential for both epididymal and sperm function, we evaluated whether exposure of epithelial cells to CRISP1 was capable of modulating ATP release into the medium, observing an increase in ATP levels within the media. Altogether, these studies indicate the ability of CRISP1 to regulate not only sperm function but also the functionality of epididymal epithelial cells through the modulation of the cAMP-PKA signaling cascade.

A52
REACTIVE OXYGEN SPECIES AND ACTIVE MITOCHONDRIA DYNAMICS DURING THE *IN VITRO* MATURATION OF PORCINE OOCYTES

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The role of reactive oxygen species (ROS) in the processes related to the culture and *in vitro* manipulation of gametes and embryos is still controversial. It has been proposed that ROS may act as mediating molecules that influence processes such as cell proliferation, gene expression, and the activation of various signaling pathways, being the mitochondria and, particularly, the electron transport chain, the main source of ROS. Therefore, the aim of this work was to determine active mitochondria and ROS production fluctuations during the *in vitro* maturation (IVM) of porcine oocytes at 0, 24, and 44 h. Immature oocyte–cumulus complexes (COCs) were obtained by the aspiration of antral follicles from slaughtered gilts ovaries and only oocytes completely surrounded by an intact and dense cumulus were used. COCs were matured in medium 199 supplemented with 50 µg/mL gentamicin sulfate, 10% (v/v) porcine follicular fluid, 0.57 mM cysteine, 0.5 µg/mL FSH, and 0.5 µg/mL of LH, under mineral oil at 39°C, 5% CO_2 in air and 100% humidity for 44 h. To determine ROS production and active mitochondria dynamics, cohorts of COCs were extracted from the maturation medium at 0, 24, and 44 h, time points in which some of the most important events for IVM of porcine oocytes occur. The groups of extracted COCs were incubated with hyaluronidase at 37°C and denuded with a fine Pasteur pipette. ROS production dynamics were evaluated by fluorescein diacetate 2',7'-dichlorodihydrodiacetate (DCH_2FDA) staining, while active mitochondria were evaluated with Mitotracker Green staining. Digital microphotographs were obtained by epifluorescence microscopy and were analyzed using ImageJ software, assessing the individual luminosity of each oocyte. Results were analyzed by means of an ANOVA followed by a Bonferroni test. A $P < 0.05$ was considered statistically significant. A decrease in ROS production was observed at 24 h maturation ($P < 0.05$), coinciding with an increase in active mitochondria at the same time ($P < 0.05$), while no significant differences were detected at 0 and 44 h of culture for any parameter. From the results obtained, it can be inferred that, as observed in bovine oocytes, a fluctuation in ROS production and in active mitochondria is observed throughout porcine oocyte IVM, where an increase in the mitochondrial activity is related to a consequent decrease in ROS production. In future studies we aim to evaluate whether the variation observed in ROS production is related to the activation of the MAPK intracellular signaling pathway, to increase the understanding of their possible role as signaling molecules during the maturation process.

A53

METABOLOMIC STUDIES IN MOUSE SPERM INCUBATED IN CONDITIONS OF STARVATION AND SUBSEQUENT RECOVERY REVEALED SIGNIFICANT CHANGES BETWEEN TREATMENTS

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Mammalian sperm require to undergo capacitation either in the female tract or *in vitro* to become fertilization competent. For *in vitro* capacitation, sperm are incubated in media containing a limited number of compounds including calcium, bicarbonate, serum albumin, and energy sources (e.g., glucose, pyruvate). Using a mouse model, we have recently reported a new methodology that improves sperm functionality including fertilization and embryo development rates. As part of this method, sperm are rendered immotile by incubation in media devoid of energy nutrients. Despite their lack of movement, sperm are viable and can be completely recovered by adding back energy substrates. How this treatment affects capacitation-associated signaling and metabolism is still not well understood. As part of this work, we investigated the effect of starvation and recovery in the capacitation-associated phosphorylation pathways; we also analyzed the effects of this treatment in glycolysis and oxidative phosphorylation levels using a combination of methodologies including Seahorse technology (by measuring extracellular acidification rate (ECAR) and oxygen consumption rate (OCR)), nuclear magnetic resonance and mass spectrometry. These experiments revealed that during starvation many metabolites are decreased, others remain stable, and, more relevant, some metabolites such as L-carnitine ($P < 0.05$) and 5'AMP ($P < 0.001$) are significantly increased. Once sperm is recovered by the addition of glucose, energy production metabolic pathways are completely restored, and this recovery is accompanied by metabolomic changes that closely mimic those observed in sperm that have not undergone starvation ($P > 0.05$). Altogether, the results of this work indicate that manipulation of sperm metabolism can affect sperm function improving fertilization.

A54

EFFECT OF TROLOX ON REACTIVE OXYGEN SPECIES AND REDOX STATE DURING *IN VITRO* MATURATION OF BOVINE OOCYTES

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The production of reactive oxygen species (ROS) is a normal process that occurs in cells, but certain stressful conditions typical of *in vitro* environments that alter the balance between ROS production and their removal by intracellular antioxidant systems can produce oxidative damage and impaired cell function. The role of ROS and/or antioxidant substances in the manipulation and culture of gametes and embryos *in vitro* is contradictory, being reported as having both harmful and beneficial effects. New antioxidants such as Trolox have emerged, and their properties are being analyzed. There is not enough evidence regarding the concentrations at which Trolox could exert an effect on *in vitro* maturation (IVM) of bovine oocytes, nor its subsequent effect on fertilization and embryonic development. The aim was to study the effect of Trolox on the levels of ROS production and the redox state in the IVM of bovine oocytes. Ovaries from slaughterhouses were transported to the laboratory, where the aspiration of the antral follicles and the recovery of the oocyte-cumulus complexes (COCs) were performed. COCs were matured in medium 199 supplemented with gonadotrophins and fetal bovine serum for 22 h at a temperature of 39°C with a humidified atmosphere of 5% CO_2 in air (control) or supplemented with 25 µM (T_1), 50 µM (T_2) or 100 µM (T_3) Trolox. Then the COCs were denuded with hyaluronidase at 37°C. To determine ROS production, denuded oocytes were incubated for 30 minutes with 5 µM 2',7'-dichlorodihydro fluorescein diacetate (DCHFDA) and mounted on slides to quantify their fluorescence. Oocyte redox state was determined by the FAD/NAD(P)H ratio, measuring the intensity of the autofluorescent endogenous compounds FAD and NAD(P)H. The digital microphotographs obtained by epifluorescence microscopy were analyzed using IMAGE J, quantifying the individual luminosity of each oocyte. Oocyte nuclear maturation was evaluated by Hoechst 33342 fluorescent staining. Quantitative data were analyzed by ANOVA and qualitative data by Chi-square ($P < 0.05$). ROS production in the oocytes matured in the presence of Trolox decreased significantly compared with the control ($P < 0.05$), while no significant differences were observed between the three concentrations studied. In addition, the FAD/NAD(P)H ratio in the oocytes gradually decreased with the increase in Trolox concentration, with significant differences being observed between control and T_3 and between T_1 and T3 ($P < 0.05$). Nuclear maturation did not vary between treatments. We can conclude that supplementation of IVM medium with Trolox decreases ROS production and shifts the redox state of oocytes towards reduction. However, its effect on cytoplasmic maturation and embryonic development *in vitro* has yet to be evaluated.

A55
CALCIUM IONOPHORE A23187 EFFECT ON ACQUISITION OF FERTILIZING ABILITY IN EQUINE CRYOPRESERVED SPERMATOZOA

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Spermatozoa must undergo three events to fertilize an oocyte *in vivo*: capacitation, hyperactivation, and acrosome reaction. Therefore, it is necessary to induce these physiological events *in vitro* in the masculine gamete during assisted reproductive techniques. Even though the *in vitro* conditions under which many species acquire their fertilizing ability are well known, to this day, there is no standard protocol to induce these events in equine cryopreserved spermatozoa. Thus, there is no available protocol for embryo production through *in vitro* fertilization (IVF) with these samples. It has been proven that brief exposure of murine and bovine spermatozoa to calcium ionophore A23187 and the consequent calcium influx to the cell cytoplasm increases their fertilizing ability *in vitro* and embryo production due to the induction of capacitation and hyperactivation. Moreover, A23187 increases events related to capacitation without inducing the classical capacitation signaling pathway such as PKA-activation (pPKA) or protein tyrosine phosphorylation (pTyr). Given the current difficulties in equine embryo production through IVF with cryopreserved spermatozoa and the previously shown effects of the calcium ionophore in spermatozoa from other species, this work aimed to study the potential fertilizing ability of equine cryopreserved spermatozoa that were previously exposed to A23187. First, we studied the effect of A23187 (1 µM) on spermatozoa. After 10 min of exposure, the ionophore decreased the spermatozoa motility (CASA, $P < 0.05$) without affecting their viability (HOS test, $P > 0.05$) or acrosome status (PSA-FITC, IF, $P > 0.05$). After removing the ionophore, the spermatozoa were incubated under non-capacitating conditions for 20 min, and motility was reassessed, and their fertilizing ability was studied. The previous incubation with A23187 increased the hyperactivated sperm population (CASA, $P < 0.05$) and the induction of capacitation-associated events such as progesterone-induced acrosome reaction (IF, $P < 0.05$) and the sperm ability to bind to bovine oocyte zona pellucida ($P < 0.001$) without activating the pTyr and pPKA pathways (IF, $P > 0.05$). Our results suggest that brief exposure of cryopreserved equine spermatozoa to calcium ionophore A23187 could be incorporated into the assisted reproductive techniques to increase spermatozoa fertilizing ability *in vitro* due to its effect on hyperactivation and capacitation in this species.

A56
EFFECT OF LOW-DOSE PHOSATE ON EMBRYO IMPLANTATION

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Although glyphosate-based herbicides are considered safe due to their low persistence, new evidence suggests that they could affect the correct embryo implantation and development even in low doses. The trophectoderm surrounding the blastocyst plays a pivotal role in the invasion, migration, and spiral arteries remodeling from the decidua. This process is high-regulated, and its alterations could carry out preeclampsia, miscarriages, and other associated pathologies. Previous studies demonstrated that concentrations of 0.2 and 2 µM of glyphosate (G) stimulated migration activity in a human endometrial carcinoma cell line (Ishikawa). This study aims to analyze *ex vivo* the effect of 2.5 µM G in murine blastocyst development. The cellular migration was also assayed using the trophoblast cell line HTR-8/SVneo with 0.625, 1.25, 2.5, 5, and 10 µM of G. E3.5 embryos, recovered from pregnant BALC/b mice, were placed on murine uterine epithelial cells monolayer with 2.5 µM of G or vehicle (V). The implantation area and hatching/attachment time were registered for six consecutive days. The wound healing assay was performed to evaluate the migration activity. The monolayer was pretreated with G concentrations for 24 h, and the medium was renewed after scratching. Then, the uncovered areas were registered at 0 and 12 h. Cell viability was determined spectrophotometrically after 24 and 48 h of treatment using WST-1 reagent and by counting cells in a hemocytometer. All the assays were performed in triplicate. The blastocyst implantation area (G: 0.47 ± 0.03 mm^2; V: 0.32 ± 0.14 mm^2) and hatching/attachment time (G: 42.3 ± 10.5 h; V: 45.2 ± 19.9 h) were similar between groups. Cellular migration was stimulated at 0.625 µM G compared to V ($P < 0.05$). These results suggest that even low concentrations of G could dysregulate some processes associated with implantation.

A57
MOLECULAR AND MORPHOLOGICAL ALTERATIONS IN THE UTERUS OF ADULT RATS FED WITH CAFETERIA DIET AND EXPOSED TO A GLYPHOSATE-BASED HERBICIDE

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The development of endometrial hyperplasia (EH), a preneoplastic lesion, is associated with environmental factors, including unhealthy diets and chemical exposure. In this sense, we have demonstrated that cafeteria diet (CAF) provoked EH in adult rats and that exposure to glyphosate-based herbicide (GBH) altered uterine development, leading to EH later in life. However, the potential interaction between these environmental factors which women are frequently co-exposed remains unclear. Thus, we evaluated whether the addition of a

subchronic low dose of a GBH enhances the CAF effects on the rat uterus. Female Wistar rats at postnatal day (PND) 21 were randomly divided into: Control group, fed with chow diet; CAF group, fed with CAF, and CAF+GBH group, fed with CAF and added a GBH from PND 140. Rats were sacrificed at PND 240. Serum samples were collected to assess 17β-estradiol (E2) and progesterone (P4) levels, and uterine samples were obtained for histological studies (morphometric and immunohistochemical analysis). The levels of E2 and P4 were similar in CAF and CAF+GBH rats with respect to Control rats ($P = 0.208$). However, the serum levels of P4 were increased in the CAF+GBH group with respect to CAF one ($P = 0.018$). In the uterus, the addition of GBH enhanced the presence of preneoplastic lesions induced by CAF alone, reflected by an increased density of glands with cellular anomalies plus glands with daughter glands in the CAF+GBH group than in CAF and Control groups ($P = 0.002$). At the protein level, higher cell proliferation and expression of estrogen receptor alpha were found in the CAF and CAF+GBH groups with respect to the Control group ($P < 0.05$). Also, the CAF+GBH group exhibited a reduced expression of phosphatase and tensin homolog (PTEN) and p27, both tumor suppressor molecules that inhibit cell proliferation, with respect to the Control group ($P = 0.049$ for PTEN and $P = 0.048$ for p27). Thus, the cell proliferation found in CAF+GBH animals could be a consequence of reduced PTEN/p27 expression. In conclusion, the addition of GBH exacerbates the CAF effects on uterine preneoplastic lesions, and the PTEN/p27 signaling pathway seems to be involved. The high prevalence of these factors in our environment makes highly possible the interactions between them, and we would like to stress that future studies focusing on their interaction are mandatory to better understand the real-world risks for women.

A58
PROGESTERONE PRODUCTION DURING *IN VITRO* MATURATION OF SWINE COC. EFFECT OF INSULIN-TRANSFERRIN-SELENIUM AND METFORMIN

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The pig is an excellent model to study the effects of supplementation of defined media for *in vitro* maturation (IVM) due to the metabolic and physiological similarities with humans, in addition to the ease of obtaining a large number of cumulus–oocyte complexes (COC) from slaughterhouse ovaries. However, there is low efficiency in the *in vitro* production of porcine embryos worldwide, mainly due to poor IVM systems. In previous work from our laboratory, we saw that insulin–transferrin–selenium (ITS) alone and combined with metformin (M) cause an increase in glucose consumption and a decrease in oxidative stress. Progesterone (P4) production by COC plays an important role during IVM. We decided to study it, seeking to internalize ourselves to the effects of ITS and M on the metabolism of COC. The aim of this study was to evaluate the effects of adding M and/or ITS to the IVM media on the production of P4 by porcine COC. They were obtained by follicular aspiration from slaughterhouse ovaries, selected according to quality, and subjected to IVM for 44 h in supplemented TCM-199 + hMG (1.5 UI/mL) + dAMPc during the first 22 h. COC were randomly distributed in IVM drops supplemented with: Group M: M (10^{-4} M), Group ITS: ITS (1 µg/mL), Group M+ITS: M (10^{-4} M) + ITS (1 µg/mL) and Group C: without supplement. Progesterone (ng/mL) was quantified by RIA in media samples before and after IVM, stored at −20°C until use (N = 3–6/experimental group, 50 µL per determination). Post-MIV P4 concentration was analyzed using a general linear model with variance modeling by potentiation, followed by ANOVA and Tuckey post-test, in R and RStudio software. The results were expressed as mean ± SEM. Differences were considered if $P < 0.05$, and tendencies if $0.05 \leq P < 0.01$. The concentration of P4 in the pre-MIV culture media was insignificant. In the treatments, there was a tendency to modify the production of P4 (C: 276.3 ± 55.1; M: 500.8 ± 147.8; ITS: 651.5 ± 144.4; M+ITS: 704.3 ± 194.6; $P = 0.045$). Although differences were not significant, ITS and M+ITS treatments showed the greatest effect on P4 production compared to Group C. This effect on P4 production is likely related to the previously observed increase in glucose consumption and increased metabolic activity due to the IGF-like effect of ITS. It will be interesting to know the effect of these supplements on early embryonic development.

PHARMACOLOGY, TOXICOLOGY AND ECOTOXICOLOGY

A59
CHARACTERIZATION AND EVALUATION OF A ROSEMARY EXTRACT WITH THERAPEUTIC PURPOSES FOR ENDOMETRIOSIS

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Endometriosis is a benign gynecological disease that affects approximately 6–15% of women during their reproductive age. Endometriosis is characterized by the presence of endometrial tissue outside the uterine cavity. Current pharmacological treatments are inefficient and have several adverse effects that disable long-term administration, allowing disease recurrence. In recent years, we have focused on investigating plant-derived agents as natural treatment options, which represent a promising option to provide to patients suffering from a chronic disease such as endometriosis, due to their low or no side effects. Among them, we studied the effect of the major active compounds of rosemary: carnosic acid (CA) and rosmarinic acid (RA). In the present study, we have proposed to work with a complete rosemary extract, studying the synergistic action of all its components. This rosemary extract is known to contain several

phytochemical compounds with proven antioxidant, antimicrobial, and anti-inflammatory properties. Our objective was to characterize the polyphenolic composition of a rosemary extract by assessing the content of CA and RA; and to evaluate its effects on the viability of two human endometrial cell lines, a stromal one (t-HESC) and an epithelial one (ECC-1). The extract was diluted in DMSO and characterized by semi-preparative high-performance liquid chromatography (HPLC), using appropriate CA and RA standards. Cell viability was evaluated by WST-1 colorimetric assay after 24 h of stimulation with different concentrations of extract (4, 5, 7, 8, 10, 12, 17, and 25 mg/mL). The HPLC results showed that the studied extract contains 60% AR, 25% terpenes unknown so far, and 15% CA. Furthermore, the extract significantly inhibited ECC-1 cell viability starting from the 5 mg/mL dose ($P < 0.01$ *vs*. Basal), whereas t-HESC cell viability was significantly inhibited starting from the 8 mg/mL dose ($P < 0.01$ *vs*. Basal). These findings are promising for the inhibition of the disease and support further investigation of these compounds as new therapeutics for endometriosis.

A60
6-O-PALMITOYL-L-ASCORBIC ACID (ASC16) AS INHIBITOR OF OPHIDIAN VENOM ENZYMES

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Snake venoms are a complex mixture of toxins as phospholipases A_2 (PLA_2) nature, serine protease, 5' nucleotidases, metalloproteinases, phosphodiesterases, glutaminylcyclase, C-type lectin, crotamine, L-amino acid oxidase, disintegrins, and others. There is currently a growing interest in the search for natural or synthetic inhibitors acting on the components of venom and allowing mitigation of the local and systemic effects of their components. In the present work, ASC16 was proposed as an inhibitor of the main toxic enzymes in the venom of *C. d. terrificus* and *Bothrops diporus*. For this, ASC16 (0.1 µg/µL) was incubated with entire venom (1 µg/µL), and substrate-specific kinetic assays were performed to measure the activity (µmol/min/mL) of PLA_2, serine protease, phosphodiesterase, and L-amino acid oxidase. In order to evaluate the metalloprotease activity, a proteolytic assay was performed with *B. diporus* venoms using azocasein as substrate. Results were expressed as mean ± SD; independent experiments were performed in duplicate. The *in vitro* experimental data were analyzed with the statistical package InfoStat version 2008. Statistical analyses were performed using the LSD Fisher test, being considered significant if $P < 0.05$. The results show that ASC16 inhibited PLA_2 by 80.16% (2:1 w/w), serine protease 61.57% (1:10 w/w), phosphodiesterase 45.13% (1:20 w/w), L-amino acid oxidase 50.64% (1:20 w/w), and metalloproteinase 50% (1:10 w/w) activities. A decrease in the activity of the enzymes tested was observed due to the non-specific inhibition of ASC16 on the ophidian enzymes. Consistent with our study, similar research was carried out with venom from the species *Echis carinatus* that ASC16 significantly inhibits PLA2 activity and acts non-specifically with other enzymes. It is concluded that ASC16 has the potential as an inhibitor by reducing the enzymatic activity of the main ophidian proteins. Future studies should be conducted to further investigate the mechanisms of ASC16 inhibition and its safety.

A61
PHYSIOLOGICAL RESPONSE OF SANTOLINA (*Santolina chamaecyparissus*) EXPOSED TO LEAD

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The objective of this work is to determine the physiological response of the santolina plants (*Santolina chamaecyparissus*) against lead (Pb) stress. The experiment was set up with seedlings in pots of 1 kg with a substrate of sand:vermiculite (3:1) in a greenhouse. 30 days later, 1 g of Nitrofull fertilizer with macro and micronutrients was applied to each of the plants. After 32 days, a $PbCl_2$ solution was used, where the final concentration of Pb in the substrate was 0, 250, 500, 1000, and 2000 ppm. After 25 weeks, the volume of each plant was estimated using image analysis software as a growth parameter. In addition, samples of each treatment were collected, and lipid peroxidation was determined as a stress indicator. Enzymatic activity of catalase, ascorbate peroxidase, and guaicol peroxidase was also measured. The data was analyzed through univariate and multivariate statistics ($P < 0.05$), which indicated that the concentrations up to 1000 ppm of the metal did not present a significant difference with respect to the control treatment in the growth parameter and in the enzymatic measurements. However, in the samples that contained 2000 ppm, it was possible to observe that there were significant differences with respect to the others, where the value of the growth parameter decreased, and the values of the enzymatic measurements increased. In the treatments from 250 to 2000 ppm of Pb, the concentration of the metal in the aerial part was lower than in the substrate, and the measured value never exceeded the value of 1000 ppm taken as a reference for the plant to be a hyperaccumulator. Significant differences in the growth parameters and in the enzymatic activity with respect to the control treatment were found from 2000 ppm. Therefore, this specie is not considered a bioaccumulator of Pb, although it can tolerate its presence in large amounts and can be used for phytoremediation.

A62

THE UV FILTER 4-MBC ALTERS TROPHOBLASTS MIGRATION AND BLASTOCYSTS IMPLANTATION WHEN ANALYZED *IN VITRO*

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The chemical substance 4-MBC (4-methylbenzylidene camphor) is a UV filter present in many personal care products. Previous studies have suggested that this compound may act as an endocrine-disrupting chemical. Therefore, the main aim of this work was to analyze the effects of 4-MBC in the pregnancy-related processes of placentation and implantation, by means of *in vitro* assays. For studying the effect on placentation, the trophoblast cell line Swan-71 was used using *in vitro* assays measuring proliferation and migration of the trophoblasts. In these assays, three different concentrations of the compound were analyzed (0.2 ng/mL, 2 ng/mL, and 20 ng/mL) and compared to the control. There were no effects of 4-MBC on the proliferation of the trophoblast in any of the concentrations analyzed. However, when analyzing the migration of the trophoblasts in a wound-healing assay, the migration was significantly impaired in the presence of 2 ng/mL and 20 ng/mL 4MBC when compared to the control (N = 8 for each condition; $P < 0.05$ by Mann–Whitney test). In order to analyze whether 4MBC acted through the androgen receptor (AR) pathway, the same assay was performed in the presence of an inhibitor of the androgen receptor named flutamide. The presence of the AR inhibitor did not modify the previously obtained results, indicating that 4-MBC did not act through the AR pathway. In another set of experiments, the implantation capacity of mouse blastocysts was analyzed by means of an *in vitro* implantation model. This model consists of the co-culture of mouse blastocysts over a monolayer of autologous uterine epithelial cells. Blastocysts were cultured in the presence of 0.2 ng/mL 4MBC (N = 4), 2 ng/mL 4-MBC (N = 7), 20 ng/mL 4-MBC (N = 7), or vehicle (N = 7), and the time at which each blastocyst implanted in the monolayer was registered. Whereas 100% of the blastocyst of the control group, as well as 100% of the blastocysts exposed to 0.2 ng/mL or 2 ng/mL 4-MBC were implanted after 96 h of culture, only 71.4% of the blastocysts exposed to 20 ng/mL were implanted. This suggests an alteration in the implantation provoked by the presence of 4-MBC. All these results show that 4-MBC has the capacity to alter the migration of trophoblasts as well as the implantation capacity of blastocysts. This indicates that 4-MBC may influence critical processes of gestation, pointing out the need to study the effects of this compound in more detail.

A63

PHYTOEXTRACTION CAPACITY AND EFFECTS OF LEAD IN ROSEMARY (*Rosmarinus officinalis*)

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Phytoremediation is a sustainable and low-cost alternative technique that allows cleaning or restoring contaminated soils using plants. Aromatic plants can be efficient phytoextractors of heavy metals. Once heavy metals are absorbed, they can cause damage to metabolism and cell structure. Therefore, plants have several cellular protection mechanisms to eliminate reactive oxygen molecules (ROS) or control their excess, including enzymatic antioxidant components. The determination of these compounds would allow evaluation of the efficiency of aromatic plants for their use in soils contaminated with heavy metals. The objective of this study was to evaluate the use of *Rosmarinus officinalis* as a phytoextracting agent and to determine the effect–response to stress in the presence of lead. 0, 500, 1000, and 2000 ppm of lead were applied to the plants, distributed in four replicates per treatment. Lead in plants and substrate was determined by atomic absorption spectroscopy. The aerial part was collected for analysis and determination of lipid peroxidation (malondialdehyde, MDA) and activity of antioxidant enzymes catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) by means of UV-Vis spectrophotometry. The results obtained showed that Rosmarinus officinalis has high survival capacity in substrates with high lead content, but it had low phytoextraction capacity, having determined low quantity or absence of lead in its aerial tissues. Regarding the evaluation of stress parameters and response to stress, no differences were observed with respect to the values obtained for MDA and enzymatic activities CAT, APX, and GPX of the treatments in relation to the control treatment (0 ppm). In conclusion, rosemary could be considered a species tolerant to lead in substrates with up to 2000 ppm, without obtaining a response to stress in the presence of lead. This could suggest that it presents some exclusion mechanism for lead. However, more studies are required to confirm this.

A64

IN OVO EXPOSURE TO ENDOSULFAN ALTERS THYROID HORMONE HOMEOSTASIS IN JUVENILE FEMALE BROAD-SNOUTED CAIMAN (*Caiman latirostris*)

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In vertebrates, thyroid hormones, triiodothyronine (T3) and thyroxine (T4) have a role in the regulation of growth, development, metabolism, and reproduction. Circulating levels of T3 and T4 are, in turn, regulated by the hepatic deiodinases dio1 and dio2 (that catalyze the conversion of T4 into T3) and dio3 (that converts T3 into reverse-T3). Endosulfan (END), a persistent pesticide reported to

be present in broad-snouted caiman eggs, has been described as a potential thyroid-disrupting compound. The aim of this study was to assess the effects of *in-ovo* exposure to a single dose of 20 ppm of END on thyroid hormone homeostasis in juvenile female caimans. Sixteen animals (8 for the CONTROL group, and 8 for the END group) were used in this work. For the thyroid histoarchitecture study, the percentage of the gland section represented by follicular epithelium, colloid, reabsorption vacuoles, and stroma was assessed by using an orthogonal grid. T3 and T4 plasma levels were assessed by electrochemiluminescence, and hepatic dio1 gene expression was assessed by qPCR using L8 as a housekeeping gene. Our results (presented as mean ± SEM) show that, although exposure to END did not induce changes in thyroid histoarchitecture, T4 circulating levels were higher in exposed animals (CONTROL 1.01 ± 0.08 µg/dL *vs.* END 1.19 ± 0.03 µg/dL; $P = 0.017$), which significantly modified the T3:T4 ratio (CONTROL 144.4 ± 5.0 *vs.* END 129.2 ± 3.3; $P = 0.030$). Regarding hepatic dio1 gene expression, although a decreasing trend was observed in END-exposed animals (CONTROL 40.85 ± 17.7 *vs.* END 9.91 ± 3.10), no statistical significance was achieved ($P = 0.456$). Our results demonstrate that exposure to END during key stages of embryo development increased the T4 plasma levels, possibly as a consequence of T4-altered hepatic metabolism. Although subtle decreased mRNA levels of dio1 could account for increased T4 circulating levels, other deiodinases, possibly dio2 could have a more relevant role in this effect. Our findings suggest that modified T4 circulating levels because of natural exposure to END could affect female broad-snouted caiman metabolism, growth, and reproduction. This, in turn, could negatively affect the caiman population dynamic and wetland ecosystem health.

A65
DERMAL APPLICATION OF THE UV FILTER 4-MBC DURING PREGNANCY PROVOKES INTRAUTERINE GROWTH RESTRICTION IN A MURINE MODEL

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The chemical substance 4-MBC (4-methylbenzylidene camphor) is a UV filter present in many personal care products. Considering our previous results showing that 4-MBC affects trophoblast migration and blastocyst implantation *in vitro*, the aim of this work is to analyze the effect of 4-MBC using an *in vivo* model. C57BL/6 female mice were dermally exposed to a daily dose of vehicle (olive oil) or 4-MBC (22 mg/kg body weight/day), from gestational day 0 (GD0) until GD10 (Veh: N = 6; 4-MBC: N = 6) or gd14 (Veh: N = 6; 4-MBC: N = 5) and euthanized 4 h after the last dermal application. HPLC analysis showed that 4-MBC can be found in serum samples of mice of GD10 and GD14 in a range of 11.0–38.0 ng/mL, similar to the concentration found in humans after dermal application of sunscreens (20 ng/mL). At GD10, no differences in the size of the fetoplacental units were found. However, at GD14, differences in the fetuses' weights were found between the groups. The weight of the fetuses exposed to 4-MBC (N = 36) was significantly lower ($P < 0.05$; Mann–Whitney) than the weight of the fetuses exposed to the vehicle (N = 39). These results suggest that 4-MBC applied during an early and middle period of mice's pregnancy provokes intrauterine growth restriction (IUGR). Considering that many studies correlate an alteration in angiogenesis with IUGR, the effect of three doses of 4-MBC (0.2 ng/mL, 2 ng/mL, and 20 ng/mL) was analyzed *in vitro*, by means of a tube formation assay using the first-trimester trophoblast cell line HT8-SvNeo cultured on Matrigel using a reduced-serum medium. Using this assay (N = 3 for each condition), it was found that the 0.2 ng/mL concentration was able to provoke a significant diminution when compared to the control (one-way ANOVA followed by a Dunnet post-test) in the number of meshes, total meshes area, number of master junctions, total branching length, as well as in the number and length of master segments, together with a significant augmentation in the number of extrems. These results suggest a possible anti-angiogenic effect of 4-MBC, which could be related to the IUGR phenotype observed *in vivo*. This opens up the possibility to study in more detail the correlation between the underlying mechanisms of the IUGR obtained after the dermal application of 4-MBC.

GENERAL, CELLULAR AND MOLECULAR BIOLOGY

A66
CHROMOSOMIC DISTRIBUTION OF RIBOSOMAL DNA IN NEOTROPICAL PRIMATES (*PLATYRRHINI*)

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The Nucleolar Organizer Region (NOR) is the chromosomal region that contains the genes coding for ribosomal DNA (rDNA). This region can be detected by silver staining (Ag-NOR), a highly selective technique. Ribosomal genes are arranged in tandem repeat clusters and grouped in one or several different regions, in one or more chromosome pairs. The number of NORs can vary from one species to another but, within a given karyotype, the number and localization of NORs are constant. In this work, NORs were characterized by silver staining in *Alouatta caraya*, *Alouatta guariba clamitans*, *Aotus azarae*, and *Cebus cay*, with the purpose to determine the number

and localization of rDNA genes and generate cytogenetic markers for chromosome identification and the study of diversification and genome organization. We analyzed 2 males and 2 females from *A. caraya*, 2 males from *A. guariba clamitans*, 1 male and 1 female from *A. azarae*, and 2 males and 2 females from *C. cay,* from zoos and breeding centers in Argentina and Brazil. More than 20 silver-stained mitotic metaphases were photographed for each individual. Ag-NOR bands were observed in the proximal region of the q arm in two acrocentric chromosome pairs in *A. caraya*, *A. guariba clamitans*, and *C. cay*. In *A. azarae*, one Ag-NOR band was observed in the interstitial/distal position in only one metacentric chromosome pair. Homeologies of NOR-bearing regions with human chromosomes were established in the four studied species: for *A. caraya* with human pairs 1 and 3 (associated to 3/21 synteny); for *A. guariba clamitans* with human chromosomes 3 (3/21 synteny) and 10; for *A. azarae* with human chromosome 1 (associated to 3/21 synteny), and for *C. cay* with human chromosome 1. When comparing these results with the literature, it is clear that a big proportion of NOR regions in Platyrrhini are located in regions with homeology to human chromosomes 1 and 3, and in this last case, associated with 3/21 synteny, of evolutionary importance in the group. Furthermore, the detected NOR regions in this work colocalize with evolutionary chromosomal breakpoints in 17 of a total of 19 species studied to this date (90%). The results of this work support the importance of analyzing the distribution and dynamic of these repetitive sequences since their variability might be correlated with chromosome evolution in primates.

A67
IDENTIFICATION OF CELLULAR PROCESSES REGULATED BY OXER1 RECEPTOR ACTIVATION IN HUMAN ADRENOCORTICAL CELLS

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The membrane receptor OXER1 belongs to the G protein-coupled 7TMS receptor superfamily. It is expressed in a wide variety of cell types and primarily binds a metabolic product of arachidonic acid, 5-oxo-eicosatetraenoic acid (5-oxo-ETE). OXER1 is considered an inflammatory receptor, involved in the chemoattraction of circulating mononuclear cells, Ca^{2+} increase in neutrophils, inflammation, steroidogenesis, and cancer. We detected the presence of this receptor and found evidence of its involvement in the regulation of steroid production in the human adrenocortical line cell H295R. The aim of this work was to identify proteins and signaling pathways involved in the activation of OXER1 by its natural ligand 5-oxo-ETE using proteomic and bioinformatic approaches. The technique of choice was a reverse phase protein array assay (RPPA) using antibodies for functional proteomics studies. We used H295R cells, in which OXER1 silencing was performed in order to analyze changes in protein expression/phosphorylation due to the effect of this agonist exclusively by binding to this receptor. Control and silenced H295R cells were stimulated with 5-oxo-ETE (500 nM). Proteomic analysis revealed that of the 496 proteins/phosphoproteins analyzed by RPPA, 16 and 30 proteins (after 5 min and 3 h treatment with 5-oxo-ETE, respectively) showed changes in their expression levels, which was also reversed by the absence of OXER1 ($P \leq 0.05$, $\log_2$ fold-change cut-off ± 0.8). With the combined use of bioinformatics tools (DAVID, STRING, PANTHER) we integrated information from different databases (KEGG, Reactome, WikiPathways). The enriched signaling pathways associated with protein profiling mainly belong to the following clusters: gene transcription (enrichment score 2.89), cellular response to stimuli and stress involving ErbB, EGFR, TGF beta signaling pathways (score 2.52), PI3K-Akt pathway and focal adhesion (score 2.42), and insulin signaling (score 2.06). In addition, PANTHER signaling pathways indicate activated ($P < 0.05$) oxidative stress response pathways and Ras and EFG receptor signaling pathways. Functional annotation revealed that the major post-translational modifications associated with this set are phosphorylation, ubiquitination-like conjugation, and acetylation ($P < 0.001$). Among the biological processes (GOTERM) enriched were positive regulation of proliferation and negative regulation of apoptosis (score 2.71) and regulation of transcription (score 1.15). With the information obtained, candidate proteins will be selected for experimental validation and to confirm the role of the OXER1 receptor in the pathophysiology of adrenocortical cells.

A68
BASIC PHOSPHOLIPASES A_2 ISOLATED FROM *Bothrops diporus* SNAKE VENOM INHIBITS ENDOTHELIAL TUBULOGENESIS *IN VITRO*

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Angiogenesis is a regulated process of growth and remodeling of new blood vessels from preexisting vasculature, which is correlated with a variety of physiological processes. However, when this process is deregulated contributes to the pathogenesis of numerous disorders such as the survival and progression of solid tumors. Snake venoms are a complex mixture that contains biologically active molecules, being phospholipases A_2 (PLA_2s) one of the most abundant components with pharmacological potential. In the present study, two basic PLA_2s (PLA_2-I and PLA_2-II) were isolated from the Argentine northeastern snake *Bothrops diporus* venom, and their potential inhibitory effect on *in vitro* tubulogenesis was evaluated. Toxins were purified by reverse-phase high-performance liquid chromatography (RP-HPLC) on a C18 column. Venom (2 mg) was dissolved in 200 µL of 0.1% trifluoroacetic acid (TFA). Elution was performed at 1 mL/min using a gradient of acetonitrile with 0.1% TFA. Protein concentrations were estimated by measuring the absorbance at 280 nm. Phospholipase activity was assessed, and purity and apparent molecular mass were determined by SDS-PAGE. Cytotoxic activity of PLA_2-I and PLA_2-II (1.25–30 µg/mL – 24 h of incubation) was determined using the tEnd murine endothelial cell

line (CVCL_6272) cultured in DMEM–5% FBS at 37°C–5% CO_2. For tubulogenesis assay, 96-well plates were coated with 50 µL of Geltrex at 4°C and incubated for 30 min at 37°C. Then tEnd cells were seeded (1×10^4 cells/well) with non-cytotoxic concentrations (25 µg/mL) of PLA_2s or culture medium (control group). The morphological changes induced at 8 h were evaluated using phase contrast microscopy. Photographs were taken and angiogenic parameters were quantified using ImageJ software. Results showed an inhibitory effect on the tubulogenesis process with both toxins assayed in comparison to controls. There was a decrease in the nodes, segments, and meshes when cells were incubated in the presence of PLA_2-I or PLA_2-II, the former having a greater inhibitory effect. Although more studies are needed to elucidate the triggered mechanisms of action, these results suggest a potential antimetastatic effect of these snake toxins.

A69
STUDY OF LAPATINIB RESISTANCE MECHANISM IN HER2 POSITIVE BREAST CANCER

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Lapatinib (L) is a dual EGFR/HER2 tyrosine kinase inhibitor used in HER2+ metastatic breast cancer (BC), but its clinical benefit is less than 30%. Previously, we showed that soluble TNFα (sTNFα) induces trastuzumab (Tz) resistance by upregulating mucin 4 (MUC4), a transmembrane glycoprotein that shields the T epitope on the HER2 molecule; and that women with HER2+MUC4+ BC have a worse survival. Furthermore, we showed that blocking sTNFα *in vivo* with INB03, a TNFα dominant-negative protein (DN), overcomes Tz and L resistance, inhibiting tumor growth and cell migration. Here, we proposed to study the molecular mechanisms by which sTNFα blockade overcomes L resistance. JIMT-1, a HER2+MUC4+human cell line, *de novo* resistant to L and Tz, was used. To corroborate the results obtained in the preclinical model, JIMT-1 cells were treated for 72 h with vehicle, L 1 µM, DN 10 µg/mL, or the combination therapy L+DN, and proliferation was determined by cell count. Moreover, HER2 expression, AKT and ERK1/2 signaling pathways were analyzed by Western Blot (WB) in cells treated for 48 h with vehicle, DN and the combined treatment L+DN. Also, Tz binding to HER2 was studied in these conditions by immunostaining and flow cytometry. Treatment with L or DN did not inhibit cell proliferation. However, the combination therapy L+DN significantly decreased cell proliferation compared to monotherapies and vehicle ($P < 0.01$). On the other hand, a decrease in AKT phosphorylation was observed in the presence of L and L+DN. However, upon these treatments, an increase in ERK1/2 phosphorylation was observed. Finally, treatment with L showed an increase in HER2 expression, while the addition of DN restored the receptor's basal levels. This behavior was validated by flow cytometry, showing an increase in Tz binding in cells treated with L compared to its control, while a decrease was observed in cells treated with L+DN. Taken together, these data show that L increases HER2 expression and that sTNFα blockade in combination with L inhibits JIMT-1 cell proliferation and overcomes L resistance. In addition, the increase in ERK phosphorylation in cells treated with L could indicate an alternative pathway that would help to elucidate a new resistance mechanism to L. Since sTNFα blockade restores basal HER2 expression levels, it will be subjected to future studies as a tumor sensitization mechanism to this tyrosine kinase inhibitor and other HER2-targeted therapies. These results open the door to a possible sequential treatment of L+DN to achieve a decrease in MUC4 expression, a HER2 overexpression, and a greater epitope availability; and then add Tz to achieve an objective response in HER2+MUC4+ BC.

A70
COMPLEX/HYBRID TYPE N-GLYCANS OF INTEGRIN ALPHA V ASSOCIATE WITH MALIGNANT BEHAVIOR OF HUMAN GLIOBLASTOMA

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Gliomas are the most common and aggressive primary brain tumors in adults. Despite the study and development of new therapeutic strategies, the prognosis of patients with high-grade glioma is still poor so the identification of novel approaches is needed. Integrin Subunit Alpha V (IαV) is a transmembrane glycoprotein that plays a key role in glioma aggressiveness since it mediates the interaction of the tumor cells with the extracellular matrix. It is known that the glycosylation profile of malignant cells participates in the interaction with the microenvironment, therefore changes in this kind of post-translational modification can determine cell behavior. Little is known about the participation of glycosylation in glioma biology and how it alters the conformation and activity of the relevant proteins. The aim of this work is to compare the IαV expression on a set of high- and low-grade human glioma cell lines and characterize its glycosylation profile. Firstly, we evaluated the expression of IαV between high- and low-grade human glioma cell lines by flow cytometry. In addition, we made an immunoprecipitation of IαV from the high-grade cell line LN229 and observed a recognition by Concanavalin A lectin, which recognizes oligomannose-type N-glycans with high-affinity and complex-type bi- antennary N-glycans with low-affinity. IαV has 13 theoretical N-glycosylation sites where these glycans could be covalently attached. The analysis of IαV glycosylation by mass spectrometry in LN229 cells showed 6 glycosylation sites on its extracellular domain, whose structures included complex-type and oligomannose glycans. The same analysis on a low-grade line, SW1088, showed only 2 glycosylation sites and the presence of oligomannose structures. In this work, we characterize the expression of IαV in high- and low-grade glioma cell lines and demonstrate that it shows a different glycosylation profile and structural heterogeneity since complex-type glycans were not observed in the low-grade glioma cell line, suggesting a role of this type of branching in the aggressive behavior.

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A71

IMPLEMENTATION OF DIGITAL PCR FOR THE DETECTION OF SARS-CoV-2 VARIANTS IN GROUPED SAMPLES

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One of the most important applications of droplet digital PCR (ddPCR) is rare mutation detection (RMD). The challenge is the differentiation between two very similar sequences, one significantly more abundant than the other. In this case, the detection of a variant of SARS-CoV-2 present at a low frequency in a medium containing high concentrations of wild-type virus (WT). This is possible because ddPCR partitions the sample into 20,000 nanodroplets per tube, increasing the relative concentration of the defective variant. This results in increased sensitivity and increased resistance to inhibitors. Our laboratory has already validated the use of ddPCR for the detection of SARS-CoV-2 in pooled samples by combining up to 34 samples. Considering that new SARS-CoV-2 variants of interest (VOI) and concern (VOC) continue to emerge, it is important to monitor their circulation through genomic surveillance. Our objective is to demonstrate the feasibility of differentiating them by working with pooled samples in order to reduce analysis times and costs. For this, the ThermoFisher TaqMan SARS-CoV-2 mutation panel was used for both RT and ddPCR to differentiate the variants. The tests were carried out from July 2021 to March 2022 with anonymous samples. The samples were selected from their initial analysis by RT-qPCR. Positive samples were individually genotyped by RT-qPCR. Finally, one sample was chosen for the omicron variant and another for delta and they were pooled with WT samples in pools of different sizes (1 variant in a group of 4, 9, or 14 WT samples) and analyzed by ddPCR. Data were analyzed with Quanta Soft analysis software (Bio-Rad). A negative test result indicates that no individual in the group has the variant being tested, while a positive result indicates that at least one individual in the group is positive for that variant. If positive pools are detected, that pool is opened and the samples are analyzed individually by RT-PCR. In the present work, we demonstrate that it is possible to identify a delta or omicron variant in pools of up to 10 samples by ddPCR. Sensitivity and specificity studies were performed following the guidelines of the European Pharmacopoeia. This assay has a sensitivity of 100% (N = 24) and a specificity of > 98% (N = 50). Therefore, we report a new diagnostic technology for the detection of SARS-CoV-2 variants in pools by ddPCR to achieve rapid results (< 24 h), with high throughput and low costs.

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