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CONFERENCES

A1
BACULOVIRUSES: ISSUES AND OPPORTUNITIES IN ECONOMY, BIOLOGY AND MEDICINE

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Ca. six thousand years ago, an unknown source of death of silkworms (蚕 *Bombyx mori* caterpillars changing into moths) set off the alarm and threatened to wipe out one of the major assets of the Chinese economy: the silk industry. Only in the 20th century, there became clear that the contagious disease that spread in silkworm farms was due to a peculiar virus included in the Baculoviridae family. The members of Baculoviridae are arthropod-specific, enveloped viruses with large circular, supercoiled double-stranded DNA genomes (80–150 kbp) and undergo an infectious cycle yielding a progeny with two different phenotypes and a single genotype: budding virus (BV) and occlusion bodies (OB) containing one or many virions embedded in a proteinaceous matrix, a conspicuous structure that facilitates their detection in infected insects. The exquisite target species specificity of baculoviruses led to exploit their potential to control insect pests in an environmentally friendly strategy compatible with other measures of integrated pest management programs. From these considerations, it is clear that the initial interest in baculoviruses was led by reasons quite different from the study of most viruses, which aimed to seek solutions for their adverse impact on human, veterinary, and plant health. Many baculoviruses have been developed as commercial products to control insect pests without affecting the environment due to their safety for plants, vertebrates, and invertebrates other than their target insects in their larval instars. Later, the utility of baculoviruses as gene expression vectors was evidenced leading to numerous applications. Several strategies are employed to obtain recombinant viruses that express large quantities of heterologous proteins, based on their ability to produce high yields of the major OB protein necessary for the completion of the infectious cycle in the larvae. A major step forward was the development of bacmid technology (the construction of bacterial artificial chromosomes –BAC– containing the viral genome) which allows the manipulation of the baculovirus genome in bacteria. With this technology, foreign genes can be introduced into the bacmid by homologous and site directed recombination or by transposition. Baculoviruses have been used to explore fundamental questions in molecular and cell biology such as the nature of programmed cell death, metastasis, etc. Moreover, the ability of baculoviruses to transduce mammalian cells led to the consideration of their use as gene therapy and vaccine vectors. Strategies for genetic engineering of baculoviruses have been developed to meet the requirements of new application areas. Display of foreign proteins on the surface of virions or in nucleocapsid structures, the assembly of expressed proteins to form virus-like particles (VLPs) or protein complexes have been explored and validated as vaccines. The aim of this talk is to update the areas of application of the baculoviruses in pest control, protein expression, alternative vaccine designs, and gene therapy for infectious diseases and genetic disorders.

A2
WHAT IS ESSENTIAL IS NOT INVISIBLE TO THE EYE

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"In this treacherous world nothing is the truth nor a lie; everything depends on the color of the crystal through which one sees it." The main aim of my lab is the study of the retina, both under physiological and pathological conditions. In recent years, we have concentrated on the study of prevalent visual diseases that are causes of blindness, such as diabetic retinopathy, uveitis, optic neuritis, glaucoma, and retinal ischemia, and for which there are not yet sufficiently effective therapies. To fulfill this objective, we have either developed new experimental models or we have validated pre-existing models, in which we have analyzed the feasibility of new therapeutic strategies. In this sense, we have demonstrated the therapeutic efficacy of ischemic conditioning for diabetic retinopathy, melatonin treatment for experimental optic neuritis, and exposure to an enriched environment or visual stimuli for retinal ischemic damage. Moreover, we have analyzed the effect of experimental glaucoma on the non-imaging visual system.

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EXPERT SCIENTISTS' SYMPOSIUM

A3
ESTABLISHING NEURONAL DIVERSITY IN THE DEVELOPING SPINAL CORD: A MATTER OF TIME AND SPACE

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Generation of neurons at the right time, location, and number is essential for building a functional nervous system. Considerable progress has been made in understanding the mechanisms that control the production of specialized neuronal types. Positional identity has emerged as a fundamental organizing principle governing neuronal subtype diversification. However, how the timing of differentiation contributes to cell diversity in the developing spinal cord is still pending. We have identified the generation of neurons during advanced embryonic stages, at the "gliogenic phase," previously considered non-neurogenic. These late neurogenic events exclusively give rise to CerebroSpinal Fluid-contacting Neurons (CSF cN), an anatomically discrete cell type of the ependymal area of the spinal cord. We identified that the transcription factors Ascl1, Gata3, and Gata2 sequentially control the specification of CSF-cNs. With fate mappings and time-controlled deletions, we demonstrate that CSF-cNs derive from progenitors expressing the proneural protein Ascl1, with Ascl1 triggering late neurogenesis in the amniote spinal cord. Ascl1 abrogation transforms prospective CSF-cN progenitors into ependymocytes, demonstrating that late spinal progenitors have the potential to produce neurons and that Ascl1 balances the neuronal and non-neuronal composition of the spinal central canal. Furthermore, downstream of Ascl1, the acquisition of the precise CSF-cN identity depends on the postmitotic action of the transcription factors Gata3 and Gata2. In summary, we demonstrate that Ascl1-Gata3/2 are essential components of the temporally restricted transcriptional program that sustains spinal cord late-born neuron specification.

A4
HOMOCASTASTERONE BIOSYNTHESIS IN THE FEMALE GAMETOPHYTE IS ESSENTIAL FOR EARLY EMBRYOGENESIS IN *Arabidopsis*

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Mitochondrial adrenodoxins (ADXs) are small iron-sulfur proteins with electron transfer properties. In animals, ADXs transfer electrons between an adrenodoxin reductase (ADXR) and mitochondrial P450s, which is crucial for steroidogenesis. Through the analyses of single and multiple mutants, we demonstrated that a plant steroidogenic pathway that depends on a mitochondrial ADXR-ADX-P450 shuttle is essential for female gametogenesis and early embryogenesis through a maternal effect. The steroid profile of maternal and gametophytic tissues of WT and *adxr* ovules showed that homocastasterone is the main steroid present in WT gametophytes and that its levels are reduced in the mutant ovules. The application of exogenous homocastasterone partially rescued *adxr* and P450 mutant phenotypes, suggesting that gametophytic homocastasterone biosynthesis is affected in the mutants and that a deficiency of this hormone was the cause of the alterations observed. These findings also indicate not only a remarkable similarity between steroid biosynthetic pathways in plants and animals but also a common function during sexual reproduction.

A5
GAMETE FUSION IN THE TREE OF LIFE AND THE VIRAL VINE

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Sexual reproduction in Eukarya consists of genome 28 reduction by meiosis and subsequent gamete fusion. The presence of meiotic genes in Archaea and Bacteria suggests that prokaryotic DNA repair mechanisms evolved towards meiotic recombination. However, the evolutionary origin of gamete fusion is less clear because fusogenic proteins resembling those found in Eukarya have so far not been identified in prokaryotes. Here, using bioinformatics, we identified archaeal genes encoding candidates of fusexins, a superfamily of fusogens mediating somatic and gamete fusion in multiple eukaryotic lineages. Crystallographic structure determination of a candidate

archaeal FusexinA reveals an archetypical trimeric fusexin architecture with novel features such as a six-helix bundle and an additional globular domain. We demonstrate that ectopically expressed FusexinA can fuse mammalian cells and that this process involves the additional domain and a more broadly conserved fusion loop. Genome content analyses reveal that archaeal fusexins genes are within integrated mobile elements. Finally, evolutionary analyses place these archaeal fusogens as the founders of the fusexin superfamily. Based on these findings, we propose a new hypothesis on the origins of eukaryotic sex where an archaeal fusexin, originally used by selfish elements for horizontal transmission, was repurposed to enable gamete fusion.

A6
NEOKIT: FROM MOLECULAR BIOLOGY TO THE DEVELOPMENT OF DIAGNOSTIC KITS

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Infectious diseases show a great impact on public health and productivity, particularly in low-income countries. Proper diagnosis access can make a great difference in reducing both human and economic losses. Currently, there is a vacancy in diagnostic methods adapted to the field possibilities of the user, the beneficiary, and the system in which they are immersed (infrastructure, equipment, etc.). Ten years ago, we have started to work to develop and transfer tests –and associated know-how– for the detection of infectious agents (in human, veterinary, and plant health), which were fast, affordable, and simple to use in any field conditions. The first development done by this group arose from the call of FITS CHAGAS, FONARSEC-National Agency for Scientific and Technological Promotion (today "Agencia I+D+i"), to develop a simplified molecular diagnostic test for vertical Chagas. The grant required us, as CONICET researchers, to form a Public-Private Partnership Consortium (CAPP) with companies. The two signatory companies were national SMEs. That developed kit, namely Chagas NeoKit, has shown adequate sensitivity and specificity, but also has resulted very easy to perform, from a blood sample in the liquid state, from purified DNA, or directly from a dry blood drop on a card from the Neonatal Screening Program (PPN). It only requires the use of a simple thermal device, since the reaction is based on a loop-mediated isothermal amplification (LAMP), and no pipettes, centrifuges, or other laboratory equipment are necessary. This kit has been the 1st ARGENTINE MOLECULAR DETECTION REAGENT APPROVED by ANMAT (Resol. 1-47-3110-1994/17-5). This experience has been the beginning of a great learning process to turn a molecular reaction into a tangible good, adding capabilities from the scientific, health, and industrial systems. The work team grew and received other funds from the Agency that allowed us to increase and consolidate this knowledge, systematizing it in a technological platform where the LAMP technique is combined with tools focused on simplifying both the processing of the sample. as well as the reading out method, to obtain effective, robust, fast and simple to apply kits to detect different infectious agents. With the advent of the pandemic, the NEOKIT COVID-19 was developed and clinically and analytically validated (with 2 presentations: TecnoAMI and Plus), in record time, obtaining the approval of ANMAT in May 2020. This pandemic scenario has driven the installation of production capacities of approx. 1 million reactions/month and generated more than 10 job positions. Currently, in addition to accompanying the production process of the kit for COVID-19, work is being done on the development of kits for the detection of other infections, including vertical syphilis, dengue, zika, and chikungunya, among others. This is an example of the convergence of researchers from CONICET, the Pablo Cassará Foundation, and the Pablo Cassará SRL Laboratory, accompanied by state policies of the MINCyT, for transforming science and technology into diagnostic kits.

SYMPOSIUM OF BIOLOGY SOCIETIES

A7
LETHAL AND SUBLETHAL EFFECTS OF NATURAL PRODUCTS OF PLANT ORIGIN ON PEST INSECTS

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The global demographic growth of the last few years has significantly increased the qualitative and quantitative demand for agricultural food products, which is why strategies aimed at an increase in productivity, for instance, the use of agrochemicals, have grown. Given that the application of synthetic pesticides causes a serious imbalance in ecosystems, current research focuses on the search for bioactive natural products, of microbial or plant origin, capable of controlling pest organisms in agricultural products. The secondary metabolites of plants constitute an interesting alternative due to their rapid biodegradability and low environmental impact. Our team works on obtaining natural products of plant origin and assessing their toxic effects on pest insects in important crops for the regional economy. Based on morphological and molecular analyses, we intend to identify compounds, which interfere especially in physiological processes typical of insects such as molting or ecdysis, as a means to reduce the undesirable effects of nonspecific pesticides on the environment and human health. From the collection of plant-based material to the formulation of a bioproduct suitable for field application, an interdisciplinary research team is required. The effects of botanical products from regional flora in the form of extracts, sub-extracts,

and essential oils, were assayed through toxicity and repellency bioassays. *Senecio rudbeckiaefolious* is one of the plant species with the most promising results due to its pyrrolizidine alkaloid (PA) content. Interesting lethal and sub-lethal effects of PA were proven on *Diatraea saccharalis* (Lepidoptera: Crambidae) larvae, the main pest in sugar cane in North-western Argentina, as well as in corn in the Pampas region, and on two species of insects which frequently infest the walnut warehouses of La Rioja and Catamarca: *Orizaephilus surinamensis* (Coleoptera: Silvanidae) and *Plodia interpunctella* (Lepidoptera: Pyralidae). It was demonstrated through microscopic studies that PA produces a high percentage of mortality in *O. surinamensis* larvae due to irreversible damage caused to the midgut epithelial lining. Among the essential oils, the one made from *Lippia turbinatta* (Verbenaceae) was found to repel *O.surinamensis* larvae and be toxic to *P. interpunctella* larvae, probably interfering with the gene regulation of the neuroendocrine system involved in the process of ecdysis. The identification of the responsible active principles of the toxic effects and the elucidation of the modes of action provide scientific proof for the incorporation of these phytosanitary products in sustainable pest management programs.

A8
FOOD PRODUCTION AND TECHNOLOGICAL CHANGES. FOOD SECURITY AND SOVEREIGNTY

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At a global level, there were great technological milestones that emerged with the purpose of feeding the world, from the Marshall plan, and the green revolution, to industrial agriculture and agribusiness. In order to rethink food production and the concepts of Food Security and Sovereignty, a bibliographic search of different authors was carried out that put in tension the impacts produced by the production models that prevail in Argentina. In this sense, we could express that at present said agro-export system is fragile and unviable due to the size of its ecological footprint, the resources that are needed to sustain it, and the level of exploitation that is required; it is systemically vulnerable and all it takes to expose it could be a shortage of oil, due to its high requirements in fossil fuels. The modes used in the food production of the industrial-exporting agricultural model produce environmental pollution mainly due to the indiscriminate use of agrochemicals and the lack of treatment of effluents from intensive animal production establishments. Although technically these problems are solvable, the technologies to respond are usually not viable in poor countries due to economic variables and the high costs they entail, among other issues. At the same time, they have produced an increase in poverty due to the loss of territory with the consequent cultural degradation of the peoples, the disappearance of the diversity of local crops and the flora and fauna of the ecosystems, added to the contamination of the soil and water. This has repercussions on people's health, causing diseases and phenomena of a different nature, which can occur acutely or chronically, in addition to contributing to antimicrobial resistance, due to their excessive use in the intensive production of cattle, pigs, and birds. It is considered of great importance, in the implementation and development of new technologies, that the academy and the sciences promote having a broader and more ethical vision of the aspects that surround and cross the agri-food systems and all their components; under the perspective of the "One Health" approach and with the results of long-term epidemiological studies related to such technological developments.

A9
INFERRING HOMOLOGOUS RECOMBINATION EVENTS IN PLANT MITOCHONDRIA USING HIGH-THROUGHPUT SEQUENCING DATA

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Homologous recombination (HR) plays a crucial role in shaping the complex architecture of plant mitogenomes. Somatic hybrids between distant species offer a remarkable model to study the genetic composition after mitochondria fusion. Recently, our lab has found highly chimeric mtDNA in two somatic hybrids between the Solanaceae *Nicotiana tabacum* and *Hyoscyamus niger* unrevealing that the vast majority of rearrangements occurred by the break-induced replication (BIR) pathway of HR. To examine the recombination map of a somatic hybrid between *N. tabacum* and *Physochlaina orientalis* (Solanaceae), we performed high-throughput sequencing and developed a novel bioinformatic strategy to infer recombination events without using the hybrid mitogenome. Using this pipeline, we inferred a total of 107 HR events occurring mostly (74.77%) by the initiation of the BIR pathway. Interestingly, the detailed data offered by the PE reads showed that independent events frequently occur in the same regions in more than half (56.60%) of the recombining tracts. In addition, we re-analyze two somatic hybrids between tobacco and *H. niger* rebelling that recombination events tend to occur in the same mitogenome regions. These results together suggest the existence of recombination hotspots in plant mitogenomes. Our technique allowed us to deeply study the somatic hybrid recombination map. Nonetheless, further analysis needs to be done to completely understand the mechanism of mitochondrial recombination.

A10
MODULATION OF SIGNALING PATHWAYS AND TRAFFIC IN NEURONAL DEVELOPMENT

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During early neuronal development, a crucial and necessary event is the process called neuronal polarity or axonal specification, regulated by the expression and localization of spatiotemporally specific proteins, cytoskeletal dynamics, and the action of extracellular signals required to induce neuronal polarity and migration. During the development of the cerebral cortex of the central nervous system (CNS). The SARA protein (Smad Anchor for Receptor Activation) was originally identified as a protein that recruits unphosphorylated Smads2/3 to activated TβRI (Transforming Growth Factor Beta-Receptor I). In primary cultures of hippocampal neurons from rat embryos (*in vitro* model), we observed SARA-positive endosomes, with domains containing endosomal markers (EEA1, Rab4 and Rab11), and when SARA was suppressed (with a specific shRNA), an alteration of the delivery to somatodendritic (transferrin) and axonal proteins (adhesion molecule L1) to the membrane. Through *in utero* electroporation in mice (*in vivo* model), we demonstrated that SARA participates in the neuronal migration process during neocortical development, regulating L1 traffic. Recently, we have identified that SARA is a negative regulator of the TGFβ pathway, since the loss of function of SARA keeps the pathway over-activated, thus participating as a modulator of neuronal development. Finally, we focus on the biological role of SARA during the development of sensory neurons in the peripheral nervous system (PNS). We analyze the participation of SARA in axonal growth in dorsal root ganglia (DRG) neurons and if this role is also associated with the TGFβ pathway. In DRG embryonic cultures, we observed that both the TβRI and SARA are endogenously expressed in the early stages of development of these neurons, showing a significant increase in expression at 12 h of culture, coinciding with the beginning of axonal development and growth in these neurons. Finally, we analyze if there is physical interaction between SARA and TβRI, by AP-FRET (Acceptor Photobleaching Förster Resonance Energy Transfer) in endosomes of DRG. The FRET efficiency under basal conditions and after aggregating the TGF® ligand, confirm such interaction. Together, these findings show that the activation of the TGFβ pathway, mediated by SARA, participates in the regulation of neuronal development in both the CNS and the SNP. This work contributes to understanding how the mechanisms and molecules manage to regulate them and allow the neurons to establish and grow the axon successfully, constituting a key and necessary link to understanding physiological and pathological processes of neurodevelopment.

YOUNG SCIENTISTS' SYMPOSIUM

A11
THE NOVEL ROLE OF TANKYRASE DURING EMBRYONIC GENOME ACTIVATION IN MAMMALS

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The "oocyte-to-embryo transition" encompasses the series of developmental events that gives rise to the totipotent cleavage-stage preimplantation embryo. Critical aspects of this transition include meiotic maturation-associated recruitment for translation of specific maternally stored mRNAs, widespread chromatin remodeling, and initiation of transcription from the embryonic genome, or "embryonic genome activation" (EGA). In this work, we show a novel role of tankyrase (TNKS), a poly(ADP-ribosyl) polymerase that regulates β-catenin levels. TNKS undergoes programmed translation during oocyte maturation and serves an essential role in mouse EGA. Newly translated TNKS triggers proteasomal degradation of axin, reducing targeted destruction of β-catenin and promoting β-catenin-mediated transcription of target genes, including *Myc*. MYC mediates ribosomal RNA transcription in 2-cell embryos, supporting global protein synthesis. Suppression of tankyrase activity using knockdown or chemical inhibition causes loss of nuclear β-catenin and global reductions in transcription and histone H3 acetylation. Chromatin and transcriptional profiling indicate that development arrests prior to the mid-2-cell stage, mediated in part by reductions in β-catenin and MYC. Our findings indicate that post-transcriptional regulation of tankyrase serves as a ligand-independent developmental mechanism for post-translational β-catenin activation and is required to complete EGA.

A12
ALTERING ARBOVIRUS SEQUENCE SPACE FROM BEING "GENERALISTS" TOWARDS BECOMING INSECT-SPECIFIC "SPECIALISTS"

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The compositional properties of viral genomes are informative about their origin and evolution. Arboviruses (arthropod-borne viruses) replicate in insects that transmit them to mammals, their second host. Thus, arboviruses have been exposed to the mutational biases of both hosts from significantly different phyla. Here, we applied principal component analysis (PCA) and multidimensional scaling (MDS)

approaches to more than 8000 viral genomes to find meaningful patterns. We observed that arboviruses present a dinucleotide under-representation in CpG and UpA, whereas insect-specific viruses (ISVs) were only under-represented in UpA. Using the Mayaro virus (MAYV) as a model, which causes a Dengue-type syndrome, we have, by computer design and synthetic biology, rationally altered this dinucleotide frequency balance in favor of insect-specific viruses (ISVs). Recoded MAYVs are currently under investigation to define their replication kinetics in insects as well as in mammalian cells. This approach suggests a previously unidentified possibility to develop new antiviral strategies against a broad spectrum of different human arboviruses.

A13
DEVELOPMENT OF AN ORAL VACCINE PLATFORM WITH SURFACE PROTEINS FROM *Giardia lamblia*

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Overwhelming evidence demonstrates the benefits of immunization as one of the most successful and cost-effective health interventions achieved to date. Although parenteral immunization is generally effective in eliminating systemic infections, it often fails to establish protective responses on mucosal surfaces, where most infectious agents initiate infection. Mucosal vaccines target the common mucosal immune system and achieve a more complete protective immune response by conferring both local and systemic immunity. Oral immunization is the form most widely used of the mucosal vaccines due to its ease of administration. However, to achieve proper bioavailability oral vaccines must avoid antigen degradation by digestive proteases. We have recently developed a vaccine platform that leverages the properties of *Giardia lamblia*'s variant-specific surface proteins (VSPs) to allow oral immunization of subunit vaccines. VSPs cover the entire surface of this parasite that inhabits the upper gastrointestinal tract where digestive enzymes have their highest concentration. Since VSPs have outstanding resistance to proteases and to changes in pH, high immunogenicity, and absence of toxicity, we engineered them to accommodate at a virus-like particle (VLP) and demonstrated that they could confer protection to a viral antigen displayed on the particle. As a proof of concept, mice were orally immunized with VLPs containing glycoproteins of influenza virus and VSP1267. Immunized mice generated an efficient humoral and cellular, mucosal and systemic immune response that protected them from infection with the virus and from tumors expressing viral antigens. These exciting results prompted us to study the application of this platform as a potential vaccine for several other viruses. In conclusion, this novel vaccine platform provides new opportunities in the field of vaccine technology to ultimately achieve more efficient vaccines with better patient compliance.

SHORT COMMUNICATIONS

DEVELOPMENTAL BIOLOGY AND REPRODUCTION 1

A14
IN OVO EXPOSURE TO ENDOSULFAN OR ATRAZINE INDUCES ALTERATIONS IN THE MOLECULAR MECHANISMS THAT GOVERN OVIDUCTAL ADENOGENESIS IN THE BROAD-SNOUTED CAIMAN (*Caiman latirostris*)

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The formation of glands (adenogenesis) is a process that characterizes the postnatal development and differentiation of the oviduct in the broad-snouted caiman (*Caiman latirostris*). Previously we reported that the oviduct of prepubertal juvenile caimans expresses proteins involved in the differentiation and invagination of the epithelial cells, such as wnt-7a, wnt-5a, β-catenin and FoxA2. Additionally, we demonstrated that early exposure to endocrine disrupting compounds (EDCs) bisphenol A and 17β-Estradiol alters the expression of these proteins. While epithelial differentiation is necessary for adenogenesis, the process may not be carried out if the surrounding subepithelium, highly rich in collagen, is not adequately rearranged. Thus, the aims of this study were: (a) to evaluate the expression of MMP2 and MMP9, proteins involved in the disaggregation and rearrangement of collagen fibers, and their inhibitors (TIMP1 and TIMP2), and the expression of β-catenin, a protein related to epithelial cells differentiation and invagination, in the oviduct of juvenile *C. latirostris*; (b) to evaluate the effects of *in ovo* exposure to EDCs endosulfan (END; 20 ppm) and atrazine (ATZ; 0.2 ppm) on the expression of the mentioned proteins. Protein expression levels were evaluated by immunohistochemistry, quantified by image analysis and reported as integrated optical density arbitrary units, while mRNA expression levels were evaluated by qPCR and reported normalized by the expression of a housekeeping gene (L8). Our results show that the studied proteins are expressed in the oviduct of *C.*

latirostris and may be playing an important role in oviductal adenogenesis by inducing changes at epithelial and subepithelial levels. *In ovo* exposure to END upregulated the expression of MMP2 (VEH 2.03 ± 1.85 *vs.* END 7.54 ± 5.94), MMP9 (VEH 1.26 ± 0.21 *vs.* END 3.66 ± 2.69) and TIMP1 (VEH 1.74 ± 0.78 *vs.* END 2.61 ± 0.67), and it downregulated the expression of β-catenin in the glandular epithelium (VEH 8.92 ± 2.20 *vs.* END 6.13 ± 2.59); *in ovo* exposure to ATZ upregulated the expression of MMP9 (VEH 1.26 ± 0.21 *vs.* ATZ 2.92 ± 1.90). Our results demonstrate that the signaling pathways that govern oviductal adenogenesis are affected by exposure to END and ATZ. Changes in the differentiation of the oviduct may alter fertility and development of eggs in *C. latirostris*.

A15
PARTICIPATION OF ENDOGENOUS LIPIDS IN CAPACITATION AND ACROSOME REACTION OF CRYOPRESERVED BOVINE SPERMATOZOA

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Studies on *in vitro* capacitation and acrosome reaction in cryopreserved bovine spermatozoa are, mostly, performed in mediums containing pyruvate and lactate as oxidative substrates. There is not enough evidence about the role played by endogenous lipids in those processes. The aim of this work was to study the role of endogenous lipids in *in vitro* capacitation and acrosome reaction of cryopreserved bovine spermatozoa. Samples were incubated in capacitating conditions (with the addition of 6mg/mL BSA and 2mM $CaCl_2$) in different mediums: TALP (with lactate and pyruvate), TA (without lactate or pyruvate), TALP + heparin (capacitation inducer), TA + heparin (capacitation inducer), TA + heparin + etomoxir (inhibitor of fatty acids oxidation) and TA + heparin + carnitine (inducer of fatty acids oxidation). After capacitation and acrosome reaction (induced by 30% bovine follicular fluid), progressive motility (by optic microscopy), viability (by trypan blue stain), capacitation (by chlortetracycline fluorescent technique), and true acrosome reaction (by differential interferential contrast with trypan blue stain) were evaluated. Data were statistically analyzed by ANOVA and compared with the Bonferroni test. A $P < 0.05$ was considered statistically significant. In TALP medium with heparin, spermatozoa capacitated and responded to the induction of acrosome reaction. In TA medium with heparin, spermatozoa preserved progressive motility but failed to capacitate or respond to acrosome reaction induction (versus TALP + heparin, $P < 0.05$). The addition of etomoxir (TA + heparin + etomoxir) significantly diminished progressive motility and sperm viability (versus TA + heparin, $P < 0.05$). The presence of carnitine significantly improved the percentage of capacitated spermatozoa and the acrosome reaction (versus TA + heparin, $P < 0.05$), without reaching the values of TALP + heparin. These results suggest that during the incubation in a capacitating medium free of oxidative substrates, cryopreserved bovine spermatozoa can use endogenous lipids as a source of energy for sperm capacitation and acrosome reaction. Future studies on the utilization of other oxidative substrates would complement these results and would contribute to elucidating the metabolic pathways used by cryopreserved bovine spermatozoa to obtain the energy required for those processes.

A16
PARTICIPATION OF ADENYLATE CYCLASE ISOENZYMES, SOLUBLE AND MEMBRANE-ASSOCIATED, IN CRYOPRESERVED BOVINE SPERMATOZOA CAPACITATED WITH HEPARIN

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Soluble and membrane-associated isoenzymes of adenylate cyclase are present in the intracellular signal system of sperm capacitation. It is interesting to determine which inducers and inhibitors can activate or block them to unchain this sperm process, since the way these enzymes modulate the signals is controversial. The objective of this study was to determine the capacitation, viability, motility, and mitochondrial activity of cryopreserved bovine spermatozoa treated with heparin and inhibitors of the soluble and membrane-associated adenylate cyclase enzymes. Heparin was used as a capacitation inducer, LRE-1 as a soluble adenylate cyclase inhibitor, and 2,5-dideoxyadenosine as a membrane-associated adenylate cyclase inhibitor. Capacitation was evaluated by the chlorotetracycline epifluorescent technique and viability and membrane integrity by the trypan blue vital staining with interferential differential contrast. Sperm motility was evaluated by microscopy and analyzed with the ISAS-Prosier software. Mitochondrial membrane potential was measured using JC-1 fluorochrome. Data were analyzed by ANOVA and Tukey's test ($P < 0.05$). Percentages of heparin-capacitated sperm and sperm with positive mitochondrial membrane potential (65.00 ± 11.14%) were significantly higher than control, samples treated with heparin/LRE-1 and heparin/2,5-dideoxyadenosine. Heparin significantly increased total motility and progressive motility compared to its control, 17.84 ± 5.70% and 11.97 ± 4.35% respectively, and to samples treated with heparin/LRE-1 and heparin/2,5-dideoxyadenosine. Amplitude of lateral head displacement and beat-cross frequency were significantly decreased in heparin/inhibitors samples compared to heparin-capacitated samples. In heparin-induced *in vitro* capacitation in cryopreserved bovine spermatozoa, adenylate cyclase isoenzymes play an important role, demonstrated when these enzymes were blocked, causing inhibition of capacitation and decreased mitochondrial function and sperm motility.

A17
EFFECTS OF HUMAN CHORIONIC GONADOTROPIN (hCG) HYPERSECRETION ON THE REPRODUCTIVE PHYSIOLOGY OF PERIPUBERAL FEMALES. STUDIES IN A MURINE MODEL

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The hypothalamic–pituitary–gonadal axis plays a fundamental role in the endocrine regulation of reproductive function in mammals. Any alteration it may suffer, either in the control of the different participating hormones or in the receptors involved, may lead to alterations in the onset of puberty, infertility, development of cancer, and other dysfunctions. A model of human chorionic gonadotrophin hormone (hCG) hypersecretory transgenic mice has been described, which overexpresses the β subunit of this hormone (hCGβ+) and causes phenotypic alterations in females that severely compromise their reproduction: infertility, obesity, elevated steroidogenesis and hyperprolactinemia accompanied by the development of prolactinomas in adulthood. To understand the alterations observed in adulthood, the phenotype of immature hCGβ+ female mice (at 3 weeks of age) was characterized. Transgenic females exhibited precocious puberty and a significant increase in the uterine weight at the age studied ($P < 0.001$). At the hormonal level, in addition to the increase in hCG levels ($P < 0.0001$), a significant increase in serum progesterone levels was observed ($P < 0.0001$). Gene expression of *Gnrhr*, *Lhb*, *Fshb*, and *Prl* in the pituitary was analyzed (by RT-qPCR), resulting in a decrease in all cases ($P < 0.01$), except for *Prl*, which showed a significant increase ($P < 0.001$). In ovary, the gene expression of steroidogenesis-related enzymes (*Star*, *Cyp11*, *Cyp17*, *Cyp19*) and luteinization markers (*Lhcgr*, *Prlr*, *Pgr*) were significantly increased in hCGβ+ females ($P < 0.0001$) at the age studied, whereas, among folliculogenesis markers (*Amh*, *Fshr*, *Inha*, *Inhba*, *Inhbb*, *Esr2*), only Amh showed significant differences ($P < 0.01$). Finally, follicular count analyzed by histological sections showed a reduction in primary follicle populations ($P < 0.0001$), and an increase in antral ($P < 0.001$), atretic ($P < 0.001$), and corpora lutea ($P < 0.05$) in hCGβ+ females compared to WT. In conclusion, hCG stimulates an excessive production of gonadal steroids and prolactin from early ages, generating precocious puberty and premature luteinization in the ovary and consequently, leading to the infertility observed in adulthood.

A18
AMINO ACIDS AS UNIQUE OXIDATIVE SUBSTRATES DURING PORCINE OOCYTE NUCLEAR AND CYTOPLASMIC MATURATION

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The aim of this work was to study the implication of amino acids (Aa) as unique oxidative substrates during porcine oocyte nuclear and cytoplasmic maturation *in vitro*. Immature cumulus–oocyte complexes (COCs) were obtained by aspiration of antral follicles from slaughtered gilts and then selected under a stereomicroscope. Oocytes surrounded by a dense cumulus were randomly distributed into five groups: NCSU-37 without pyruvate or glucose, NCSU-37 + glucose, NCSU-37 + Aa, NCSU-37 + Aa + glucose, and NCSU-37 + Aa + salicylate (Aa catabolism inhibitor). All the groups were matured for 44 h at 39ºC, 5% CO_2 and 100% humidity. To determine meiotic maturation percentages oocytes were denuded and then stained with Hoechst 33342. The nuclear status of each oocyte was analyzed using an epifluorescence microscope with 330–380 (excitation) and 420 (emission) filters at 400*x*. To evaluate Aa catabolism, we used a spectrophotometric assay based on NADPH oxidation by glutamate dehydrogenase, quantifying the residual ammonia in each maturation medium. Data was expressed as ammonia production/COC/min. To determine cytoplasmic maturation, COCs were co-incubated with 1×10^6 motile sperm/mL for 3 h in modified Tris Buffer medium with 0.4% serum bovine albumin. Then zygotes were transferred to NCSU-23 medium under mineral oil at 39ºC, 5% CO_2 and 100% humidity, and the blastocyst rates were evaluated at day 7. Maturation percentages and blastocyst rates were compared using a Chi-square analysis for non-parametric data. The levels of residual ammonia in the maturation media were expressed as mean ± standard error mean, and their interactions were analyzed by two-way ANOVA, using post-hoc general contrasts for comparison among treatments. Values with a $P < 0.05$ were considered significant. Oocytes matured in media supplemented with Aa as unique oxidative substrates or in combination with glucose presented higher nuclear maturation rates than those incubated with glucose or without supplementation ($P < 0.05$). In coincidence, the medium containing Aa as unique oxidative substrates resulted in a higher level of residual ammonia compared with the other groups ($P < 0.05$). On the other hand, the addition of salicylate generated a reduction in the nuclear maturation percentage and in the residual ammonia in the maturation medium ($P < 0.05$). The medium with Aa + glucose presented a higher blastocyst rate compared the medium with glucose ($P < 0.05$), but it didn't differ respect to the medium supplemented with Aa. In conclusion, our results suggest that amino acids could be used as unique oxidative substrates for porcine oocyte meiotic and cytoplasmic maturation *in vitro*.

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BIOCHEMISTRY, PHYSIOLOGY, AND PATHOLOGY

A19
ASSESSMENT OF ESSENTIAL OILS CHEMICAL PROFILE of *Baccharis spartioides* IN THREE ANNUAL CONSECUTIVES HARVESTS

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The complexity of the chemical composition of essential oils may be due to biological characteristics of the plant species and/or the edaphoclimatic conditions where they grow. The standardized quality of an essential oil is difficult to achieve, since the largest number of variables have to be managed before, during and after harvest. The objective of the work was to compare the chemical composition of essential oils of *Baccharis spartioides* in three harvests carried out in consecutive years (2017–2018–2019). The harvests were made in December each year, in a low saline area located at the GPS coordinates: 37°10'42.73" (S) and 64°17'09.32" (W). Yield percentage of oil essentials, physicochemical properties, and chemical profile by CG–MS were measured. The results obtained in physicochemical characterizations and in the chemical profile of the years 2017–2018 were similar. However, the 2019 harvest differed from the other years both in yield, physicochemical properties, and the chemical profile of the oil. The visual appearance (color), the essential oil component profile, and other measured parameters seem to indicate a greater susceptibility to oxidation in the material harvested in 2019. The abundance of non-oxygenated terpenes practically disappeared, which implies a reduction in the oil quality. Although it is not possible to determine the reasons why the material from this harvest was affected, it can be suggested that specific edaphoclimatic conditions of that year influenced such susceptibility. For this reason, it is important to investigate the environmental factors that may be influencing the growth of plants during the corresponding year, which affect the stability and quality of the essential oil.

A20
COMPOSITIONAL PROFILE OF ESSENTIAL OILS OF *Origanum vulgare* IN TWO DRYING CONDITIONS

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Origanum vulgare L. is an aromatic herbaceous plant that belongs to the Labiaceae family. The essential oil obtained from this species is considered of commercial interest by the gastronomic, pharmaceutical, cosmetic and food industries due to its organoleptic characteristics and its use as an antimicrobial, antifungal, antioxidant, among others. One of the aspects that determines the quality of the product is the post-harvest treatment, particularly the drying stage. Plant material not properly dried is damaged and has no commercial value and, therefore, it is discarded. The objective of the work was to evaluate the physicochemical properties, the compositional profile of the essential oils and yields of *O. vulgare* in two drying conditions of the plant material. The chemical profile was determined by CG–MS and the percentage yield of essential oils from flowers of the Don Bastia variety. The plant material was dried for 10 days, in an environment with shelter from light and natural air circulation, rotation every 24 h and without rotation. The yield of the essential oil decreased by 40% when plant rotation was not carried out. However, the essential oil obtained in both drying conditions showed similar physicochemical properties and relative abundance of the major components, thymol and cis-β-terpineol, characteristic of the chemotype. This result indicates that material that has not been properly dried and is disposed of, can be used to obtain essential oils with similar quality to that obtained from properly dried materials. Therefore, it is possible to generate added value in materials that are discarded due to their appearance and no commercial value.

A21
IDENTIFICATION OF GENES MODULATED BY PROBIOTICS IN COLORECTAL CANCER

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Probiotics are recognized for their beneficial effect in intestinal pathologies, but their use in therapeutic schemes is still under study due to the complexity of the molecular mechanisms involved in their action. We previously observed that *Lactobacillus casei* (*L. casei*) and *Bifidobacterium breve* (*B. breve*) orally administered in a mixture of probiotic strains to rats with colorectal cancer (CRC) and treated with capecitabine delayed tumor development, improved clinical manifestations and overall survival of animals. The aim of this work was to identify genes and molecular functions associated with the observed effect of both probiotic strains on CRC through an analysis

of gene expression profiles and their integration into protein–protein interaction (PPI) networks. The Gene Expression Omnibus (GEO) database and GEO2R analysis software were used to analyze differentially expressed genes (DEG) in microarray expression profiles of CRC patient samples, including GSE41258, GSE37364, GSE68468, and GSE44076. The DEGs ($log_2FC > 1$ and p adj < 0.05) were incorporated into the FunRich software, and 174 overexpressed genes (Up) and 218 common negatively regulated genes (Do) were identified in the tumor samples from the four CRC datasets. Furthermore, the expression profiles of the Caco-2 cell line derived from CRC co-cultured with *L. casei* (DN-114001) or with *B. breve* (DN-156007) (GSE37369) were evaluated. The expression profile analyzed in the Caco-2 cell line resulted in 129 genes Up and 57 genes Do under the effect of *L. casei*, and 379 genes Up and 310 genes Do under the effect of *B. breve*. The functional enrichment by Enrichr showed that the genes Up by the effects of both strains are involved in cancer signaling pathways (p adj < $8.37e^{-05}$) and the Do genes in the FOXO signaling pathway (p adj < $2.76e^{-04}$), among others. The overlap between the DEG in CRC and the DEG in the cell model co-cultured with probiotics resulted in 2 Up genes and 12 Do genes in CRC that reverted their expression due to the effect of both strains, finding the major change in the growth/differentiation factor-15 (GDF-15) gene. This factor is also known as the macrophage inhibitory cytokine-1 (MIC-1). In addition, using Cytoscape software, the integration of DEG expression in the PPI network showed that GDF-15 is associated with the epithelial membrane protein 1 (EMP1), a tumor suppressor in CRC, and with the activating transcription factor 3 (ATF3) involved in the regulation of immune responses. This bioinformatic analysis indicates that these probiotics could have an immunomodulatory effect through the regulation of GDF-15 in CRC. Experimental studies are necessary to validate this data.

A22
CYTOTOXICITY OF *Prosopis flexuosa* ALKALOID-ENRICHED EXTRACTS ON GLIAL CELLS

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Genus *Prosopis* spp., commonly known as “algarrobo”, is a tree found in different regions of the world. In Argentina, *P. flexuosa* are geographically distributed throughout different ecoregions, including Chaco, Espinal Norte, and Pampeana. *Prosopis* pods are among the ancient food resources used for animal feed, due to their nutritional value. Although its consumption is quite widespread, in Brazil, neurological symptoms have been observed in animals associated with long-term intake of pods of another species of this plant, *P. juliflora*. In Argentina, intoxications with *P. flexuosa* have been reported in goats and cattle. Astrogliosis is considered an early marker of neurotoxicity as it is generally detected before any other toxic effects on neurons. This could be associated with the presence of the juliprosine and juliprosopine alkaloids previously identified in the total extract of *P. flexuosa* pods by HPLC–HRMS. The aim of the present work was to determine the cytotoxicity on glial cells of alkaloid enriched extracts of *P. flexuosa* collected in three different years, to evaluate the stability/variability of secondary metabolites present. Briefly, C6 Glioma cells (ATCC:CCL-107™) were resuspended and seeded in 96-well microplates, at initial density of 3.0–3.5 $\times 10^4$ cells per well in growth medium (DMEM-SFB 10%). When monolayers reached 80% confluence, different concentrations (10–50 µg/mL) of *P. flexuosa* extracts collected in the years 2019, 2020, and 2021 were added to the cells (200 µL/well). After 48 h of incubation at 37°C and 5% CO_2, cell viability was quantified by crystal violet staining. Results showed that *P. flexuosa* extracts from the three years studied, decreased cell viability in a dose-dependent manner. However, the extracts obtained from the years 2019 and 2020 proved to be more toxic (50 ⌈g/mL: 74% and 76% cytotoxicity, respectively) than those from 2021 (50 ⌈g/mL: 36% cytotoxicity). These results show a possible variation in the stability and /or concentration of the alkaloid components present in *Prosopis flexuosa* pods according to the year of collection.

A23
IN OVO EXPOSURE TO ATRAZINE ALTERS THYROID HISTOMORPHOLOGY IN LATE POSTNATAL BROAD-SNOUTED CAIMAN (*Caiman latirostris*)

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Previously, we demonstrated that prenatal exposure to environmentally relevant doses of the herbicide atrazine (ATZ) induce hypothyroid-like alterations in female *Caiman latirostris* thyroid gland, being the female caimans more sensitive to the exposure than males. However, if those alterations occur during juvenile stage of development or are the result of subtle changes that occur at earlier developmental stages remains unknown. Our objective was to assess if exposure to ATZ induces alterations in the thyroid gland of caimans at earlier developmental stages and to confirm sex-related effects due to the exposure. Eggs were incubated at female or male producing temperature (30°C or 33°C, respectively) and before sex determination takes place, they were exposed to 0.2 ppm of ATZ or ethanol (vehicle). When caimans reached the late postnatal stage (3 months), thyroid glands were excised and processed to paraffin embedding. Histomorphological studies (% of the gland occupied by stroma, epithelium, and colloid; follicular density; follicular area; follicular epithelial height; percentage of microfollicles and percentage of follicles showing grade 1, 2, or 3 of hyperplasia) and epithelial proliferative activity were performed on 5-µm thick tissue sections stained with PAS or immunomarked for proliferating cell nuclear antigen, respectively. Our results were expressed as mean ± SEM for vehicle *vs.* ATZ. In female caimans, ATZ exposure decreased follicular size (5902 ± 1301 *vs.* 2444 ± 260 μm^2) and follicular epithelial height (11.31 ± 1.88 *vs.* 6.32 ± 0.36 µm) but increased the percentage of follicles showing hyperplasia grade 2 (4.33 ± 1.36 *vs.* 13.98 ± 2.65 %), the percentage of microfollicles (0.003 ± 0.003 *vs.* 0.075 ± 0.030), and the proliferative activity of the follicular epithelium (1.450 ± 0.661 *vs.* 2.985 ± 0.069 %). In males, ATZ exposure increased the proliferative activity of the follicular epithelium (1.202 ± 0.248 *vs.* 3.850 ± 0.483 %). The results regarding proliferative activity would explain the increased incidence of hyperplastic follicles (type 1 in males, types 2 and 3 in females) previously observed

in juvenile caimans. On the other hand, these results confirm that female caimans are more sensitive to ATZ exposure than males and that the alterations in thyroid gland observed at juvenile stage begin at earlier developmental stages, suggesting that exposure to ATZ induce organizational effects that become evident as caimans grow and mature.

A24
STAT3 BLOCKADE IN COMBINATION WITH CHEMOTHERAPY: TOWARDS A RATIONAL TREATMENT IN TRIPLE NEGATIVE BREAST CANCER

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Breast cancer (BC) is the most frequent tumor in women (2.250.000 cases/year) and the leading cause in mortality worldwide (685.000 cases/year) and in our country. Triple negative breast cancer (TNBC) accounts for 15–20% of the cases and shows the worst survival. TNBC is associated with young Hispanic or African American women (< 35 years), advanced stages at diagnosis, increased risk of metastasis and poor response to treatment, which consists solely in systemic chemotherapy (CT). Stat3 is an oncogene constitutively activated in ~60% of BC and promotes tumoral progression, immune system evasion, and CT resistance. We studied Stat3 blockade in combination with CT in TNBC and demonstrated that inhibition of Stat3 induces cellular senescence in the murine TNBC model 4T1 and releases a senescence-associated secretome phenotype (SASP) enriched in cytokines, induced by type I interferon (IFN I), which are key to the antitumoral response. We evaluated the effect of conditioned medium produced by cells transfected with control siRNA (Ctrl-SASP) or siRNA targeting Stat3 (Stat3-SASP) and measured angiogenesis through HUVEC cells proliferation, proliferation of T lymphocytes and tumor cells by [^{3}H]-thymidine incorporation, and cell migration using the wound healing assay. Stat3-SASP exhibited antitumoral and immunostimulatory properties given that induced T lymphocyte proliferation and inhibited proliferation and migration of tumor cells and decreased the angiogenic potential. We established 4T1 tumor in Balb/C mice (10000 cells/mouse s.c.) and were treated with 1 mg/kg i.p. JSI-124 daily (pharmacological inhibitor of Stat3), 5 mg/kg i.p. doxorubicin (Doxo) twice a week, Doxo + JSI or vehicle (V) as a control. Doxo or JSI alone reduced tumor growth ($P < 0.001$ *vs.* V) but simultaneous administration showed a synergistic effect with a 50% of tumor regression. Organotypic cultures of 4T1 tumors were treated with 10 μM JSI or 100 nM Doxo, and samples were collected after 24 h for Western blot. Doxo induced an increase in Stat3 activation measured by Tyr705 phosphorylation, whilst JSI diminished it. Stat3 blockade induced Stat1 activation, main mediator of IFN I production. In conclusion, we consider that the combination of Stat3 blockade with CT is a promising treatment for TNBC and we propose that measurement of Stat1/Stat3 activation in organotypic cultures of patient's tumors could be used to predict CT response leading to the implementation of rational treatments based in precision medicine.

A25
ATTACK RATE OF *Spodoptera frugiperda* SMITH AND ITS NATURAL ENEMY COMPLEX IN THE SANTIAGO DEL ESTERO IRRIGATION AREA

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In Santiago del Estero, the fence is a traditional production system that associates several crops. Studies have shown that *S. frugiperda* is one of the most harmful insect pests for this system. However, there are not enough bibliographic references to studies carried out in Santiago del Estero that indicate the levels of attack of *S. frugiperda* in corn crops, monocultures or polycultures, or its natural enemies. The objective of this research was to analyze the attack rate of *S. frugiperda* under two planting systems and determine the presence, relative importance, and frequency of natural enemies (parasitoids and predators) that affect the populations of S. *frugiperda* in zones I and III of the irrigation area of Río Dulce. The trial was planted in the first week of February 2021. For the attack rate, the association between the *Cucurbita moschata* (Duchesne ex Lam.) and the corn variety (Leales 25 plus) was evaluated. The treatments evaluated were: T1, Monoculture Leales 25; T2, Intercalary Leales 25-Anquito. For the evaluation of the damage, 1 m was taken at random, and the total number of plants and plants damaged was recorded; a subjective scale ranging from 0 to 3 was used. The attack rate was calculated as follows: affected plants / plants checked, if the larva was present, the larvae captured in the corn plants were artificially fed until the emergence of the adult or parasitoid; predatory insects were recorded by Barber-type soil traps. The highest attack rate values corresponded to the polyculture system with a maximum value of 51%, coinciding with the first true leaf of the crop, while in the monoculture the maximum value was 26% and occurring in the V2 stage of the crop. Of the captured larvae, the highest parasitoidism value was recorded in stage V3 (third true leaf). The most frequent parasitoids were: *Chelonus insularis* (Cresson) (Hymenoptera: Braconidae) with an IR of 23% and *Archytas marmoratus* (Townsed) (Diptera: Tachinidae) with an IR of 33%. The percentage of total parasitoidism in *S. frugiperda* larvae was 15.63%. In the Barber traps, a total of 162 predatory and parasitoid insects were caught, of which the most abundant predator family was the family Carabidae (Coleoptera) with a relative frequency of 31.48%.

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GENERAL, CELLULAR, AND MOLECULAR BIOLOGY

A26
STUDY OF FGD6 PROTEIN AS A NOVEL REGULATOR OF NEURONAL ACTIN CYTOSKELETON DINAMIC ORGANIZATION

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In the CNS, Rho GTPases regulate and coordinate the actin cytoskeleton dynamics, contributing to the regulation of several processes in neurons. FGD6 belongs to a family of proteins with similar domain organization, which suggests that they may function as cross-linkers between plasma membrane and the actin cytoskeleton. Although FGD6 is associated with Cdc42 and Rac Rho GTPases, its function in CNS is still unknown. This work aimed to characterize FGD6 function in neurons as a new modulator of intracellular pathways that impact in actin cytoskeleton dynamics. Using RT-qPCR, we studied FGD6 expression during SK-N-SH neuronal cell line differentiation and in rat primary cortical neurons. Cdc42 and Rac activities were assessed by FRET and Rho GTPase activity biosensors in SK-N-SH cells transfected with a specific siRNA for FGD6. Finally, we analyzed the effect of FGD6 knockdown in actin state and the expression of some actin-related cytoskeleton proteins downstream Cdc42/Rac by IF and WB, respectively. We observed modulation of FGD6 expression during neuronal differentiation. In SK-N-SH neuroblastoma cells, FGD6 knockdown results in Rac1 activation, a decrease in Arp2 expression and F-actin disassembly with no changes in the total actin pool. In neurons FGD6 expression is tightly regulated during cell maturation, and FGD6 knockdown affects actin cytoskeleton dynamics. This may in turn hamper axonal growth, spine formation, and neuronal activity, among others.

A27
IDENTIFICATION AND PHENOTYPICAL CHARACTERIZATION OF WILD STRAINS OF *C. elegans* IN ARGENTINA

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The free-living nematode *Caenorhabditis elegans* is a widely used invertebrate model in biomedical sciences. Most of the studies on this animal come from a single laboratory-adapted strain obtained in Bristol, England (N2 Strain). To identify fundamental biological processes that are masked in this laboratory strain of *C. elegans*, in recent years, several scientists have directed their efforts to the isolation and characterization of wild strains. In the present work, we performed the isolation and phenotypic characterization of the first *C. elegans* strain obtained from soil samples from Argentina. Our phenotypic analyses reveal substantial differences with the laboratory strain. First, the velocity of movement in liquid of the isolated strain (OAR137) is significantly higher than that of laboratory strain N2 ($P < 0.001$, ANOVA test). This result, together with the fact that the OAR137 strain is more resistant to the paralytic action of the nicotinic agonist levamisole, suggests important differences in neuromuscular transmission. Besides the differences in movement, we found that OAR137 individuals exhibit a clumping population pattern, with social aggregation of animals (compared to the solitary and dispersed pattern characteristic of N2) and leave fewer offspring (213.50 ± 29.689 animals/mother for OAR137 *vs.* 292.75 ± 35.704 animals/mother for N2). Taking advantage of the availability of several strains isolated in other regions of the world, whose genome is sequenced, the feasibility of performing Whole Genome Sequencing in the strain that we isolated, and our experience in the analysis of neural circuits and neuromuscular junction of *C. elegans*, we will try to identify the molecular basis of the phenotypic differences observed in locomotion. Given the conservation of fundamental processes throughout the animal kingdom, this study could contribute to the universal understanding of nerve transmission.

A28
DIFFERENTIAL SENSITIVITY OF CRC CELLS TO CHEMOTHERAPEUTIC DRUGS: STUDY OF MECHANISMS INVOLVED

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Colorectal cancer (CRC) is one of the leading causes of cancer death worldwide, and chemoresistance is the main obstacle for the treatment. Parathyroid hormone-related peptide (PTHrP) is a cytokine that is involved in the initiation, growth, and invasion of various carcinomas. In CRC cells, we found that this peptide promotes events related to the aggressive behavior of tumor cells such as chemoresistance to irinotecan (or CPT-11, a drug used for CRC treatment). Based on these antecedents, this work aimed to evaluate whether PTHrP induces resistance to chemotherapeutic agents in the HCT116 cell line derived from CRC, and the molecular mechanisms

involved in this process. HCT116 cells were treated with oxaliplatin (10 µM), doxorubicin (5 µM) or 5-fluorouracil (10 µM) in the presence or absence of PTHrP. Trypan blue dye exclusion test showed that oxaliplatin and doxorubicin significantly decrease the number of viable cells. However, PTHrP treatment attenuates the cytotoxicity induced by both drugs. Besides, the antitumor effect of 5-fluorouracil was effective in HCT116 cells but PTHrP did not interfere with its cytotoxicity. We previously observed that, in HCT116 cells, PTHrP activates the signaling pathways of β-catenin and Met (a receptor with tyrosine kinase activity), which are key in the progression of CRC. The inhibition of Met and β-catenin pathways using specific inhibitors restored the cytotoxicity of CPT-11, oxaliplatin, and doxorubicin even in the presence of PTHrP, suggesting that this cytokine decreases the sensitivity of CRC cells to these three drugs through both pathways. To evaluate the impact of the tumor microenvironment on this chemoresistance, we performed the same experiments using a conditioned medium (CM) from the stromal endothelial HMEC-1 cells. Preliminary studies suggested that the CM of these cells, previously treated with PTHrP, attenuates the cytotoxic effect of the drug CPT-11. This work expands the knowledge of the molecular mechanisms associated with PTHrP-induced chemoresistance in CRC cells.

A29
EARLY EFFECTS OF FACTORS RELEASED BY TUMOR CELLS DERIVED FROM CERVICAL CANCER ON THE MORPHOLOGY AND PROTEIN PROFILE OF VASCULAR ENDOTHELIAL CELLS

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Tumor angiogenesis plays a crucial role in cervical cancer (CC) from early stages. Research for potential biomarkers and therapeutic targets has been hampered by heterogeneity, plasticity, and molecular differences of the endothelial cells that form the tumor vasculature. This is due, in part, to the effect of factors released by tumor cells. The aim of this work was to investigate the early effects of factors released by CC-derived tumor cells on the morphology and protein profile of endothelial cells. Treatment with CC-derived HeLa cells conditioned medium (TCM) for 3 h increased the number of HMEC-1 endothelial cells with cytoplasmic processes and a more elongated shape. Furthermore, qRT-PCR analysis revealed that HMEC-1 cells exposed to TCM decreased VE-cadherin endothelial marker mRNA and increased α-smooth muscle actin mRNA, a cancer-associated fibroblast marker. These findings could be associated with early stages of tumor angiogenesis characterized by increased cell migration and a partial transition from endothelial to mesenchymal phenotype (EndoMT). Next, the proteome response of HMEC-1 cells was studied under these experimental conditions, performing a Label-Free quantitative (LFQ) mass spectrometry (MS) (CEQUIBIEM Proteomics Center). Proteins were identified and quantified with the Proteome Discoverer software and the Uniprot database. Using the Perseus software, 9 negatively regulated proteins and 24 positively regulated proteins were obtained in HMEC-1 cells treated with TCM ($P \leq 0.05$ and fold change > 1.5), where clusterin (CLU) presented the highest fold change. CLU is a glycoprotein with a key role in cellular stress response and cancer, regulating processes such as cell migration. In addition, the classification of positively regulated proteins, according to their class, with the PANTHER bioinformatics tool, showed 2 cytoskeletal proteins, actin alpha cardiac muscle 1 (ACTC1) and tubulin beta-8 chain (TUBB8). ACTC1 can promote cell migration and modulate the length of actin tension fibers. qRT-PCR additional analysis also revealed an increased mRNA expression of CLU and ACTC1 in HMEC-1 cells treated with TCM. These results suggest that the recently identified proteins are involved in the early stages of biological processes leading to angiogenesis or EndoMT in CC and could be considered as potential biomarkers.

A30
THE DOPAMINERGIC AGONIST CABERGOLINE INDUCES AUTOPHAGY IN PANCREATIC BETA CELLS *IN VITRO*

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The dopamine D2 receptor (D2R) plays an important role in glucose homeostasis. Acting at the central nervous system and pituitary level, D2R agonists have shown an inhibitory effect on hyperglycemia in patients with type 2 diabetes, while the administration of neuroleptics, which antagonize dopamine receptors, causes hyperinsulinemia in normal subjects, or is associated with diabetes in psychiatric patients. Concordantly, D2RKO mice show hyperglycemia and decreased glucose-stimulated insulin secretion, highlighting a role of pancreatic D2Rs. *In vitro* studies point to the participation of pancreatic D2R not only in insulin secretion, but also in β-cell proliferation and apoptosis. On the other hand, autophagy constitutes a mechanism for maintaining cellular homeostasis through the constant elimination of potentially toxic ubiquitinated proteins and damaged organelles. Since its dysregulation, in the pancreatic β-cell, contributes to the development of diabetes, modulators of autophagy could be novel therapeutic agents for the treatment of human diabetes. Previous data indicate that D2R activation can alter autophagy in various cell types, though no data have been presented in pancreatic β-cells. Therefore, we studied the effect of the D2R agonist cabergoline (CAB) on the autophagy process in the MIN6B1 murine pancreatic β-cell line. Cell cultures were stimulated with CAB 10^{-5} M for 1, 6, and 24 h in order to evaluate the kinetics of autophagic vesicle formation, and on the other hand, with CAB 10^{-5} M for 24 h in the presence or absence of chloroquine (CQ), a late-stage autophagy inhibitor, in order to analyze autophagic flux. The autophagy markers LC3 (autophagic vesicle marker) and p62/SQSTM1 (receptor of cargo to be degraded by autophagy, and substrate degraded by autophagy) were analyzed by immunofluorescence and confocal microscopy. The results showed an increase in LC3 nucleation as a function of the stimulation time

with CAB, observing significant differences relative to the control after 6 h (repeated measures ANOVA, $P < 0.05$; Tukey: CAB 6 h *vs*. Control, $P < 0.05$; CAB 24 h *vs*. Control, $P < 0.05$). In addition, the number of p62 *puncta* did not show significant changes in the time period studied, although it showed a tendency to decrease at 24 h compared to the control condition, suggesting an increase in its degradation. Autophagic flux studies showed a significant increase in LC3 *puncta* in the presence of CQ (two-way repeated measures ANOVA, $P < 0.005$; Tukey: CQ *vs.* Control, $P < 0.001$), as expected, and an even greater increase when cells were incubated simultaneously with CQ and CAB (CQ-CAB *vs.* CAB, $P = 0.0005$). Therefore, we conclude that CAB is able to increase the formation of autophagosomes and to induce autophagic flux after 24 h of stimulation in the pancreatic β cell line MIN6B1. *[This work was financially supported by CONICET, ANPCyT, Fundación René Barón, and Fundación Williams.]*

A31
EFFECT OF *Bothrops diporus* VENOM ON THE VASCULAR AND RENAL SYSTEM OF *Gallus gallus domesticus* EMBRYOS

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The majority of snakebites in northeastern Argentina are caused by *Bothrops diporus* ("yarará chica"). The venom proteome of this snake comprises PI- and PIII (SVMPs), phospholipases A_2 (PLA_2s), serine proteases (SVSP), L-amino acid oxidases (LAOs), and vasoactive peptides responsible for its myotoxic, proteolytic, and hemorrhagic effects. One of the animal models used to evaluate the toxic effects of biological compounds is the chicken embryo (*Gallus gallus domesticus*). Eggs of this species are used since it was proved that the pathways responsible for pain receptivity are not fully formed until embryonic day 13. Thus, the aim of this work was to evaluate the changes induced by *B. diporus* venom in the vascular (heart and chorioallantoic membrane, CAM) and renal systems on this animal model. Briefly, fertilized chicken eggs of *G. g. domesticus* were disinfected and then incubated at 37°C and 65% relative humidity, rotating them periodically to prevent embryos from sticking to the shell membranes. The viability of the embryos and the vasculature of the CAM were visually inspected. On day 8 of incubation, the eggs were removed from the incubator and disinfected with 70% ethanol. Then, a 0.5 × 0.5 cm hole was made in the blunt end of the eggs shell, above the embryo and 1 mL of venom solution (1 mg/mL) or physiological solution (controls) were injected and sealed with parafilm to avoid contamination and desiccation. After 24 h incubation, parafilm paper was removed, the orifices were enlarged, and embryos were removed for histological evaluation. Histopathological results evidenced, specifically in the cardiac tissue of the atrial region, the disorganization and loss of muscle fibers. The capillaries showed abundant prolongations and the light partially occluded; the thickness of the endothelial wall was variable. In the ventricular region, areas of necrosis and infiltrate of leukocytes were observed. In chorioallantoic membranes, an increase in capillary thickness was observed, accompanied by cytoplasmic acidophilicity and the presence of abundant hydropic and swollen cells. Endothelial vascular damage caused intense edema, which can be associated with hypoxic and ischemic processes that would generate cell death. Kidney tissue also revealed areas of disorganization in the tubular ducts with dilation and marked increase in acidophilicity. The renal corpuscles showed alterations and loss of their normal structure. Regarding the glomeruli, damage was evidenced as rupture of the capillaries accompanied by generalized renal congestion. These observed effects are probably due to the synergistic action of SVMPs that induce bleeding, blistering, dermonecrosis, and degradation of the components of the extracellular matrix and cell membrane proteins, and PLA_2s that induce myonecrosis and affect lymphatic vessels. In conclusion, *Bothrops diporus* venom induces alterations that contribute to ischemia and necrosis of the vascular and renal tissue of *Gallus gallus domesticus* embryos.

A32
SUGARCANE: ANATOMIC COMPARISON OF THE VASCULAR BUNDLES OF TWO CONTRASTING BIOTYPES

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Sugarcane (*Saccharum* spp.) is the most important industrial crop planted and the main source of sugar in Argentina. Recently, the interest as an energy crop has increased as it combines several desirable features for the production of cellulosic ethanol. For this reason, the study of stalk tissues is key to understanding lignin deposition, an important aspect in the deconstruction of biomass. The aim of this work was to compare the anatomy of the internodes of LCP 85-384 (LCP384), the cultivar with the largest area currently planted in Argentina, and INTA 05-3116 (INTA3116), a promising energy-cane biotype. The length, width, and diameter of metaxylem of vascular bundles (VB) were measured at four developmental stages (T: tillering, GG: grand growth, ER: early ripening, and LR: late ripening) and different internode positions above soil level (IP1, IP5, IP10, IP15, and IP20). Internodal sections were fixed in FAA solution (formaldehyde–alcohol–acetic acid) solution for 48 h and preserved in 70% ethanol. Freehand sections were manually cut and stained with a phloroglucinol-HCl solution for 5–10 min; the stained sections were rinsed in distilled water, mounted on a slide, and viewed with a light microscope (DMRXP, Leica). It was observed that in the medulla, the VB had an organized distribution, while in the cortex, under the epidermis, a higher density of superimposed VB was observed. For this reason, only the medullary VB were analyzed, showing that IP1 was responsible for the longest VB for each genotype. INTA3116 showed significantly longer VB (372.7–536.8 µm) than LCP384 (350.6–434.3 µm) in three development stages, except for in GG. In LCP384, the width of the VB increased from higher to lower IP, while in INTA3116 it decreased in the opposite direction. LCP384 had wider VB (364.9–420.2 µm) than INTA3116 (327.5–362.9 µm), from GG onwards. In LR, diameter of both metaxylems revealed differences, being the upper internodes

responsible for carrying higher values than the lower internodes. LCP384 presented larger metaxylem diameter (109.8–129.5 µm) than INTA3116 (100.6–118.4 µm) in the four developmental stages. This analysis showed clear anatomical differences between genotypes, since the VB were shorter, wider, and the diameter of the metaxylems was longer in LCP384 than in INTA3116.

A33
POTENTIAL ROLE OF THE PI3K/AKT PATHWAY IN VASCULOGENIC MIMICRY FORMATION IN CERVICAL CANCER

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The alternative microvascular system in which highly invasive tumor cells mimic endothelial cells by forming blood vessel-like structures is known as vasculogenic mimicry (VM). This process is strongly correlated with poor prognosis, metastasis, and resistance to anti-angiogenic therapy in various cancers, including cervical cancer (CC). The objective of this work was to identify potential molecular mechanisms involved in the formation of VM in CC, through *in silico* and *in vitro* analysis. Cytoscape 3.8.2 stringApp was used to visualize molecular networks with a confidence score greater than 0.9 from the STRING database related to CC and VM. VE-cadherin (CDH5) and the EPH A2 receptor (EPHA2) are the two proteins with the highest text-mining score of the VM-related network. The intersection of both networks showed 12 proteins shared by both terms, including the serine/threonine kinase AKT1. In addition, using the GEPIA2 online platform, we analyzed the correlation between the expression of CDH5 with AKT1 in human samples from patients with CC and obtained a *P*-value < 0.01 (R = 0.22). From this *in silico* analysis, *in vitro* studies were carried out to investigate the potential role of AKT1 in the formation of VM in CC cells. Using RT-qPCR, we observed that the treatment of CC-derived HeLa cells with LY294002 (5 µM), a phosphatidylinositol-3-kinase (PI3K) inhibitor, which is an enzyme involved in the activation of AKT1, decreases the mRNA levels of CDH5. Furthermore, preliminary studies revealed that exposure of these cells to LY294002 inhibits tube-like structure formation of HeLa tumor cells. These results suggest a potential role for PI3K and its most probable effector AKT1 in VM formation in CC. Expanding the knowledge of the molecular mechanisms associated with VM in CC will help identify potential biomarkers that contribute to predicting the prognosis and predetermining resistance to anti-angiogenic therapy.

A34
HUMAN DERMAL FIBROBLASTS ISOLATED FROM ADULT DONORS ARE *IN VITRO* MODULATED BY MANGANESE

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Skin aging is characterized by a progressive loss of regenerative potential. At the cellular level, that means dermal fibroblasts are disabled to proliferate, migrate, release growth factors, and synthesize extracellular matrix. Thus, it is of biomedical interest to study different therapeutic strategies to revert the fibroblasts' aged phenotype and to optimize the repair capacity of skin layers. These strategies may include the use of bioactive ions with geroprotective actions, able to reactivate and reset cellular and molecular mechanisms involved. Manganese (Mn^{2+}) is a bioactive ion linked to oxidative stress-related molecular process. However, to date, its geroprotector potential has not been studied. With this in mind, the present study aimed to stablish a protocol for the isolation and culture of dermal fibroblasts from adult donors (three donors, age range 40–60 years), and to analyze the *in vitro* geroprotector potential of Mn^{2+}. Skin explants were histologically characterized using hematoxylin–eosin and the fibroblasts were isolated. The cells were analyzed under optical microscopy, and their proliferation and migration capacities in response to Mn^{2+} were measured. Histological evaluation demonstrated that isolated fibroblasts were mostly papillary dermis residents and had a lean, spindle-shaped morphology. Flow cytometry results showed a decreased G2/M phase in the aged fibroblasts compared to their younger counterpart (two donors, age range 20–30 years). Furthermore, the aged fibroblasts showed a significant reduction in the proliferative and migratory responses compared to young fibroblasts, but this reduction could be reverted under Mn^{2+} stimulus. This work allowed to stablish a human dermal fibroblast suitable model to identify bioactive ions with geroprotective function and, additionally, to set the basis for the study of the Mn^{2+} geroprotector potential. These findings may be relevant for the development of new technologies for skin care and rejuvenation.

A35
GASTROINTESTINAL PARASITE EFFECTS ON THE MAMMARY PUBERTAL DEVELOPMENT AND ON THE FIRST LACTATION IN HOLSTEIN BOVINE

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Gastrointestinal nematode infections delay the growth and fattening in bovine. Our previous investigations evidenced negative effects on the onset of puberty and the mammary gland development in parasitized Holstein heifers. In the present work, we aimed to study the influence of gastrointestinal parasites on the proliferation and angiogenesis of the peripubertal mammary gland and analyze the productive and reproductive parameters of these heifers in their adult life. Twenty Holstein calves were randomly assigned, at birth, to an untreated control group (C) or to the treated group (T), which received, from birth onward, monthly anthelminthic treatment rotating different drugs. At 20 (prepuberty), 30 and 40 (peripuberty), and 70 weeks of age (postpuberty) biopsies from the mammary gland were taken. In order to evaluate mammary IGF system, the expression of IGF-1 and IGFR mRNAs was assayed. We determined an increment in IGFR mRNA in peripubertal C heifers ($P = 0.002$), probably as a compensatory mechanism due to systemic IGF-1 decrease we had described at that age. Instead, no differences were observed on IGF-1 mRNA expression levels. Cell proliferation was evaluated with PCNA marker by Western blot (WB), and we found an increased expression in T 40 weeks heifers respect to C ($P = 0.014$). In order to study angiogenesis involvement, the levels of CD34 endothelial cell marker were determined by WB, and an increased expression in T peripubertal heifers ($P = 0.01$) was observed. The microvascular density was analyzed with the smooth muscle cell marker αSMA, by immunohistochemistry. A tendency to increase the microvascular density was found in T peripubertal heifers. Moreover, a decrease in VEGF levels of C 30 weeks heifers was observed ($P = 0.0025$) while no differences were found in Endocan mRNA levels. On the other hand, when productive and reproductive parameters were analyzed on these heifers in their adult life, an increment in DIM (days in milk) at first lactation was observed in C (434 d) *vs.* T (351 d) ($P = 0.0075$), while there were no differences in total liters produced. Besides, birth to conception interval was increased in C ($P = 0.018$). We conclude that gastrointestinal nematode infections affect the proliferation and angiogenesis in peripubertal mammary development, which is probably related to lower productive and reproductive success of these cows.

BIOTECHNOLOGY, GENETICS, AND NEUROSCIENCES

A36
ASSEMBLY OF MITOCHONDRIAL COMPLEXES IN THE BRAIN OF A RAT MODEL OF ALZHEIMER

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Mitochondrial respiratory complexes (CI, CII, CIII and CIV) are associated in super complexes (SCx) (SCx1: I + III2 + IIn; SCx2: I + III2 + IV1, SCx3: I + III2 + IV2, SCx4: I + III2 + IV3 and SCx5: I2 + III2). Although its role in mitochondrial respiration is still controversial, the study of SCx (dis)assembly is relevant to understand the causes of brain bioenergetic dysfunction reported in Alzheimer's disease (AD). In this work, we evaluate the organization of SCx in an animal model of AD-like brain amyloidosis, McGill-Thy1-APP transgenic rats (Tg) and controls (CNT). Mitochondria from the hippocampus of 12-month-old animals (N = 3 per group) were isolated, and the organization and abundance of SCx were analyzed by electrophoretic runs in native gels (BN-PAGE) stained with Coomassie Brilliant Blue G-250. The functionality of the CI was assessed by *in-gel* activity. The densitometric analysis of the bands showed that both Tg and CNT do not present SCx2 and SCx3, unlike that reported in mice. In Tg, we observed a statistically significant decrease in CI abundance ($P = 0.01$) and increase in SCx1 ($P = 0.01$) and SCx5 ($P = 0.02$). CI activity was higher in Tg ($P = 0.008$), however, not significant differences between genotypes were detected for SCx4. We did not detect CI activity on the SCx1 or SCx5. These preliminary results suggest that (1) the organization of SCx is different between species; (2) the total abundance of CI is the same between genotypes (Tg and CNT); and (3) in Tg, CI is active only disassembled or associated with SCx4. We speculate that, in Tg, a greater activity of CI alone could be responsible for a greater production of reactive oxygen species (ROS). We postulate that, in Tg, there could be structural alterations in CI mediated by brain amyloid deposition that impact on its functionality once associated with SCx, challenging the hypothesis that assembly *per se* promotes functionality.

A37

CHARACTERIZATION OF A TRYPSIN-LIKE ENZYME ISOLATED FROM CAECA PYLORIC OF *Pygocentrus nattereri* (PALOMETA) AND ITS USE FOR COLLAGEN HYDROLYSIS

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A large amount of protein waste is generated by the fishing industry, such as skin and viscera; however, the latter constitutes an important source of proteases, such as trypsin. This serine protease exhibits high activity over a wide range of pH and temperature; these characteristics have made it suitable for different applications in industry, e.g., production of hydrolyzed proteins. The Argentine Northeast has a big variety of fish species; among them, *Pygocentrus nattereri* ("palometa") is a freshwater fish with a carnivore-type diet and voracious habits, so its viscera are a rich source of proteases. Thus, the goal of this work was to isolate this alkaline protease from the viscera of the "palometa" fish, in order to characterize the enzyme and evaluate its hydrolytic activity on the collagen obtained from the skin of the same fish species. The pyloric caeca were extracted, ground, and homogenized with 50 mM Tris-HCl buffer (pH 7.8), sonicated, centrifuged, and the supernatant was submitted to affinity chromatography (Benzamidine-Sepharose), eluted with 25 mM acetic acid, pH 4.5 and 3.2. Isolated protein concentration was evaluated using $Abs_{280\ nm}$, the specific activity on BApNA-10 mM and its purity verified by SDS-PAGE. The effect of pH (from 2 to 14) and temperature (from 0 to 100 °C) was determined using azocasein (5 mg/mL). Protease inhibitors (TBSI, PMSF, and EDTA-Na2) were assayed on BApNA. The proteolytic activity of trypsin was evaluated at 0.5, 1, 2, 3, and 24 h, on "palometa" collagen extracted by acid treatment. Enzyme activity was stopped by heating in a boiling water bath for 5 min. The cleavage of collagen was analyzed by SDS-PAGE. The trypsin-like serine protease was purified, and the molecular mass was 30 kDa, the optimum activity was a pH range of 8.0–10.0, and 60°C was the optimum temperature. The purified enzyme was partially inhibited by PMSF and fully inhibited by SBTI, but it was not inhibited by EDTA. SDS-PAGE analysis showed complete degradation of the α-chains of collagen from "palometa" skin. These results suggest that this trypsin-like enzyme present in *P. nattereri* viscera extract exhibits chemical and kinetic properties (optimal activity at high pH and temperatures, ability to hydrolyze collagen) that make it an attractive candidate for industrial applications.

A38

Crotalus VENOM AS A SOURCE OF BIOLOGICALLY ACTIVE ENZYMES FOR LYSOLECITHIN PREPARATION

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Enzyme-catalyzed processes in industry are more and more numerous, as they have a number of advantages over conventional non-biological catalysts. Phospholipases A2 (PLA2s) are receiving a lot of attention due to their biotechnology potential. It is the family of enzymes most used for the enzymatic modification of lecithin. 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, particularly suitable for use in many industrial applications, such as food technology and the cosmetic and pharmaceutical industry. They are found in invertebrates (bees), mammals (bovine and porcine pancreas), and some microorganisms and in the snake venoms. The aim of this work was to evaluate the use of *Crotalus durissus terrificus* venom (rich in PLA2 enzymes) for lysolecithins production to be used later in the formulation of safe and effective emulsions with industrial potential. The crude lecithin extracts (CLE) were carried out by solvent extractions from egg yolk (20 g): first step with ethanol (96%), then, the soluble fraction was treated with acetone; finally, the precipitated was dried and weighed. Lecithins present in CLE were detected by two tests: (a) precipitation with saturated solution of cadmium chloride 2% in ethanol, and (b) by the relative mobility on thin layer chromatography (TLC), on silica gel 60 F254 using acetone/hexane (1:3) v/v; Cl_3CH/MeOH/acetic acid/water (50:25:8:2) v/v as mobile phase and Dragendorff's reagent as developer solution. Lysolecithins (crude lysolecithins extract, CLyE) were obtained by the action of *C. durissus terrificus* venom (1 mg/mL PBS, 2 mL) on CLE (30 mg/mL), for 30 min at 37°C. The lysofosfolipids presents in CLyE were detected by: (a) TLC and (b) hemolytic test, by measuring of absorbance at 530 nm of supernatant solution after incubation (30 min, T_{amb}) of lysolecithins solution with erythrocytes suspension. The results showed that: (a) solvents extraction was effective to obtain a solid extract (0.32 g) rich in lecithins. An abundant white precipitate with cadmium solution and a spot with Rf 0.47 by TLC assay confirmed the lecithins' presence. (b) *C. durissus terrificus* venom was capable of hydrolyze phospholipids from CLE, obtaining an extract rich in lysolecithins (CLyE). This extract exhibited hemolytic activity and the spot in TLC showed a low retention factor value (Rf 0.20), typical of lysolecithins. These preliminary results demonstrate the ability of crotalic venom to produce lysolecthins capable to disrupt the erythrocyte membrane, as "strong" detergents do. Further studies are required to assess the potential use of these biomolecules to produce lysolecithins able to be used in industry as emulsifiers and detergents.

A39

LACTIC ACID BACTERIA ISOLATION FROM VINAL PODS (*Prosopis ruscifolia*) AND PRELIMINARY CHARACTERIZATION FOR BIOTECHNOLOGICAL PURPOSES

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Lactic acid bacteria (BAL) fermentation increases nutritional and organoleptic properties of foods and extends their shelf life. Plant matrices have a native microbiota that can be used to improve biological and functional properties of foods manufactured with them. In this regard, *Prosopis ruscifolia* is an underused shrub species with nutritional properties that could be improved through fermentation. However, its microbiota and biotechnological potential have not been studied at all. In order to isolate and characterize autochthonous BAL strains from vinal, pods were collected from plants grown in Ibarreta (Formosa). The fresh pods were ground, mixed with sterile water in a 1:1 ratio and incubated under microaerophilic conditions at 37°C for 48 h. Then, the fermented doughs were plated on MRS agar and incubated under the same conditions. Colonies compatible with BAL were isolated, and bacteria were characterized by their morphology, mobility, Gram staining and catalase activity. Acidification capacity and pH reduction were evaluated 24 h after development in a culture medium containing 4% (w/v) pod flour. Amylolytic and proteolytic activities were observed by streaking in MRS agar with 1% (w/v) starch instead of glucose and 1% (w/v) gelatin, followed by the plates staining with Lugol and Coomassie blue, respectively. Significant differences between mean values were determined by Tukey's test ($P < 0.05$) using the InfoStat statistical program. The isolated bacteria were non-motile Gram-positive bacilli, without catalase activity. The pH and acidity were significantly lower in the inoculated broths compared to uninoculated controls. No significant differences were observed between the different isolates. Three isolates showed extracellular proteolytic activity, evidenced by the formation of a halo around the colony after staining the agar with a Coomassie blue solution. None of them showed amylolytic activity after being stained with Lugol. Our results represent the first report of the presence of BAL in *Prosopis ruscifolia*, a species considered an invasive pest, but which could help solve the nutritional problems of the region. More in-depth studies are being carried out on the metabolic activities of the native vinal microbiota in order to evaluate the application of selected microorganisms to the nutritional and functional improvement of vinal and its incorporation into food formulations.

A40

AIR POLLUTION IMPACT ON BRAIN: ANTIOXIDANT RESPONSE IN A RAT MODEL OF UNDERNUTRITION

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Air pollution (gases and particulate matter- PM) can lead to central nervous system (CNS) neuroinflammation and neurodegeneration, which ultimately impair cognitive function, especially in children. Exposure to high levels of ambient PM is associated with neuro and vascular inflammation, being inflammation and oxidative stress two potential biological mechanisms for these adverse health effects. Metal-carrying particles that reach the brain can directly damage neurons and can also cause extensive harm by dysregulating the activation of the immune cells in the brain. Furthermore, child malnutrition is recognized as a major problem with devastating effects on children's health. Thus, children encompass a subpopulation highly susceptible to the adverse effect of environmental pollutants and vulnerable to malnutrition. Therefore, the aim of this study was to characterize morpho-chemically particles of *Residual Oil Fly Ash* (ROFA, an ambient air PM surrogate) and to evaluate, in the brain of malnourished rats, the possible oxidative effect caused by acute exposure to ROFA. The morpho-chemical characterization was carried out by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX). For the nutritional growth retardation (NGR) animal model, Wistar male weanling rats were divided at random in two groups. NGR animals, were fed for 4 weeks a restricted diet 20% compared to *ad libitum* intake of control (C) animals. NGR and C rats were intranasally instilled with either 1 mg/kg BW of ROFA or its vehicle. The brain was isolated, and the oxidative metabolism was spectrophotometrically measured. Antioxidant enzymes activity (catalase-CAT and superoxide dismutase-SOD) and lipoperoxidation (thiobarbituric acid reactive substances-TBARS) were assessed. ROFA proved to be heterogeneous both in size and shape, and its spectral composition confirmed that is mainly composed of amorphous carbon with metallic sulfates and metallic oxide materials, with significant quantity of heavy metals such as Ti, Zn, V, Cr, and Ni. Exposure to ROFA and chronic malnutrition caused a decrease in antioxidant enzymes activity. NGR rats showed a lower capacity to respond to an oxidative stressor such as ROFA when compared to C. In conclusion, air pollution affects antioxidant defenses suggesting that oxidative stress could be in part a pathway of air PM brain dysfunction in malnourished individuals.

A41
SEASONAL VARIATION IN CHOLESTEROL LEVELS IN TYPE 2 DIABETIC PATIENTS AND ITS RELATIONSHIP WITH ENVIRONMENTAL FACTORS

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The seasonality of biochemical parameters in humans has been documented at different latitudes. Among these parameters is cholesterol, transported in macromolecular complexes, the most important of which are LDL and HDL. For its part, diabetes is a chronic metabolic disorder characterized by alterations in metabolism, including lipid. Both diabetes and excess cholesterol are considered risk factors for the development of cardiovascular disease. For this reason, it is relevant to observe whether people with diabetes have seasonal fluctuations in their cholesterol levels that may affect the risk of cardiovascular disease. In the present study, an association was sought between the potential seasonality of cholesterol in type 2 diabetic patients, their habits, and the main environmental variables that characterize the seasons of the year. 467 type 2 diabetic patients, 138 men and 329 women (35–90 years of age) participated in the study. The characterization of the population was carried out through surveys (eating habits, physical activity, lipid-lowering treatment, etc.). In addition, the controls of their biochemical parameters were accessed for a period of 4 years (2016–2019), all this after signing informed consent. The environmental variables (monthly average temperature, cloud cover, and amount of incident solar energy) were described by data extracted from the Argentine National Meteorological Service. Linear mixed models under normal distribution were proposed for the association, which in turn were adjusted for age and sex. It was observed that total cholesterol (TC) and LDL have significantly higher levels during the winter, in relation to the values taken in other seasons (221.63 mg/dL *vs*. 216.43 mg/dL and 122.74 mg/dL *vs*. 118.36 mg/dL, respectively). On the other hand, HDL levels did not show significant differences between stations. The behavior observed in CT and LDL could be explained, partially, through the variations of the environmental factors that characterize the seasons. This is supported by different studies that report a significant increase in cholesterol levels when the body is exposed to periods of lower environmental temperatures and less sun exposure. The latter because there is an inverse relationship between the synthesis of vitamin D and the production of cholesterol. It can be concluded that it is important to deepen the effect of seasonality on the metabolism of diabetic patients and to intensify the lipid profile controls in winter in order to reduce cardiovascular risk.

A42
DETECTION OF GENETIC VARIANTS IN SUGARCANE

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Sugarcane (*Saccharum* spp.) is used in Argentina to produce sugar and bioethanol by fermentation of juices or molasses. It has a highly complex genome (polyploid, aneuploid and genome size ~10 Gb). The use of genomic tools is promising to help the development of multipurpose cultivars (sugar and biomass production). This work aims to reduce the level of complexity of the sugarcane hybrids genome in order to obtain panels of molecular markers that facilitate the study of genetic variability, with special interest in bioenergetic features, in individuals of INTA sugarcane breeding program. For SNPs detection, the partial sequencing protocol ddRADseq (*PstI-MboI*) was applied to four sugarcane cultivars and a high fiber biotype. For variant calling, the monoploid sugarcane genome (cvR570) was used as reference, aligning with Bowtie2, and the Stacks package. An Illumina Novaseq6000 sequencer (150 bp matched end, 10 M reads/individual) was used. Subsequently, filters were applied (quality, reading depth between 56x–200x, MAF 0.1, and no missing data). The Variant Effect Predictor (VEP) from the Ensembl Plants was used to evaluate the potential implications of SNPs. A total number of 460890 variants was obtained. After filtering, 47964 SNPs were retained. Downstream plus upstream gene variants accomplished 61.4%, standing for more than half the variants detected. Intergenic and intron variants hit 9834 (13%) and 9662 SNPs (12.7%), respectively. Around 10% SNPs were localized in coding regions, representing 7756 variants. From this amount: (1) 4071 SNPs were missense variants (52.5%), with possible implications in protein functionality; (2) 3586 SNPs were detected as synonymous variants (46.2%); (3) 19 SNPs caused loss of start codons; and (4) 80 SNPs modified stop codons (55 were gained, 14 were lost, and in 11 codons at least one base was changed, but the terminator codon remained). This study demonstrates the relevance and viability of developing SNP-type molecular markers, using ddRADseq protocols in sugarcane, in the assistance of breeding programs. The 4071 genetic variants with changes in amino acids and, consequently, with putative alterations in protein functionality, are highlighted.

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DEVELOPMENTAL BIOLOGY AND REPRODUCTION 2

A43
EFFECT OF DIFFERENT STRATEGIES OF ARTIFICIAL OOCYTE ACTIVATION ON EARLY EMBRYONIC DEVELOPMENT IN MOUSE MODEL

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The fertilizing sperm triggers the activation of the egg. Artificial oocyte activation (AOA) techniques emerged as an alternative in cases of fertilization failure after ICSI. Since $SrCl_2$ induces Ca^{2+} oscillations in mouse oocytes, but not in humans, the most widely used activating agent for human eggs is the Ca^{2+} ionophore A23187, which induces a single Ca^{2+} peak. The aim of the present work was to study the impact of different AOA strategies on different cellular events that occur during both egg activation and early embryo development, using the mouse as a model. For this, we used different AOA strategies: eggs activated with A23187 in medium without extracellular Ca^{2+}, with one or two stimuli of A23187 in medium with extracellular Ca^{2+}. We used eggs activated with $SrCl_2$ and by *in vitro* fertilization (IVF) as controls. In the first place, we observed that the percentage of activated eggs was similar in all strategies ($P > 0.05$), although a lower proportion of those eggs activated with A23187 presented extrusion of the second polar body after 1 h ($P < 0.05$), suggesting a delay in the activation process. Next, we study the kinetics of cortical granule (CG) exocytosis, using LCA-TRITC. In eggs activated with A23187 with extracellular Ca^{2+} (one or two stimuli), the LCA fluorescence intensity was the highest at 1 h after activation that significantly decreased 6 h post-activation, similar to what was previously observed with $SrCl_2$ ($P > 0.05$). In eggs activated with A23187 without extracellular Ca^{2+} at 1 h, a lower fluorescence intensity ($P < 0.01$) was observed, without changes until 6 h. We then studied the mitochondrial membrane potential using the TMRE specific probe. In all the studied strategies, an increase in the membrane potential was detected, compared to MII eggs ($P < 0.05$), without significant differences among groups ($P > 0.05$). To evaluate early embryo development, the eggs activated with the different protocols were diploidized with cytochalasin D. With any of the activation strategies using A23187, the number of blastocysts obtained was lower than that obtained with eggs activated with $SrCl_2$ or by IVF ($P < 0.01$). In particular, we observed a lower percentage of two-cell embryos in the three activation schemes with A23187 ($P < 0.01$ *vs.* $SrCl_2$ and $P < 0.05$ *vs.* IVF). Likewise, the blastocyst development of the two-cell embryos was affected when the oocytes were activated with an A23187 stimulus with or without extracellular Ca^{2+} ($P < 0.05$), while with two A23187 stimuli it was similar to that of those activated with $SrCl_2$ and by IVF ($P > 0.05$). Taken together, these results indicate that although activation with A23187 does not generate changes in the early events evaluated so far, it could generate a delay in the progression of meiosis, which would lead to defects in early embryo development.

A44
ANANDAMIDE AND CYCLOOXYGENASE-2 REGULATE THE INTERACTION BETWEEN ENDOMETRIAL FIBROBLASTS AND ENDOTHELIAL CELLS

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During pregnancy, an adequate uterine blood flow is required to ensure the formation of the placenta and the delivery of oxygen and nutrients to the developing embryo. This change in blood flow is the result of the process of angiogenesis and remodeling of the maternal vessels. The transformation of these vessels requires a dynamic interaction between different cell types of the maternal–fetal interface, such as trophoblast cells, endometrial fibroblasts, and endothelial cells, among others. Anandamide (AEA) is a lipid molecule from the group of endocannabinoids. AEA shows pro-implantation characteristics and participates in the processes that take place at the maternal–fetal interface during implantation. Furthermore, AEA has been shown to regulate cyclooxygenase-2 (COX-2) pathway, and that COX-2-derived prostaglandins play a crucial role at implantation sites. However, the role of AEA during vascular remodeling of the maternal–fetal interface has not been studied. Therefore, the first objective of the present work was to investigate the effect of AEA on the vascular behavior of endometrial stromal fibroblasts (wound healing assay). For this, T-hESC cells (cell line derived from human endometrial stromal fibroblasts) were seeded in 24-well plates until confluence. The wound was made, and cells were incubated with 1 nM, 10 nM, 100 nM, or 1000 nM AEA for 12 h. The percentage of wound closure was measured as [(T0h cell-free area − T12h cell-free area) × 100] / T0h area. Once the effective concentration of AEA was determined, the participation of COX-2 was evaluated, using a selective COX-2 inhibitor (meloxicam). Subsequently, the effect of AEA in the interaction between endometrial stromal fibroblasts and maternal endothelial cells was evaluated. The supernatants from T-hESC migration assay performed in the presence of AEA were collected, and used as conditioned media in endothelial cell migration (EA.hy926). First, we observed that AEA stimulated the migration of T-hESC cells in a concentration-dependent manner, and COX-2 mediated this effect. On the other hand, the conditioned media from the migration of T-hESC cells incubated with AEA stimulated the migration of EA.hy926 cells. We propose that AEA stimulates the release of soluble factors derived from the COX-2 pathway that regulate the migration of stromal fibroblasts, as well as the interaction between this cell type and the endothelium. Taken together, our results suggest the participation of AEA in the vascular remodeling that takes place in the uterus during early gestation by a mechanism that involves the COX-2 isoform.

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VETERINARY, ANATOMY, HISTOLOGY, AND ANIMAL PHYSIOLOGY

A45
EFFECTS OF AN ABRUPT SALINITY INCREMENT ON GILL NA^+/K^+-ATPase ACTIVITY IN THE "FRESHWATER" SHRIMP *Palaemon argentinus* (CRUSTACEA: DECAPODA: PALAEMONIDAE)

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The "freshwater" shrimp *Palaemon* (= *Palaemonetes*) *argentinus* is able to live and reproduce in fresh water, but also in salinities near sea water. This shrimp is thus a good model to assess the underlying mechanisms that allows tolerance to high salinity, which are still unknown for most aquatic taxa. In the present study, we assessed the Na^+/K^+-ATPase (NKA) activity in isolated gills of *P. argentinus* adults kept in fresh water (1‰) and suddenly transferred to concentrated salinities (15 and 25 ‰) for a short (6 h), a medium (48 h), and a long (≥ 3 weeks) period. Control group (T0) were individuals kept in 1‰ and not transferred. NKA activity was estimated as nmol Pi × min^{-1} × mg $prot^{-1}$. We used the supernatant (10000×*g*, 30 s) from pooled gills homogenate (homogenization medium: 0.25 M sucrose / 0.5 mM EGTA-Tris (pH 7.4) (4 mL buffer × g of $tissue^{-1}$). Total activity was determined by measuring ATP (5 mM) hydrolysis in a reaction medium: 100 mM NaCl, 30 mM KCl, 10 mM $MgCl_2$, 0.5 mM EGTA in 20 mM buffer imidazole (pH 7.4). Basal activity was measured in the same reaction medium, but without KCl and in presence of 1 mM ouabain. NKA activity was the difference between both assays. We analyzed the activity changes (NKA and basal) through exposure times using ANOVAs within each salinity, and Holm-Sidak test for *a posteriori* comparisons *vs.* T0. The activity changes for an exposure time were compared between salinities by Student's *t*. Abrupt transfer of *P. argentinus* from 1 to 15 ‰ induced changes in NKA activity (ANOVA: $F_{(3;\ 34)} = 5.5$; $P = 0.002$), causing a decrement of 35% after 48 h (T0 = 1002.85 ± 172.18 *vs.* 48 h = 345.54 ± 49.69; Holm-Sidak, $P < 0.05$). Abrupt transfer from 1 to 25 ‰ induced changes in NKA activity depending on exposure time (ANOVA: $F_{(3;\ 35)} = 4.802$; $P = 0.006$), although there was no differences from T0. NKA activity at 48 h differed between salinities, it was lower at 15 than at 25 ‰ (345.54 ± 49.69 *vs.* 596.31 ± 77.26, respectively; $t = -2.73$; $P = 0.015$). After long exposure time to concentrated salinity, NKA activity reached values similar to those obtained at T0. Abrupt transfer of *P. argentinus* to concentrated salinities induced changes in basal activity between exposure times (ANOVAs 15‰: $F_{(3;\ 34)} = 3.69$; $P = 0.022$; 25‰: $F_{(3;\ 37)} = 4.43$; $P = 0.01$), but there was no changes compared to T0 (Holm-Sidak $P > 0.05$). After 6 h of exposure, the basal activity was lower in 15 than in 25 ‰ ($t = -2.31$; $P = 0.036$), suggesting that other ATPases, in addition to NKA, are involved in rapid physiological adjustments in response to sudden salinity increment.

A46
MEAT TENDER ANALYSIS BY RAMAN SPECTROSCOPY

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The aim of the present work was to evaluate the physical properties of beef tenderness in Braford bovine steers raised in the northwestern region of Argentina using Raman spectroscopy to determine if this technique could be applied as a fast and accurate tool for evaluating meat quality. Thirty samples of the *longissimus thoracis et lumborum* muscle were analyzed. The hardness of the samples was determined by the texture profile analysis, dividing them into groups: (G1) meat with lower hardness values ("tender") and (G2) meat with higher hardness values ("hard"). Other meat quality parameters were evaluated such as cohesiveness, chewiness, water holding capacity (WHC), intramuscular fat content (IMF); as well as structural parameters by scanning electron microscopy (SEM) such as fiber diameter and sarcomere length. Raman spectroscopy analysis was performed using a 785 nm laser as excitation source to avoid intrinsic fluorescence signals from tissues. No differences were observed in chewiness and fiber diameter between both groups of samples, although higher values of cohesiveness and lower values of WHC, IMF, and sarcomere length were obtained for the G1 samples than for the G2 group. The comparison of Raman measurements together with the principal component analysis (PCA) allowed to differentiate the tender and tough meat samples. The correlation between the sensory attributes obtained from the Raman spectra and the physical measurements resulted in values of R2 = 0.698 for hardness, 0.035 for IMF, 0.006 for WHC, 0.032 for chewiness, and 0.076 for cohesiveness. The high value of Pearson's correlation coefficient ($r = 0.84$) of hardness parameter with the Raman signal at 1003 cm^{-1} assigned to the symmetric vibration frequency of the phenylalanine ring, suggests that the content of this amino acid explains the differences between groups of samples, and allows us to conclude that Raman spectroscopy is an adequate and precise technique to identify Braford beef samples with differential tenderness attributes.

A47

CLUB CELLS: INTRA-SPECIFIC CHEMICAL ALARM SIGNALS AND ANTI-PREDATORY BEHAVIORS IN *Paracheirodon axelrodi* (CHARACIFORMES, CHARACIDAE)

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The cardinal tetra *Paracheirodon axelrodi* (Schultz, 1956) is a species of Characiformes with high commercial value as an ornamental fish in South America, which habits mainly in the Negro and Orinoco rivers. The Characidae family is an important and diverse family of fish, in which until now information on intraspecific chemical communication and associated behaviors is scarce. In previous studies carried out in this group, the presence of Club Cells (CCs) was observed in the epidermis of *P. axelrodi*. Among the functions attributed to CCs is that of containing substances that, when released due to damage to their structure, release substances that alert conspecifics to a possible risk of predation. Taking this into account and the few descriptions in this family, two objectives were set: First, to identify the presence and distribution of CCs in the skin of the adult body of *P. axelrodi* of both sexes, by means of classical histology and immunohistochemistry. Second, to analyze the behavioral displays of individuals subjected to conspecific skin preparations, by setting up an experimental laboratory context and analysis of footage using the Ethovison Software. The CCs in *P. axelrodi* are spherical or ellipsoidal cells, with a diameter between 20–35 µm, which are in the epidermis and present positive immunoreactivity for serotonin, as reported for other species. Regarding the distribution: (1) The presence in the skin of the whole body was confirmed. This distribution shows no changes between males and females; (2) a high density per skin area was observed in the dorsal area continuously from head to tail; (3) in the inserts of the fins and on the epidermis that covers them; (4) a higher density was also observed than in other areas of the body in the ventral area, but with a more discontinuous distribution than in the dorsal area. Regarding behavioral studies, the application of conspecific skin preparations caused changes in the behavior of the animals: (1) They presented erratic swimming movements; (2) showed avoidance of the area of application of the stimulus, significantly reducing the time of permanence in it; and (3) significantly increased the time of inactivity after the addition of the conspecific stimulus. These results allow us to conclude that *P. axelrodi* adults have CCs in the epidermis throughout the body with a more abundant distribution in specific areas of high exposure to predators (dorsal area and fins). Animals exposed to conspecific skin preparations showed a significant increase in behaviors described as antipredatory in other species. The latter supports the hypothesis that CCs present in the skin contain chemical alarm signals that are passively released when skin damage occurs and that alert the rest of the group to a risk of predation.

A48

MUSCLE ONTOGENY IN TELEOST FISH *Odontesthes bonariensis*

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The “pejerrey” (*Odontesthes bonariensis*) is a euryhaline species native to the Pampa region in Argentina whose larval muscle ontogeny has not been described yet. The skeletal muscle in teleost fish is composed of a red muscle and a white one. During larval and early stages of development, both a transition between two different patterns of white muscle fibers generation and a change in the localization of the red muscle take place. To evaluate the transition of this pattern and the processes (hyperplasia and hypertrophy) that determine post-hatching muscle growth, cross-sections of larvae and juveniles, stained with hematoxylin–eosin, were analyzed at different days (1, 2, 3, 4, 7, 17, 22, 60, and 81) post-hatching (dph). The general structure of the skeletal muscle was observed using histological preparation of body cross-sections, and the number, diameter, and area of whole white fibers of a specific myomere in the right epaxial muscle were measured. Micrographs were analyzed with ImageJ Fiji software. At each time point, the white muscle is structured by myomeres. This myomeres arrangement changes at 81 dph, when it acquires the characteristic layout of juvenile fish. A single layer of cells, that will give rise to the mature red muscle, is located under the skin bordering the whole muscle tissue until 22 dph. At 60 and 81 dph the red muscle is found under the lateral line. The study of white fibers size (diameter/area) distribution along the different sampling times showed that the smallest fibers are distributed on the periphery of the myomeres, suggesting that stratified hyperplastic growth has occurred during larval development. In addition, fibers with smaller area are located in the dorsal myomeres of epaxial muscle. However, the number of fibers did not differ significantly (68.6 ± 7.0) between 1 and 22 dph suggesting that stratified hyperplasia took place before hatching. At 60 and 81 dph, fibers of small diameter (< 70 µm) were observed among bigger ones (70–190 µm), a characteristic pattern of muscle growth by mosaic hyperplasia. At the same time, an increase in mean size (diameter) of white fibers between 3 dph (9.7 ± 2.5 µm) and 4 dph (26.4 ± 6.3 µm) was observed, inferring that during this period muscle growth was carried out by hypertrophy. These results demonstrate that muscle development in “pejerrey” and the production of the vast majority of white muscle fibers take place during embryonic growth. Also, these findings suggest that post-embryonic growth is achieved by hypertrophy until 22 dph, resuming the generation of new fibers through mosaic hyperplasia at 60 dph.

A49

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE OLFACTORY EPITHELIUM IN *Paracheirodon axelrodi* (CHARACIFORMES: CHARACIDAE)

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Although the Characidae family is an important and diverse family of fish, information about their olfactory system remains scattered and scarce. Among teleost fish, there are differences in the shape, number, and arrangement of olfactory lamellae, in the distribution of sensory and non-sensory epithelium, as well as in the abundance of various types of olfactory receptor neurons (ORNs). An ultrastructural and immunohistochemical study of the olfactory system of the cardinal tetra *Paracheirodon axelrodi* was carried out (Schultz, 1956). For this, complete heads were dissected for scanning electron microscopy (SEM) studies, as well as for immunohistochemistry, high-resolution optical microscopy (HROM), and transmission electron microscopy (TEM). The olfactory epithelium of *P. axelrodi* is found covering the 12 lamellae that form the olfactory rosette. By SEM, it was observed that the apical surface of the olfactory epithelium presents a dense layer of mucus, and an abundant density of non-sensory ciliated cells (cNSCs) was also observed. From HROM and TEM, all types of sensory and non-sensory cells characteristic of the olfactory epithelium of teleost fish were identified. Three types of ORNs were identified: ciliated (cORN), with microvilli (mORN), and cryptic cells (CC). Immunoreactivity was detected for two neuronal markers: OMP (olfactory marker protein) in the mid-apical region of the olfactory epithelium, and GαO in the mid-basal region of the olfactory epithelium. Based on immunodetections (OMP and GαO) and TEM images, the distribution of sensory and non-sensory epithelium in the olfactory rosette of *P. axelrodi* was determined. The medial area of the lamellae was always sensory, while the distal area was always non-sensory, covered only by cNSC. On the other hand, the interlamellar zone was non-sensory in central areas of the rosette (close to the raphe) and sensory towards the periphery (zone farthest from the raphe). The distribution of sensory and non-sensory cell types in *P. axelrodi* is like that described in our laboratory for *Aphyocharax anisitsi*, another species of the Characidae family, as well as for *Channa punctatus* and *Carassius auratus*. All these species inhabit bodies of water with slow or no flow, and with abundant vegetation. Therefore, it is possible to speculate that this spatial distribution could be related to the type of habitat in which they are found, however, to confirm these patterns, more studies should be carried out on other species in similar environments.

A50

CHARACTERIZATION OF EQUINE TESTICULAR AND EPIDIDIMARY TRANSCRIPTOME

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Spermatogenesis and sperm maturation are complex processes and take place in testes and epididymis, respectively. They are regulated by multiplex genes. The aim of this work was to characterize the transcriptome of equine testes and epididymis for further study of differential expressed genes (DEGs). Testes and epididymis were collected from three stallions. Stallions were more than two years old and without history of reproductive disease or infertility. Samples of 1 cm^2 from each anatomical section (testes, head, body, and tail of epididymis) were taken from each individual (N = 3). mRNA was extracted and purified using the RNeasy mini plus kit (Qiagen). The quantity (ng/µL), quality (agarose 1%), and integrity (RNA Integrity Number, kit RNA Nano 6000 Agilent Technologies CA USA) of isolated RNA were determined. Eleven of twelve samples resulted adequate for the sequencing (NGS, Illumina, Novogene, Durham NC USA). A total amount of 1 µg RNA per sample was used as input material. The bioinformatics analysis was performed with: FASTQ (Quality control), HITSAT (Reads mapping), FeatureCounts (Quantification of gene expression level), DESeq2 (Principal Components Analysis and Pearson Correlation). Samples from each tissue resulted homogeneous with high degree of association among them. This study allowed to identify a library for analyzed tissues: (i) Testes: 232.400.400 clean data reads with 69.35% of reads mapped to exons; (ii) Head epididymis: 96.892.388 clean data reads with 60.50% of reads mapped to exons; (iii) Body epididymis: 191.696.308 clean data reads with 59.41% of reads mapped to exons; (iv) Tail epididymis 155.619.178 clean data reads with 59.04% of reads mapped to exons. These results are the first part of the data analysis and show theoretical basis for futures investigations on stallion's fertility. *[The work was founded by University of Georgia Competitive Intramural Equine Programs Research Initiative.]*

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DIFFERENTIAL EXPRESSED GENES ON EQUINE TESTICULAR AND EPIDIDIMARY TISSUE

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Horses have the lowest reproductive rate of all farm animals (from 43 to 60 % pregnancy rate per cycle). Understanding the mechanisms involved in spermatogenesis and sperm maturation could allow the development of strategies to improve fertility. A key tool for study physiologic process is the analysis of differential expressed genes (DEGs). The aim of this work was to evaluate the DEGs in testes and epididymis (head, body, and tail) in stallions. Four libraries were generated according to each tissue (testes, head, body, and tail of

epididymis) using NGS (Illumina, Novogene; Durham, NC, USA). Data were normalized. Differential expression analysis was performed using the edgeR R package. We find 12158 genes independent-tissue that represented 74.5% of the total genes expressed in testes, 81.8% of the total genes expressed in head, 83.3% of the total genes expressed in body, and 85.8% of the total genes expressed in tail. Moreover, distribution of specific-tissue genes was: (i) 16.3% in testes, (ii) 2.7% in head, (iii) 1.4% in body, and (iv) 1.8% in tail of epididymis. Testes in comparison with epididymis showed clusters of DEGs detailed bellow: (i) head *vs*. testis: DEGs 13349 (5595 up-regulated and 7754 down-regulated), (ii) body *vs*. testis: DEGs 14207 (5884 up-regulated and 8323 down-regulated), (iii) tail *vs*. testis: DEGs 14822 (5842 up-regulated and 8980 down regulated). This experiment provides key information for futures studies about pathways involved on spermatogenesis and sperm maturation. *[The research was founded by University of Georgia Competitive Intramural Equine Programs Research Initiative.]*

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